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Taxonomy and systematics of shallow-water and deep-sea nemerteans (phylum Nemertea) from the western Pacific region

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This dissertation is composed of both published and unpublished taxonomic works. The works presented in Chapters 2.1, 2.2, 3.1, 3.3, and 3.4 have been published in the following papers. Taxon names mentioned in these chapters can be cited, with reference to the original publications. The remaining chapters in this dissertation are not yet published at the time of drafting. According to Article 9 of the International Code of Zoological Nomenclature (4th edition), those chapters or works are not to be regarded as published. Therefore, names mentioned in those unpublished chapters should not be cited in any form.

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Chapter 3.3. Abato, J.C., Yoshida, R., Kajihara, H. 2023. Species description and phylogenetics of *Oerstedia fuscosparsa* sp. nov. (Nemertea: Monostilifera: Oerstedidae) from Japan. *Zootaxa*. 5249(5), 589–597.

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Table of Contents

Citation and disclaimer	ii
Acknowledgements.....	iv
List of Tables	xiv
List of Figures	xvi
Chapter 1. General introduction	1
References	3
Chapter 2. Taxonomy and systematics of shallow-water nemerteans from the Philippines.....	7
References	8
Chapter 2.1. Ribbons under the rocks: intertidal ribbon-worm fauna (phylum Nemertea) from selected islands in central and southern Philippines.....	10
Introduction.....	10
Materials and methods.....	11
Sample collection and processing	11
DNA extraction, PCR amplification, and sequencing.....	12
Species delimitation and haplotype network analyses.....	14
Maximum likelihood analyses	14
Results and systematic remarks	26
Intertidal ribbon worm fauna.....	26
Taxonomy.....	26
<i>Dushia nigra</i> (Stimpson, 1855) species complex.....	26
Material examined	26
Description	27
Taxonomic remarks	28
Distribution.....	28
Lineidae sp. PH23-01	28
Material examined	29
Description	29
Taxonomic remarks	29
Lineidae sp. PH23-02	30
Material examined	30
Description	30
Taxonomic remarks	30
<i>Prosadenoporus spectaculus</i> (Yamaoka, 1940).....	31
Material examined	31
Description	31
Taxonomic remarks	33
Distribution.....	33
<i>Prosadenoporus</i> sp. PH23-01	33

Material examined	33
Description	34
Taxonomic remarks	34
<i>Prosadenoporus</i> sp. PH23-02	34
Material examined	34
Description	34
Taxonomic remarks	35
<i>Monostilifera</i> sp. PH23	36
Material examined	36
Description	36
Molecular analyses on <i>Heteronemertea</i>	36
Molecular analyses on <i>Monostilifera</i>	39
Discussion	41
Conclusions	42
References	43
Chapter 2.2. Occurrence of <i>Baseodiscus quinquelineatus</i> (Quoy & Gaimard, 1833) (Pilidiophora: <i>Heteronemertea</i>) in Mindanao, Philippines	52
Introduction.....	52
Materials and methods.....	53
Results and systematic remarks	54
Conclusions	55
References	56
Chapter 2.3. Diversity of pilidiophorans from the intertidal zones of selected sites in Mindanao, Philippines	59
Introduction.....	59
Materials and methods.....	60
Results and systematic remarks	60
Taxonomy.....	60
<i>Eousia verticivaria</i> Gibson, 1990	61
Material examined	61
Description	61
Taxonomic remarks	62
Distribution.....	62
<i>Notospermus</i> sp. PH24-03	62
Material examined	62
Description	62
Taxonomic remarks	70
<i>Quasilineus pulcherrimus</i> Gibson, 1981	71
Material examined	71
Description	71
Taxonomic remarks	72
Distribution.....	72

Lineidae sp. PH24-01	73
Material examined	73
Description	73
Taxonomic remarks	74
Lineidae sp. PH24-02	74
Material examined	74
Description	74
Taxonomic remarks	74
<i>Baseodiscus jonasii</i> Strand, Hjelmgren & Sundberg, 2005.....	76
Material examined	76
Description	76
Taxonomic remarks	76
Distribution	77
<i>Baseodiscus</i> sp. PH24-01	77
Material examined	77
Description	77
Taxonomic remarks	77
Results and systematic remarks	78
Conclusions	80
References	80
Chapter 3. Taxonomy and systematics of shallow-water nemerteans from Japan	84
References	85
Chapter 3.1. Histology-free description and phylogenetics of <i>Tetrastemma</i> <i>parallelus</i> sp. nov. (Nemertea: Eumonostilifera) from Japan	88
Introduction.....	88
Materials and methods.....	89
Sample collection and processing	89
DNA extraction, PCR amplification, and sequencing.....	90
Maximum likelihood analysis and Bayesian inference	91
Results and systematic remarks	94
Taxonomy.....	94
<i>Tetrastemma parallelus</i> sp. nov. Abato, Yoshida & Kajihara.....	94
Material examined	94
Diagnosis.....	94
Etymology.....	94
Type locality, distribution, and habitat.....	94
Sequences.....	94
Description	94
Molecular phylogeny.....	97
Taxonomic remarks	97

Conclusions	100
References	100
Chapter 3.2. Taxonomy and molecular phylogeny of <i>Tetrastemma</i>	
<i>luteum</i> sp. nov. (Nemertea: Eumonostilifera: Tetrastemmatidae) and	
first record of <i>Tetrastemma phaeobasisae</i> Kulikova, 1987 from Japan	107
Introduction.....	107
Materials and methods.....	108
Sample collection and morphological observation.....	108
DNA extraction, PCR amplification, and sequencing.....	109
Genetic distance and phylogenetic analyses	110
Results and systematic remarks	116
Taxonomy.....	116
<i>Tetrastemma luteum</i> sp. nov. Abato & Kajihara	116
Material examined	116
Diagnosis.....	116
Etymology.....	117
Type locality, distribution, and habitat.....	117
Sequences.....	117
Descriptions of the holotype and paratypes.....	117
Remarks	122
<i>Tetrastemma phaeobasisae</i> Kulikova, 1987	123
Material examined	123
Type locality, distribution, and habitat.....	123
Sequences.....	123
Description	123
Remarks	124
Genetic distance and phylogenetic analyses	125
<i>Tetrastemma luteum</i> sp. nov. taxonomic remarks.....	125
<i>Tetrastemma phaeobasisae</i> taxonomic remarks	128
Phylogeography	129
Conclusions	130
References	130
Chapter 3.3. Species description and phylogenetics of <i>Oerstedtia</i>	
<i>fuscosparsa</i> sp. nov. (Nemertea: Monostilifera: Oerstedtiidae) from	
Japan	137
Introduction.....	137
Materials and methods.....	138
Sample collection and processing	138
DNA extraction, PCR amplification, and sequencing.....	138
Maximum-likelihood analyses and Bayesian inference	139
Results and systematic remarks	143
Taxonomy.....	143

<i>Oerstedtia fuscosparsa</i> sp. nov. Abato, Yoshida & Kajihara	143
Material examined	143
Diagnosis.....	143
Etymology	143
Type locality, distribution, and habitat.....	143
Sequences.....	143
Description	144
Genetic distance and molecular phylogeny	146
Taxonomic remarks	147
Conclusions	148
References	148
Chapter 3.4. Delimiting the polymorphic congeners of the genus	
<i>Oerstedtia</i> Quatrefages, 1846 (Nemertea, Hoplonemertea), and	
descriptions of three new species from the Northwest Pacific	153
Introduction.....	153
Materials and methods.....	155
Sample collection and morphological examination	155
DNA extraction, PCR amplification, and sequencing, species	
delimitation, and phylogenetic analyses	156
Results and systematic remarks	162
Taxonomy.....	162
<i>Oerstedtia pseudoculata</i> sp. nov. Abato, Chernyshev, Hookabe,	
Tsuyuki, Kaushik & Kajihara	162
Material examined	162
Diagnosis.....	163
Etymology	163
Type locality, distribution, and habitat.....	163
Sequences.....	164
Description	164
<i>Oerstedtia rugosa</i> sp. nov. Abato, Chernyshev, Hookabe, Tsuyuki,	
Kausik & Kajihara	166
Material examined	167
Diagnosis.....	167
Etymology	167
Type locality, distribution, and habitat.....	167
Sequences.....	167
Description	167
<i>Oerstedtia viridifusca</i> sp. nov. Abato, Chernyshev, Hookabe,	
Tsuyuki, Kausik & Kajihara	169
Material examined	169

Diagnosis.....	169
Etymology.....	169
Type locality, distribution, and habitat.....	170
Sequences.....	170
Description	170
<i>Oerstedtia phoresiae</i> (Kulikova, 1987)	171
Material examined	171
Type locality, distribution, and habitat.....	172
Sequences.....	172
Description	173
Genetic distance, species delimitation, haplotype network, and phylogenetic analyses	173
<i>Oerstedtia</i> in the Northwest Pacific region	177
Taxonomic history of <i>Oerstedtia pseudoculata</i> sp. nov.	178
<i>Oerstedtia pseudoculata</i> sp. nov. and <i>O. oculata</i> taxonomic remarks	180
<i>Oerstedtia rugosa</i> sp. nov. taxonomic remarks	181
<i>Oerstedtia viridifusca</i> sp. nov. taxonomic remarks	182
Clades <i>Oerstedtia</i> and <i>Paroerstedtiella</i> in the genus <i>Oerstedtia</i> ..	183
<i>Oerstedtia venusta</i> s.str. taxonomic remarks.....	183
Polymorphism in <i>Oerstedtia</i> and its taxonomic implication	184
Conclusions	185
References	186
Chapter 3.5. Species diversity of <i>Tetranemertes</i> (Nemertea: <i>Monostilifera</i>) in Japan	192
Introduction.....	192
Materials and methods.....	193
Results and systematic remarks	200
Taxonomy.....	200
<i>Tetranemertes</i>	200
Taxonomic remarks.....	200
<i>Tetranemertes aurantia</i> sp. nov. Abato, Hookabe & Kajihara ...	202
Material examined	202
Diagnosis.....	202
Etymology.....	202
Type locality and distribution.....	202
Sequences.....	202
Description	202
Taxonomic remarks	204
<i>Tetranemertes kandai</i> (Kato, 1930) comb. nov.	205
Diagnosis.....	205
Taxonomic remarks	205

Remarks	204
<i>Tetranemertes unistriata</i> Cherneva, Ellison, Zattara, Norenburg, Junoy & Maslakova, 2023.	207
Taxonomic remarks	207
<i>Tetranemertes</i> sp. AHK-2024-1	208
Material examined	208
Sequence	208
Description	208
Remarks	208
<i>Tetranemertes</i> sp. AHK-2024-2.....	209
Material examined	209
Sequence	209
Description	209
Taxonomic remarks	210
Results and systematic remarks	210
Phylogeny and genetic distance analysis	210
Species diversity.....	211
Systematics	211
Bioluminescence	216
Conclusions	216
References	216
Chapter 4. Taxonomy and systematics of deep-sea nemerteans from Japan	223
References	224
Chapter 4.1. Ribbons from the deep: deep-sea nemerteans collected along the Japan Trench and the adjacent Kuril-Kamchatka Trench onboard the R/V <i>Hakuho-maru</i> with descriptions of <i>Nemertovema kojimai</i> sp. nov. and <i>Profundonemertes hakuhoae</i> gen. et sp. nov. in Hoplonemertea	226
Introduction.....	226
Materials and methods.....	229
Specimen collection	229
DNA extraction, PCR, amplification, and sequencing.....	230
Genetic distance calculation and species delimitation analysis	231
Phylogenetic analysis.....	231
Results and systematic remarks	255
Palaeonemertea	255
Tubulanidae Bürger, 1904.....	256
Carininidae Chernyshev, 2011	262
Pilidiophora	262
Lineidae McIntosh, 1874	263
Valenciiniidae Hubrecht, 1879	266

Hoplonemertea	266
Polystilifera Brinkmann, 1917	267
Monostilifera Brinkmann, 1917	273
<i>Nemertovema kojimai</i> sp. nov. Abato & Kajihara	276
Material examined.....	276
Etymology	276
Type locality and distribution	276
Sequences	276
Description.....	276
Taxonomic remarks	277
Genus <i>Profundonemertes</i> gen. nov. Abato & Kajihara .	277
Type species	277
Diagnosis	277
Etymology	277
Remarks.....	278
<i>Profundonemertes hakuhoae</i> sp. nov. Abato & Kajihara	278
Material examined	278
Type locality and distribution	278
Sequences.....	278
Description	278
Taxonomic remarks.....	281
Diversity of deep-sea nemerteans.....	282
Palaeonemertean diversity.....	283
Pilidiophoran diversity.....	284
Hoplonemertean diversity.....	286
Conclusions	287
References	288
Chapter 5. Summary.....	300

List of Tables

Table

1	List of the ribbon worm species included in the maximum-likelihood analyses, specimens, localities, cytochrome c oxidase subunit I (COI) GenBank accession numbers, and references.	16
2	Genetic distance (%) summary based on p-distance within and between the two clusters of <i>D. nigra</i> species complex.	39
3	List of <i>Tetrastemma</i> species included in the phylogenetic analysis, GenBank accession numbers for 16S, 18S, 28S rRNA, cytochrome c oxidase subunit I (COI), and histone H3 gene markers, and their references.	93
4	List of <i>Tetrastemma</i> species included in the phylogenetic analyses, collection location, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, and their references.	112
5	List of <i>Oerstedia</i> species included in the phylogenetic analysis, GenBank accession numbers for 16S, 18S, 28S rRNA, cytochrome c oxidase subunit I (COI), and histone H3 gene markers, and their references.	141
6	List of <i>Oerstedia</i> species sequenced in the present study and/or included in the phylogenetic analyses, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, catalogue numbers, and their references.	159
7	Uncorrected pairwise genetic distances (<i>p</i> -distances) (%) between the <i>Oerstedia</i> species collected in the present study and with some previously described species using the 657-bp cytochrome c oxidase subunit I (COI) barcode sequence. Individuals with <i>p</i> -distance $\geq 3\%$ are treated as different species.	174
8	List of Oerstediina and Amphiporina species included in the phylogenetic analyses, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, and their references.	196
9	Uncorrected pairwise <i>p</i> -distances (%) in terms of 519-bp COI sequences between the current <i>Tetranemertes</i> congeners.	214
10	Known benthic nemertean species collected from depths ≥ 1000 meters since 2013 to present. In bold are new species established in this chapter.	228
11	Locations (coordinates and depths), type of gears, and sampling dates of the 58 stations surveyed during the cruises KH-22-8 and KH-23-8 of the R/V <i>Hakuho-maru</i>	233
12	List of species included in the phylogenetic analyses with 16S, 18S, 28S, COI, and H3 GenBank accession numbers and their references.	236
13	Sequenced species, specimen (catalogue number), and GenBank accession number of the sequences generated in the present study.	251
14	The main parameters of model-based inferred phylogenies.	253

15	Species, specimen (catalogue number), location (coordinates and depths), type of gear, and sampling date of the 24 stations where ribbon worms were collected during the cruises KH-22-8 and KH-23-5 of the R/V <i>Hakuho-maru</i>	257
16	Uncorrected COI <i>p</i> -distance (%) between the described and undescribed <i>Nemertovema</i> congeners. <i>Nemertovema</i> with <i>p</i> -distance $\geq 3\%$ were treated as different species	275

List of Figures

Figure

- 1 Selected sampling sites surveyed for ribbon worms in 2023. (a), Brgy. Panilongan, Buruanga, Aklan; (b), Brgy. Naasag, Mambajao, Camiguin Island; (c), Brgy. Liong, Guinsiliban, Camiguin Island; (d), Brgy. Pampangon, Talisayan, Misamis Oriental; (e), Brgy. Tagcatong, Carmen, Agusan del Norte, and (f), Brgy. Payasan, Lianga, Surigao del Sur.. 12
- 2 Map of the Philippines showing the 16 nemertean sampling sites during the 2023 faunal survey (black dots). (a), Western Visayas region; (b), the Northern Mindanao region, and the (c), Caraga region. Pie charts represent the taxa collected in each sampling site; the areas of the pie-charts are proportional to the number of specimens collected, which are also indicated with the numerals. 13
- 3 Representatives of heteronemerteans collected in the 2023 faunal survey. Members of one of the species in the *Dushia nigra* complex showing the (a), black form, ICHUM 8667 from Medina, Misamis Oriental; (b), brown form, ICHUM 8696 from Lianga, Surigao del Sur; and (c), orange form, ICHUM 8681 from Sagay, Camiguin Island; (d), Lineidae sp. PH23-01, ICHUM 8654 from Carmen, Agusan del Norte; (e), Lineidae sp. PH23-02, ICHUM 8664 from Lianga, Surigao del Sur. Scale bar: 1 cm. 28
- 4 *Prosadenoporus spectaculus* from the Philippines. (a–f), ICHUM 8713, from Brgy. Payasan, Lianga Surigao del Sur; (g–l), ICHUM 8716, from Brgy. Lawigan, San Joaquin, Iloilo; (m, n), ICHUM 8717; and (o), ICHUM 8718, from Brgy. Liong, Guinsiliban, Camiguin Island. Abbreviations: ao, anterior ocellus; as, accessory stylet; ba, basis; br, brain; cs, central stylet; id, intestinal diverticulum; of, cerebral organ furrow; pf, posterior cephalic furrow; po, posterior ocellus; ps, prosorhochmid smile; rh, rhynchocoel; st, stomach. Scale bar: a, b k, l, m, n = 1 cm; c–e, g–i = 1 mm; f = 200 µm; j = 100 µm; o = 2 mm. 32
- 5 Representatives of monostiliferans collected from the Philippines. (a), *Prosadenoporus* sp. PH23-01, ICHUM 8707 and (b), ICHUM 8708, from Catarman, Camiguin Island; (c), *Prosadenoporus* sp. PH23-02, ICHUM 8711, from Talisayan, Misamis Oriental; (d), Monostilifera sp. PH23, ICHUM 8704, from Lianga, Surigao del Sur. Abbreviations: as, accessory stylet; ba, basis; cs, central stylet. Scale bar: 1 cm. 35
- 6 A rooted maximum-likelihood tree for Heteronemertea generated by IQ-Tree based on the best-fit model TIM + F + I + G4 model with 696-bp partial sequence of the COI gene from members of the Lineage A and Lineage N+P of the family Lineidae, and *Dushia* spp. Number at each branch represents branch support derived from 1000 ultrafast bootstrap value. Species in the present study are represented in boldface. *B. mexicanus* and *B. unicolor* were used as outgroup. 37
- 7 Delimiting the 39 *Dushia nigra* species complex from the Philippines. (a), A TCS haplotype network generated in PopART using partial sequences (629-bp) of the COI showing two clusters of the species complex. The number of black bars

	corresponds to the number of nucleotide substitutions between haplotypes. (b), ASAP COI-based delimitation tree for the Philippine <i>D. nigra</i> specimens with <i>D. wijnhoffae</i> as outgroup. Vertical bars show the species delimitation result of the ABGD, ASAP, and bPTP method.....	38
8	A rooted maximum-likelihood tree for Monostilifera generated by IQ-Tree based on the best-fit model TIM3 + F + I + G4 model with 655-bp partial sequence of the COI gene from 15 GenBank entries that showed the highest max/total scores after the MegaBLAST search of Monostilifera sp. PH23's COI sequence and <i>Prosadenoporus</i> COI sequences available in GenBank. Number at each branch represents branch support derived from 1000 ultrafast bootstrap value. Species in the present study are represented in boldface.	40
9	Updated distribution of <i>B. quinquelineatus</i> in the Philippines. Gaspar Island, Marinduque (Stimpson 1857), Sogod, Cebu (Poppe & Poppe 2009) and Brgy. Vinapor, Carmen, Agusan del Norte (present study).....	53
10	Collection site of <i>B. quinquelineatus</i> . (a), the seagrass bed from which the species was extracted; (b), the intertidal zone collection site in Brgy. Vinapor, Carmen, Agusan del Norte.	54
11	<i>Baseodiscus quinquelineatus</i> (Quoy & Gaimard, 1833), BIOCSU: 0001 from Carmen, Agusan del Norte. (a), decapitated specimen showing most of the dorsal body and (b), most of the ventral body. Arrow pointed on the decapitated anterior end of the worm. Inset is a photograph of the specimen taken on site after being extracted from its habitat. Scale bar: 2 cm.....	55
12	<i>Eousia verticivaria</i> collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), dorsal, (b), ventral, and (c), ventrolateral views, (d), magnification of the head at dorsal and (e), ventral views. Abbreviations: br, brain, cc, caudal cirrus, cs, cephalic slit. Scale bar: a, b = 5 mm; c–e = 1 mm. 62	62
13	<i>Notospermus</i> sp. PH24-03a, collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli; pr, proboscis. Scale bar: a= 5 mm; b–d = 1 mm.....	63
14	<i>Notospermus</i> sp. PH24-03b, collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), entire body at dorsal view, (b), entire body at ventral view. Scale bar: a, b= 10 mm... ..	64
15	<i>Notospermus</i> sp. PH24-03c, collected from Brgy. Vinapor, Carmen, Agusan del Norte at anaesthetized condition. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at dorso-lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 3 mm.....	65
16	<i>Notospermus</i> sp. PH24-03d, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized condition. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d),	

- magnification of the head at ventro-lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 1 mm.... 66
- 17** *Notospermus* sp. PH24-03f, collected from Brgy. Poblacion, Lianga, Surigao del Sur at living state. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at ventro-lateral view. Abbreviations: cr, cephalic ring; cs, cephalic slit; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 1 mm.... 68
- 18** *Notospermus* sp. PH24-03g, collected from Kawayan Reef, Initao, Misamis Oriental at anaesthetized state. (a), entire body at dorsal view, (b), entire body at ventral view, (c), magnification of the head at dorsal view, (d), magnification of the head at ventral view. Scale bar: a, b = 10 mm; c, = 5 mm; not available for d.... 68
- 19** *Notospermus* sp. PH24-03h, collected from Kawayan Reef, Initao, Misamis Oriental at anaesthetized state. (a), entire body at dorsal view, (b), entire body at ventral view, (c), magnification of the anterior end at lateral view, (d), magnification of the anterior end at dorsal view, (e), magnification of the anterior end at ventral view, (f), magnification of the head at dorsal view. Scale bar: a, b = 10 mm; d, e = 5 mm; not available for c and f..... 70
- 20** *Quasilineus pulcherrimus*, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view, (d), magnification of the anterior end at lateral view. Abbreviations: cs, cephalic slit; mo, mouth. Scale bar: a = 10 mm; b–d = 1 mm.... 72
- 21** Lineidae sp. PH24-01, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cs, cephalic slit; mo, mouth; pp, proboscis pore. Scale bar: a = 10 mm; b, c = 2.5 mm..... 74
- 22** Lineidae sp. PH24-02, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end after worm begins fragmentation; (c), magnification of the head at dorsal view, (d), magnification of the head at ventral view, (e), magnification of the head at lateral view. Abbreviations: br, brain, cs, cephalic slit; dh, dorsal head, mo, mouth; pp, proboscis pore, vh, ventral head Scale bar: a = 10 mm; b = 3mm; c –e = 2 mm..... 75
- 23** *Baseodiscus jonasii*, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cf, cephalic furrow; mo, mouth.... 76
- 24** *Baseodiscus* sp. PH24-01, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cf, cephalic furrow; mo, mouth; pp, proboscis pore..... 78

25	Conditions of the bottom surface where <i>Tetrastemma parallelus</i> sp. nov. was collected.....	95
26	<i>Tetrastemma parallelus</i> sp. nov., holotype ICHUM 8300. (a), anaesthetised specimen, dorsal view; (b), anaesthetised specimen, ventral view; (c), magnification of the head, squeezed specimen, dorsal view; (d), magnified stylet apparatus. Scale bars: a, b = 1 mm; c, d = 50 μ m....	96
27	Rooted Bayesian phylogenetic tree showing the position of <i>Tetrastemma parallelus</i> sp. nov. within the <i>Tetrastemma</i> Asian–Australian Pacific subclade (Chernyshev et al. 2021) based on concatenated sequences of five gene markers (16S, 18S, 28S, COI and H3). Numbers near each node indicate Bayesian posterior probability (PP)/RAxML maximum-likelihood bootstrap value (BS)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot). Solid circles indicate full support (1.00 PP/ 100% BS/100% UFBoot). Open circles indicate 1.00 PP....	97
28	<i>Tetrastemma luteum</i> sp. nov., holotype, ICHUM 8456 (a–c), dorsal view at living state and (h, j), dorsal and (i, k), ventral views at anaesthetised condition. (j), magnification of the head at dorsal and (k), ventral views at anaesthetised state; (d, e), structure of the stylet apparatus and (f, g), the accessory stylets. Dorsolateral brownish markings and one at mid ventral body are suspected to be parasites infecting the species. Abbreviations: ao, anterior ocelli; as, accessory stylet; ba, stylet basis; cs, central stylet; pf, posterior cephalic furrow; po, posterior ocelli; pr, proboscis. Scale bars: a, b, h, i = 3 mm; c, j, k = 1 mm; d = 100 μ m; e, f, g = 50 μ m....	118
29	<i>Tetrastemma luteum</i> sp. nov., paratype, ICHUM 8559. (a), freehand drawings depicting the general shape of the body in dorsal view, and (b), the anterior tip of the body in ventral and (c), lateral views, showing that the anterior cephalic furrows are invisible in dorsal view.....	119
30	<i>Tetrastemma luteum</i> sp. nov., photographs showing the variations in body color and shape of the anterior end of the body in some paratypes in dorsal and ventral views at anaesthetised conditions. (a, b), ICHUM 8457; (c, d), ICHUM 8458; (e, f), ICHUM 8459; (g, h), ICHUM 8460; (i, j), ICHUM 8461; (k, l), ICHUM 8462; (m, n), ICHUM 8463; (o, p), ICHUM 8464; (s, dorsal only) ICHUM 8465; (t, u), ICHUM 8466; (q, r), ICHUM 8467. Dorsolateral brownish markings on ICHUM 8463, 8467, and 8466 are suspected to be parasites infecting the species. Scale bars: a–p = 3 mm; q–s = 2 mm; t, u = 5 mm.....	120
31	<i>Tetrastemma luteum</i> sp. nov., photographs showing the variations in the color and shape of the anterior end of the body in some paratypes in dorsal and ventral views at anaesthetised (a, b, e, f) and non-anaesthetised (c, d) conditions. (a, b), ICHUM 8457; (c, d), ICHUM 8458; (e, f), ICHUM 8462. Scale bars: a–f = 1 mm....	120
32	<i>Tetrastemma luteum</i> sp. nov., photomicrographs showing the variations of the stylet apparatus in some paratypes. (a), ICHUM 8457; (b), ICHUM 8458; (c), ICHUM 8459; (d), ICHUM 8460; (e), ICHUM 8461; (f), ICHUM 8463; (g), ICHUM 8464; (h), ICHUM 8467; (i), ICHUM 8466. Scale bars: a–l = 50 μ m.....	121

- 33** *Tetrastemma luteum* sp. nov., photomicrographs of transverse histological sections, ICHUM 8561, paratype. (a), proposcis pore; (b), apical organ; (c), cerebral glands; (d), brain; (e), mid dorsal vessel; (f), magnification of the proboscis showing the 10 proboscis nerves (black arrows); (g), stomach region; (h), intestine and pylorus junction; (i), magnification of the ovary. Abbreviations: ao, apical organ; br, brain; cg, cerebral gland; co, cerebral organ; es, esophagus; id, intestinal diverticulum; in, intestine; md, mid-dorsal vessel; ov, ovary; pp, proboscis pore; pr, proboscis; rh, rhynchocoel. Scale bars: a–d, g, h = 100 µm; e, f, i = 40 µm..... 122
- 34** *Tetrastemma phaeobasisae*, non-type (ICHUM 8508), photographs at anaesthetised state, collected in Oshoro Bay, Hokkaido, Japan. Entire worm at (a), dorsal and (b), ventral views; magnification of the head at (c), dorsal and (d), ventral views; (e), photomicrograph of the stylet apparatus. Inserted in f is the same species collected in Peter the Great Bay, Russia (adopted from Chernyshev 2014). Abbreviations: ac, anterior cephalic furrow; ao, anterior ocelli; as, accessory stylets; ba, basis; cs, central stylet; pc, posterior cephalic furrow; po, posterior ocelli. Scale bars: a, b = 2 mm; c, d = 0.5 mm; e = 25 µm.... 124
- 35** Rooted Bayesian phylogenetic tree showing the position of *Tetrastemma luteum* sp. nov. and *Tetrastemma phaeobasisae* from Oshoro Bay within Clade C of the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent Bayesian posterior probability (PP)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support (1.00 PP/100% UFBoot/100% BS); closed circles indicate 1.00 PP; and open circles indicate 0.99 PP.... 126
- 36** *Oerstedia fuscosparsa* sp. nov., photographs (a–h) and photomicrographs (i, j) taken in anaesthetised state. (a), entire body, dorsal view; (b), entire body, ventral view; (c), entire body, left lateral view; (d), entire body, ventral view; (e, g), magnification of head, dorsal view; (f, h), magnification of head, ventral view; (i), head in squeezed preparation; (j), stylet apparatus. (a, b, e, f), ICHUM 8405 (holotype); (c, d, g, h–j), ICHUM 8406 (paratype). Abbreviations: ao, anterior ocelli; as, accessory stylet; b, basis; br, brain; cs, central stylet; lb, lateral blood vessel; p, proboscis; po, posterior ocelli. Scale bars: a–d = 2 mm; e–h = 1 mm; i, j = 50 µm.... 144
- 37** *Oerstedia fuscosparsa* sp. nov., photomicrographs of transverse histological sections, ICHUM 8405 (holotype). (a), posterior foregut region; (b), intestinal region. Abbreviations: al, accessory lateral nerve; ic, intestinal caecal lumen; pr, proboscis; py, pylorus. Scale bars: a, b, 50 µm.... 145
- 38** Rooted Bayesian phylogenetic tree showing the position of *Oerstedia fuscosparsa* sp. nov. within the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Number at each node represents Bayesian posterior probability (PP)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS).

- Solid circles indicate full support (1.00 PP/100% UFBoot/100% BS); open circles indicate 1.00 PP; solid diamonds mean 1.00 PP/100% UFBoot; open diamonds show 0.99 PP..... 146
- 39** *Oerstedtia pseudoculata* sp. nov., photographs and photomicrographs of the non-banded form, holotype, ICHUM 8587 (a–h); paratype, ICHUM 8584 (i); and the banded form, paratype, ICHUM 8582 (j–n); taken in living and anesthetized states. (a, j), entire body at dorsal view and (b, k), ventral view in non-anesthetized condition; (c), entire body at dorsal view and (d), ventral view in anesthetized condition; (e, l), magnification of the head at dorsal view and (f, m), ventral view in anesthetized state; (g), squeezed specimen at dorsal view; (h, l, n), stylet apparatus. Abbreviations: af, anterior cephalic furrow; ao, anterior ocelli; as, accessory stylet; ba, stylet basis; br, brain; cs, central stylet; es, esophagus; id, intestinal diverticula; ln, lateral nerves; oc, ocelli; pf, posterior cephalic furrow; po, posterior ocelli; pp, proboscis pore; pr, proboscis; st, stomach. Scale bars: a, b, e, f, l, m = 0.5 mm; c, d, g, j, k = 1 mm; h, i, n = 50 μ m.... 165
- 40** *Oerstedtia pseudoculata* sp. nov., banded form, paratype, ICHUM 3288, photomicrographs of transverse histological sections through the foregut region showing the (a), pyloric cecum above the stomach; (b), the posterior foregut region at the stomach–pylorus junction; and (c), the pyloric region. Abbreviations: an, accessory nerve; ic, intestinal cecum; ip, intestinal-cecum anterior dorsolateral pouch; pr, proboscis; py, pylorus; rh, rhynchocoel, st, stomach. Scale bars: a–c = 100 μ m..... 166
- 41** *Oerstedtia rugosa* sp. nov., photographs of the entire worm at living state, (a–d), holotype, ICHUM 8590; (e), paratype, MIMB 4718222; and (f–h), photomicrographs of the transverse histological sections, paratype, MIMB 4718222. (a, b, e), entire body at dorsal (c), ventral, and (d), right lateral view. Transverse histological section at (f), precerebral region; (g), brain region; and (h), anterior pylorus region. Abbreviations: an, accessory nerve; bg, basophilic glands; br, brain; es, esophagus; ic, intestinal cecum; ip, intestinal caecum pouches; py, pylorus; rg, red-stained glands. Scale bars: a = 10 mm; b = 5 mm; c, d = 2.5 mm; f–h = 100 μ m 168
- 42** *Oerstedtia viridifusca* sp. nov., holotype, ICHUM 8589, photographs of the entire worm in (a), non-anesthetized state at dorsal view; (b–e), anesthetized state, entire animal at (b), dorsal and (c), ventral views, and magnification of the head at (d), dorsal and (e), ventral views; and (f), photomicrograph of the specimen compressed under a coverslip. Abbreviations: af, anterior cephalic furrow; oc, ocelli; ov, ovary; pf, posterior cephalic furrow; pp, proboscis pore; pr, proboscis; st, stomach. Scale bars: a–c = 2 mm; d–f = 1 mm 171
- 43** *Oerstedtia phoresiae* (Kulikova, 1987), non-type specimen, ICHUM 8588, (a), photograph of the entire specimen at dorsal view in an anesthetized state; (b), photomicrographs of the compressed specimen showing the anterior half of the dorsal body and (c), its stylet apparatus. Abbreviation: ai, anterior pouch of the intestinal cecum; ao, anterior ocellus; as, accessory stylets; ba, stylet basis; br,

	brain; cf, cephalic furrow; cs, central stylet; id, intestinal diverticulum; po, posterior ocellus; pr, proboscis. Scale bars: a, b = 1 mm; c = 50 μ m.....	172
44	Delimiting <i>Oerstedtia oculata</i> , <i>O. pseudoculata</i> sp. nov., <i>O. venusta</i> and <i>O. zebra</i> sensu Thollessen & Norenburg. (a), a TCS haplotype network generated in PopART using partial sequences (657-bp) of the COI. The number of black bars corresponds to the number of nucleotide substitution between haplotypes; numbers inside each circle show the number of specimen(s) representing each haplotype; p-distance value represents the uncorrected pairwise genetic distance calculated within and between species; dashed boxes represent the species. (b), a COI-based ML tree. The number near each node represents the RAxML maximum-likelihood bootstrap value (BS) with closed circles indicated by 100%BS. Vertical bars show the results of the ABGD, ASAP, and PTP species delimitation analyses.....	176
45	Rooted Bayesian phylogenetic tree showing the position of <i>Oerstedtia pseudoculata</i> sp. nov., <i>Oerstedtia rugosa</i> sp. nov., and <i>Oerstedtia viridifusca</i> sp. nov. within the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Number at each node represents Bayesian posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Solid circles indicate full support (1.00 PP/100% UFBoot/100% BS); open circles indicate 1.00 PP; solid diamonds indicate 1.00 PP/100% UFBoot; open diamonds indicate 0.99 PP....	177
46	Taxonomic names applied to some banded and non-banded <i>Oerstedtia</i> from the Northwest Pacific according to several taxonomic works. Solid box represents nominal species (each denoted by a name bearing type specimen and an available name), and dashed box represents the species concept proposed by the author(s)	179
47	<i>Ommatoplea taeniata</i> Ehrenberg, 1828. (a), anterior end of body (Ehrenberg 1828–1831: fig. III f); (b, c), entire animal (Ehrenberg 1828–1831: fig. III a, b); (d), magnification of the head (Ehrenberg 1828–1831: fig. III d). Reproduced from a digitalized copy at Biodiversity Heritage Library (https://www.biodiversitylibrary.org/item/189273#page/85/)....	201
48	<i>Tetranemertes aurantia</i> sp. nov., holotype (ICHUM 8719), photographs in living state. (a, b), before anesthetization; (c), anesthetized state. Scale bars: b, c, 1 = cm (not available for a)	203
49	<i>Tetranemertes aurantia</i> sp. nov., holotype (ICHUM 8719). (a), magnification of the anterior end of body in anesthetized state, dorsal view; (b), same as A, ventral view, showing ‘A’-shaped cephalic furrow; (c), stylet apparatus; (d), magnification of central stylet and basis; (e, f), accessory stylets; (g), pigments around the diaphragm. Scale bars: a, b = 2 mm; c, d = 200 μ m; e–g = 100 μ m	204
50	<i>Tetranemertes kandai</i> (Kato, 1939) comb. nov. (a), anterior end of body; (b), stylet apparatus. Reproduced from Kato (1939, figs. 1, 2). Copyright © 1938 Japanese Society of Zoology	207

51	<i>Tetranemertes unistriata</i> Cherneva et al. 2023, drawings made by Svetlana A. Maslakova based on three specimens collected from among calcareous algae taken at Engetsu Island, Shirahama, Wakayama, Japan in July 1999. Reproduced from Cherneva et al. (2023, fig. 7a–e). Copyright © 2023 Irina Cherneva et al. (CC BY 4.0) 208
52	<i>Tetranemertes</i> sp. AHK-2024-1, anterior end of body, dorsal view..... 209
53	<i>Tetranemertes</i> sp. AHK-2024-2. (a), entire specimen; (b), magnification of head, dorsal view; (c), central stylet and basis (deformed after fixation), cleared in xylene, taken from Bouin-fixed specimen. Scale bar: c = 50 µm 210
54	Rooted Bayesian phylogenetic tree showing the position of <i>Tetranemertes aurantia</i> sp. nov. within the genus <i>Tetranemertes</i> based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers represent branch support—Bayesian posterior probability/IQ-Tree maximum-likelihood ultrafast bootstrap value 215
55	Sampling stations in the Japan Trench and Kuril-Kamchatka Trench where ribbon worms were collected (a–d). (a), general map highlighting the location of the five sampling stations along the Japan Trench (JT) and the Kuril-Kamchatka Trench (KKT); (b), map showing the detailed sampling stations in ‘A, C, and D lines’; (c), detailed sampling stations in ‘F line’; and (d), the ‘G station’. The sampling stations northeast the Erimo Seamount (ES) are situated in the KKT while stations to its south are in the JT 255
56	Some of the palaeonemerteans collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–e). (a–c), Tubulanidae sp. KH-2024-01, ICHUM 8899, from JT, (a), whole body at right-lateral view, (b), magnification of the anterior end at dorsal view, (c), same in b but at ventral view, white arrowheads pointed to the head; (d, e); Carininidae sp. KH-2024-01, ICHUM 8888, from KKT, (d), whole body at right-lateral view, (e), magnification of the anterior end at ventrolateral view, white arrowhead pointed to the head. Abbreviations: ab, anterior body; cf, cephalic furrow; go, gonads; pb, posterior body; tr, tubulanid ring..... 256
57	Rooted Bayesian phylogenetic tree for the Palaeonemertea supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support, 1.00 PP/100% UFBoot/100% BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones. Species in the present study are in bold. The habitat depths of deep-sea species are indicated in parentheses 261
58	Some of the pilidiophorans collected along the Kuril-Kamchatka Trench (a–e). (a–c), Lineidae sp. KH-2024-01, ICHUM 8874, (a), whole body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), same in b but at ventral view; (d), Lineidae sp. KH-2024-02, ICHUM 8876, anterior body fragment; (e)

- Baseodiscus profundus*, ICHUM 8885, anterior body fragment, white arrowheads pointed to the thin cephalic furrow. Abbreviations: cf, cephalic furrow; cs, cephalic slit; mo, mouth; pp, proboscis pore; pr, proboscis 263
- 59 Rooted Bayesian phylogenetic tree for the Pilidiophora supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support, 1.00 PP/100% UFBoot/100% BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones. Species in the present study are in bold. The habitat depths of deep-sea species are indicated in parenthesis..... 265
- 60 Rooted Bayesian phylogenetic tree for the Hoplonemertea supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS) Thickened branches indicate full support, 1.00 PP/100 UFBoot/BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones..... 268
- 61 Some of the polystiliferous hoplonemertean collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–s). (a, b), *Dinonemertes shinkaii*, ICHUM 8866, from JT, (a), dorsal and (b), ventral views; (c, d), *Nectonemertes* cf. *mirabilis*, ICHUM 8867, female, from JT, (c), dorsal and (d) ventral views; (e, f), *N. cf. mirabilis*, ICHUM 8896, male, from KKT, (e), dorsal and (f), ventral views; (g, h), *Phallanemertes murrayi*, ICHUM 8868, female, from KKT, (g), dorsal and (h) ventral views; (i, j), *Protopelagonemertes beebei*, ICHUM 8872, from JT, (i), dorsal and (j), ventral views; (k, l), *Pelagica* sp. KH-2024-01, ICHUM 8860, from KKT, (k), dorsal and (l), ventral views; (m, n), *Pelagica* sp. KH-2024-02, ICHUM 8871, from JT, (m), dorsal and (n), ventral views; (o–s), *Reptantia* sp. KH-2024-01, ICHUM 8862, from KKT, (o), magnification of the anterior body, (p), anterior body fragment, (q), posterior body fragment, (r), proboscis region containing the stylet apparatus at squeezed preparation, (s), same in r but central (pointed by white arrowheads) and accessory stylets (pointed by red arrowheads). Abbreviations: as, accessory stylets; ba, basis; sa, stylet apparatus 270
- 62 Some of the monostiliferous hoplonemertean collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–p). (a–d), *Nemertovema kojimai* sp. nov., ICHUM 8857, from KKT, (a), whole body at right-lateral view, (b), magnification of the anterior end at right-lateral view, (c), same in b but at ventrolateral view, (d), same in a but at left-lateral view, posterior body covering detached; (e–h), *Profundonemertes hakuhoae* gen. et sp. nov., ICHUM 8854, from KKT, (e), whole body at dorsal view, (f), same in e but at ventral view, (g),

magnification of the anterior end at dorsal view, white arrowheads trace the shape of the cephalic furrows, (h), same in g but at ventral view; (i–l), *P. hakuhoae* gen. et sp. nov., ICHUM 8855, from JT, (i), whole body at dorsal view, (j), same in i but at ventral view, (k), magnification of the anterior end at dorsal view, (l), same in k but at ventral view, cephalic furrows not visible since epidermis of the specimen has been detached from the body; (m–o), *Monostilifera* sp. KH-2024-02, ICHUM 8853, from KKT, (m), anterior body fragment with released proboscis at lateral view, (n), magnification of the anterior end at lateral view, (o), stylet apparatus, white arrowhead pointed to the broken central stylet; (p), *Nemertovema norenburgi*, ICHUM 8856, body fragment, from KKT. Abbreviations: ab, anterior body; as, accessory stylets; ba, basis; br, brain; cf, cephalic furrow; es, esophagus; ov, ovary, pb, posterior body; pp, proboscis pore; pr, proboscis 274

- 63** *Profundonemertes hakuhoae* gen. et sp. nov., holotype, ICHUM 8854, transverse histological sections (a–i). (a), anterior end of the head; (b, c), pre-cerebral region, red arrowhead pointed to the cerebral organ canal; (d), proboscis insertion, red arrowhead pointed to the cerebral organ canal, black arrowhead to the rhynchocoel; (e), midgut region, black arrowhead pointed to the rhynchocoel, red arrowheads on the cephalic blood vessels protruding into the rhynchocoel; (f), proboscis armature, black arrowhead pointed on the circular stylet basis, red arrowheads on the accessory stylet pouches; (g), stomach-pyloric junction; (h), mid-dorsal blood vessel (pointed by red arrowhead) in the posterior pyloric region; (i), ovaries containing primary oocytes. Abbreviations: as, acidophilic sub-dermal glands; br, brain; cg, cephalic gland lobules; cf, cephalic furrow; co, cerebral organ; es, esophagus; ic, intestinal cecum; ld, lateral diverticulum of the intestinal cecum; ln, lateral nerve; ov, ovary; pi, proboscis insertion, po, primary oocytes; pr, proboscis; py, pylorus; rh, rhynchostomodaeum. Scale bars: a–i = 100 μ m 280

Chapter 1. General introduction

Nemerteans, or ribbon worms, are classified under the phylum Nemertea. They are soft-bodied, non-truly-segmented worms, all of which (with the exception of *Arhynchonemertes axi* Riser, 1988) are characterized by an eversible proboscis housed within a fluid-filled rhychocoel (Döhren 2015). To date, the phylum is composed of 1,355 named species (Norenburg et al. 2024). Nemerteans are distributed worldwide, with most known species inhabiting benthic marine environments, although some have adapted to pelagic, freshwater, and terrestrial ecosystems. Despite being common in marine environments, nemerteans remain relatively understudied, with most of the phylum's diversity still uncharacterized (Mahon et al. 2010; Chernyshev 2011; Kajihara 2017; Maslakova et al. 2022).

An earlier classification of the phylum grouped nemerteans into classes Anopla and Enopla (Schultze 1851; Stiasny-Wijnhoff 1936). Presently, this classification system has been dismissed by nemertean researchers, now recognizing the three classes Palaeonemertea, Pilidiophora, and Hoplonemertea (Strand et al. 2019). Based on phylogenetic evidence, several orders are now recognized within each class—Carinomiformes, Tubulaniformes, and Archinemertea in Palaeonemertea; Hubretchiiformes and Heteronemertea in Pilidiophora; and Polystifera and Monostilifera in Hoplonemertea (Chernyshev 2021).

Aside from the caudal cirrus in pilidiophorans and tentacles in *Nectonemertes* and *Phallonemertes*, nemerteans do not possess any other external body appendages, and the external diagnostic characters are often restricted to size, shape, position of mouth and proboscis pore, number and pattern of eyes, and coloration. In effect, for several decades, the golden standard for nemertean species description and even identification included the detailed accounts of external and internal characters, the latter only traditionally accessible through histological sectioning (Gibson 1985). It is not surprising that descriptions of most species, particularly the old ones, were based on tissue sections. This made subsequent species identification difficult, particularly when one is not able to generate tissue sections of his/her unknown species. However, proposals have been made to divert from this tradition to facilitate rapid naming (therefore, species discovery) to reveal the actual diversity within the phylum (Sundberg

et al. 2016). As a result, several nemertean researchers started to adopt the histology-free or turbotaxonomy approach in the new era of nemertean taxonomy (Kajihara 2015; Gonzalez-Cueto et al. 2017; Simpson et al. 2017; Kajihara et al. 2018, 2022; Chernyshev et al. 2020; Hookabe et al. 2021a, 2021b, 2023). With this approach, species description (and identification) can be made based on external and internal characters, coupled with DNA sequence information, without the necessity of generating tissue sections for histological descriptions. DNA-based techniques are increasingly employed to identify and define species, clarify phylogenies, and uncover biogeographical patterns in the phylum (Chernyshev et al. 2021; Mendes et al. 2021; Kajihara et al. 2022a, 2022b).

This dissertation deals with the taxonomy and systematics of nemerteans from the western Pacific region. It focuses on the diversity of the phylum in the Philippine and Japanese marine waters, investigating species from shallow-water and deep-sea habitats. New species are established in Hoplonemertea (order Monostilifera), the most studied, and commonly sampled groups. Several accounts of species in Pilidiophora and Palaeonemertea are also presented in this dissertation. I employed either a histology-free approach alone or the traditional standard in combination with DNA sequence data in describing and identifying the documented nemerteans. A few taxonomic issues are also addressed in this dissertation.

Chapter 2 deals with the intertidal hoplonemerteans and pilidiophorans collected from the Philippines during faunal surveys conducted in 2023 and 2024—to date, the most comprehensive investigation of the phylum in the Philippines. These Philippine nemertean faunal surveys resulted in the discovery of 21 nemertean species, 11 of which are potentially new to science, six representing new distribution records in the Philippines. This chapter partially presents the results of these surveys.

Chapter 3 deals with taxonomy and phylogenetics of monostiliferous hoplonemerteans in the genus *Tetrastemma* Ehrenberg, 1831, *Oerstedia* Quatrefages, 1846, and *Tetranemertes* Chernyshev, 1992 from Japanese waters. New species are established in this chapter (i.e., two in *Tetrastemma*, four in *Oerstedia*, and one in *Tetranemertes*). The species identity of one highly polymorphic *Oerstedia* previously known as *O. venusta* sensu Thollessen and Norenburg, 2003 and *O. zebra* Thollessen

and Norenburg, 2003 and of *Emplectonema kandai* Kato, 1939 are settled in this chapter.

Chapter 4 presents the species diversity of deep-sea nemerteans collected in the Japan Trench and Kuril-Kamchatka Trench during the R/V *Hakuho-maru* research expeditions in 2022 and 2023. This chapter represents the first inclusive deep-sea nemertean investigation in Japanese waters. The expedition yielded a collection of 80 nemertean specimens composed of 30 putative species. The phylogenetic positions of the collected species were inferred within the classes Palaeonemertea, Pilidiophora, and Hoplonemertea. DNA sequences were generated from the collected species and were submitted to GenBank, which increased genetic information from deep-sea representatives in public databases. Two deep-sea species in Monostilifera, one placed in a new genus, are established in this chapter, in addition to several first-record species in Japanese waters.

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Chapter 2. Taxonomy and systematics of shallow-water nemerteans from the Philippines

The Philippine nemertean fauna is currently poorly documented. The lack of experts specializing in the taxonomy and systematics of this taxon has led to their unexplored and undocumented diversity in the country. The first documentation of a Philippine ribbon worm was made by Stimpson (1857). The succeeding nemertean reports from the country were authored again by foreign nemertean researchers. From 1942 to 2020 only eight species of ribbon worms were reported from the Philippines, two in Palaeonemertea and five in Hoplonemertea (Humes 1942; Gibson & Moore 1998; Kajihara 2007, 2020; Kajihara et al. 2011). This implies the lack of attention to this taxon in the Philippines despite active ribbon worm studies in other parts of the globe. Being in the ‘coral triangle’, the Philippine marine waters are expected to be home to a diverse marine-invertebrate fauna including nemerteans. To address the inadequacy of the nemertean diversity in Philippine marine waters, a faunal survey was conducted in 2023 and 2024.

In this chapter, the results of the 2023 and 2024 nemertean faunal survey conducted in the intertidal zones of the selected sites in the Visayas and Mindanao Islands of the Philippines are presented. The main goal of this chapter is to preliminarily reveal the under-explored and unreported diversity of the phylum in tropical regions such as the Philippines. The surveys resulted in the discovery of 22 nemertean species, 12 of which are potentially new species and six represent new distribution records in the country. Combining the previous species data, a total of 24 nemertean species are now recorded from the Philippines.

Species that are first reported in the country include *i) Baseodiscus hemprichii* (Ehrenberg, 1831), *ii) Baseodiscus jonasii* Strand, Hjeltgren & Sundberg, 2005, *iii) Dushia nigra* (Stimpson, 1855) species complex, *iv) Eousia verticivaria* Gibson, 1990, *v) Prosadenoporus spectaculus* (Yamaoka, 1940), and *vi) Quasilineus pulcherrimus* Gibson, 1981. The other remaining Philippine nemertean species that are currently left unidentified include *i) Baseodiscus* sp. PH24-01, *ii) Drepanophoridae* sp. PH24-01, *iii) Drepanophoridae* sp. PH24-02, *iv) Notospermus* sp. PH24-03, *v) Lineidae* sp. PH23-01, *vi) Lineidae* sp. PH23-02, *vii) Lineidae* sp. PH24-01, *viii) Lineidae* sp. PH24-02, *ix)*

Ototyphlonemertes sp. PH24-01, x) *Prosadenoporus* sp. PH23-01, xi) *Prosadenoporus* sp. PH23-02, and xii) *Tetrastemma* sp. PH24-01.

The identity of these unidentified Philippine nemertean species will be confirmed in the future using DNA sequence and morphological data. Those species that will be confirmed as new will be given formal taxonomy in future papers.

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Chapter 2.1. Ribbons under the rocks: intertidal ribbon worm fauna (phylum Nemertea) from selected islands in central and southern Philippines

Introduction

Ribbon worms are unsegmented, bilaterally symmetrical metazoans comprising the phylum Nemertea (Döhren 2015). Nemerteans are primarily marine, but some freshwater, terrestrial, and brackish water species are also known (Gibson 1995; Kajihara et al. 2008). Most species are benthic and found from the intertidal to the hadal zones (Chernyshev & Polyakova 2019) while others dwell in water columns from mesopelagic to the abyssopelagic regions (Maslakova & Norenburg 2001; Kajihara & Lindsay 2010). In the intertidal zone, ribbon worms can be collected by turning rocks at low tide and by examining sandy sediment material along the shoreline. Meanwhile, in the littoral zone, ribbon worms can be extracted from coral debris, kelp holdfast, filtering sargassum, and seagrasses among others (Gibson 1994).

The Philippine ribbon-worm fauna is currently poorly documented. The lack of experts specializing in the taxonomy and systematics of this taxon has led to the unexplored and undocumented diversity of Philippine nemerteans. The first documentation of a Philippine ribbon worm was made by Stimpson (1857). The succeeding nemertean reports (i.e., published in scientific papers) from the country were authored by foreign researchers. To date, eight named ribbon-worm species published in scientific journals are known mostly from Luzon and Visayas, namely *Balionemertes* cf. *australiensis* Sundberg, Gibson & Olsson, 2003; *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1833); *Callinera emiliae* Kajihara, 2007; *Carcinonemertes mitsukurii* Takakura, 1910; *Coella gloriae* Kajihara, 2020; *Diplomma serpentinum* (Stimpson, 1855); *Geonemertes philippinensis* Gibson & Moore, 1998; and *Prosadenoporus olympiae* Kajihara, 2020 (Humes 1942; Gibson & Moore 1998; Kajihara 2007, 2020; Kajihara et al. 2011). However, this number is relatively small in comparison with those of other regions where research has advanced; for instance, ~130 species have been reported from Japan (Kajihara 2017).

This research aims to enhance the knowledge of the Philippine ribbon worm diversity. To explore further the intertidal ribbon worm composition of Panay and Mindanao Islands, a nemertean faunal survey was performed. The morphological

features of the collected ribbon worms are briefly presented in this chapter. Also, the cytochrome c oxidase subunit I (COI) gene sequence of the collected specimens is determined for molecular species identification, genetic distance, species delimitation, and maximum-likelihood (ML) analyses.

Materials and methods

Sample collection and processing

Prior to collection, Gratuitous Permits (GP No. 0253-23 and GP No. R10 2023-09) were secured from the Bureau of Fisheries and Aquatic Resources, Department of Environment and Natural Resources, and from the Protected Area Management Board (for Initao-Libertad Protected Landscape and Seascape in Brgy. Tubigan). Ribbon worms were collected from March to April 2023 along slightly silty to sandy and rocky shores in the intertidal zone (Fig. 1) of the 16 collection sites (Fig. 2) distributed in Western Visayas, Northern Mindanao, and Caraga regions: 1) Brgy. Panilongan, Buruanga in Aklan, Panay Island; 2) Brgy. Lawigan, San Joaquin in Iloilo, Panay Island; 3) Brgy. Naasag, Mambajao in Camiguin Island; 4) Brgy. Catibac, Catarman in Camiguin Island; 5) Brgy. Hubangon, Mahinog in Camiguin Island; 6) Brgy. Bugang, Sagay in Camiguin Island; 7) Brgy. Liong, Guinsiliban in Camiguin Island; 8) Brgy. Tubigan, Initao in Misamis Oriental, Mindanao Island; 9) Brgy. Pampangon, Talisayan in Misamis Oriental, Mindanao Island; 10) Brgy. Duka, Medina in Misamis Oriental, Mindanao Island; 11) Brgy. Vinapor, Carmen in Agusan del Norte, Mindanao Island; 12) Brgy. San Agustin, Carmen in Agusan del Norte, Mindanao Island; 13) Brgy. Cahayagan, Carmen in Agusan del Norte, Mindanao Island; 14) Brgy. Tagcatong, Carmen in Agusan del Norte, Mindanao Island; 15) Brgy. Payasan, Lianga in Surigao del Sur, Mindanao Island; and 16) Brgy. Anibongan, Lianga in Surigao del Sur, Mindanao Island.

Intertidal rocks in the 16 collection sites were randomly turned and nemerteans sighted were hand-picked using tweezers, forceps, or plastic pipettes. Collected worms were placed in plastic containers with seawater and photographed using a Nikon D5700 camera attached to either an AF-S Micro NIKKOR 40 mm lens or a stereomicroscope before and/or after they were anaesthetized in a $MgCl_2$ solution isotonic to seawater (Abato et al. 2022). Hoplonemerteans were dissected to extract the proboscis, which was then mounted onto a glass slide and observed under a compound microscope for

the documentation of the stylet apparatus. After morphological observations, the worms were cut in halves. The posterior halves were preserved in 99% ethanol for genomic DNA extraction, while the anterior halves were preserved in Bouin's fluid then in 70% ethanol for future serial sectioning.

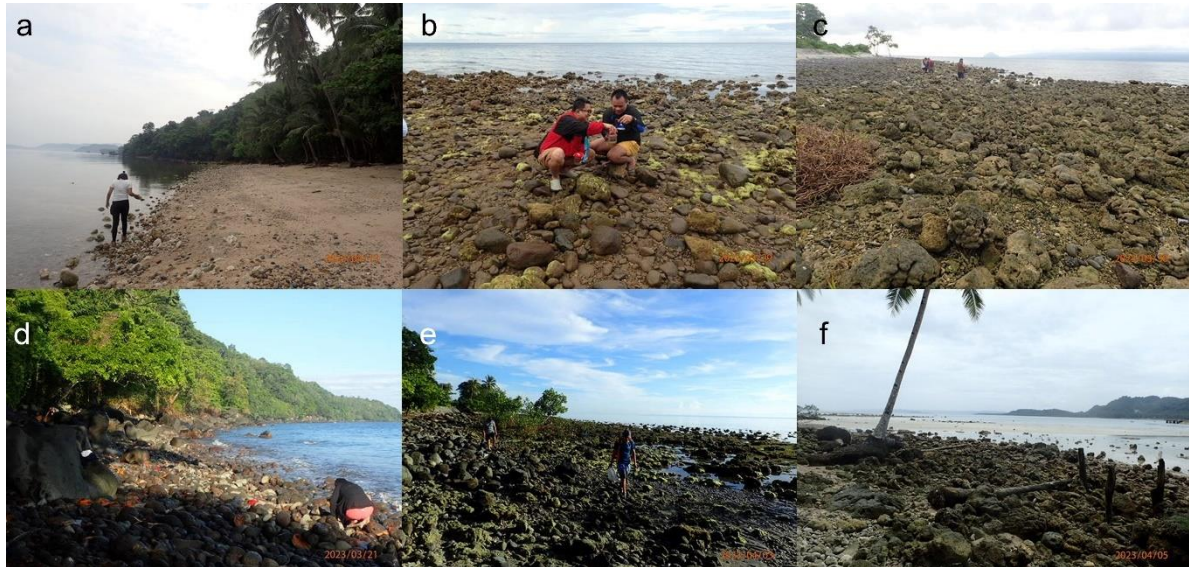


Figure 1. Selected sampling sites surveyed for ribbon worms in 2023. (a), Brgy. Panilongan, Buruanga, Aklan; (b), Brgy. Naasag, Mambajao, Camiguin Island; (c), Brgy. Liong, Guinsiliban, Camiguin Island; (d), Brgy. Pampangon, Talisayan, Misamis Oriental; (e), Brgy. Tagcatong, Carmen, Agusan del Norte, and (f), Brgy. Payasan, Lianga, Surigao del Sur.

Voucher materials have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan.

DNA extraction, PCR amplification, and sequencing

DNA extraction, TaKaRa Ex Taq PCR amplification of the COI gene, and sequencing followed Abato et al. (2022). However, when standard conditions in Abato et al. (2022) did not work, a touch-down PCR was performed: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 45°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 35°C, 40 sec at 72°C), followed by 7 min at 72°C. MegaBLAST searches (Morgulis et al. 2008) on the NCBI website (<http://www.ncbi.nlm.nih.gov/>) were performed to check for possible sequence contamination and to initially determine the putative species and/or supra-specific

higher taxa identification of the collected specimens. COI sequences were aligned using MUSCLE algorithm (Edgar 2004) implemented in MEGA ver. 11 (Tamura et al. 2021) while uncertainly aligned sites were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al. 2020). The genetic variation between the taxa represented by more than one individual was determined by calculating the uncorrected pairwise genetic distance (p -distance) based on the aligned and trimmed COI sequences.

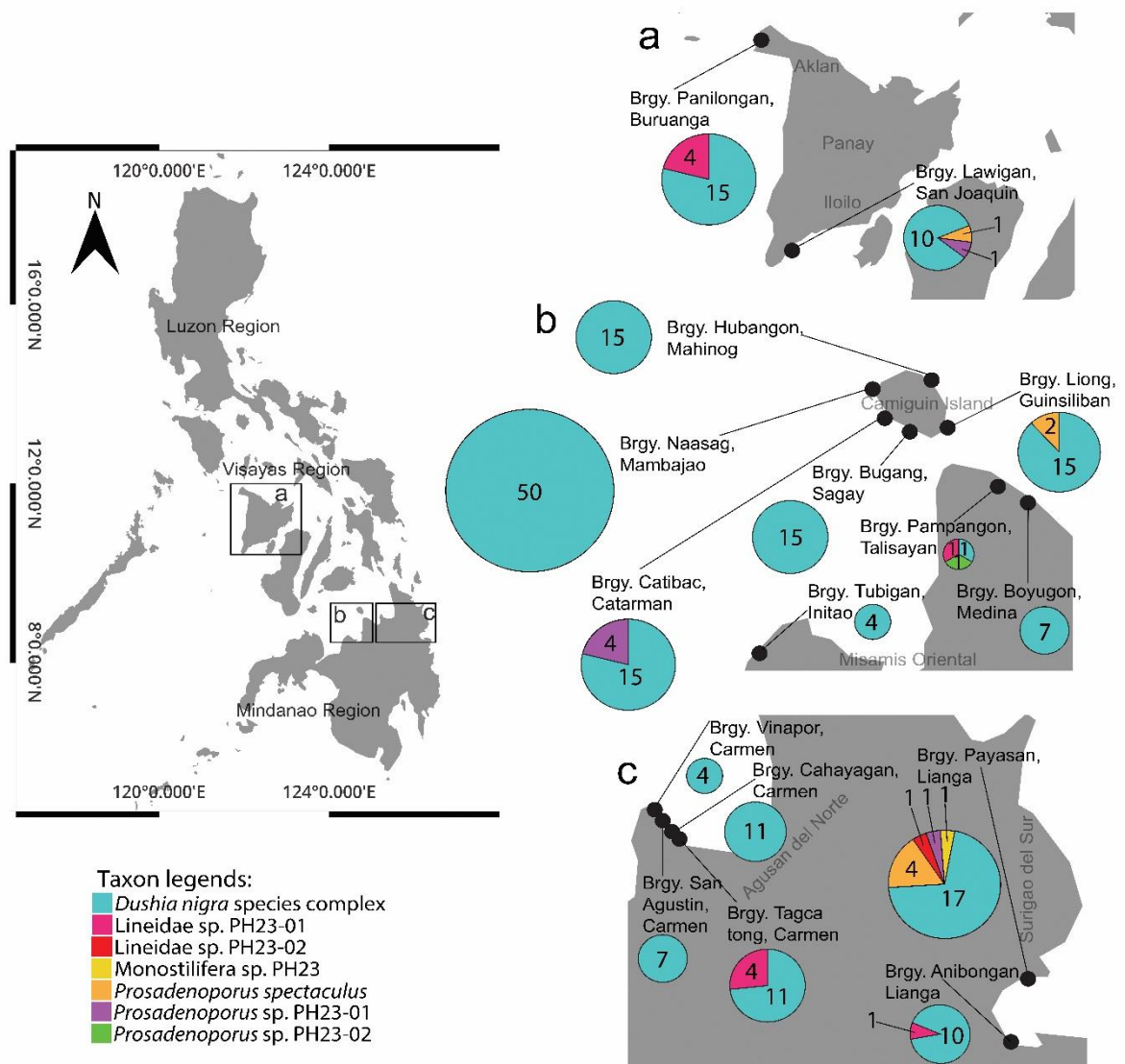


Figure 2. Map of the Philippines showing the 16 nemertean sampling sites during the 2023 faunal survey (black dots). (a), Western Visayas region; (b), the Northern Mindanao region, and the (c), Caraga region. Pie charts represent the taxa collected in each sampling site; the areas of the pie-charts are proportional to the number of specimens collected, which are also indicated with the numerals.

Species delimitation and haplotype network analyses

A COI-based molecular species delimitation analysis for *Dushia nigra* (Stimpson, 1855) using the sequences generated from the present specimens and those available in public databases was performed using three methods: *i*) Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021), *ii*) Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), and *iii*) Poisson Tree Processes (PTP) (Zhang et al. 2013). Using the default settings, ASAP was done in webserver <https://bioinfo.mnhn.fr/abi/public/asap/>; ABGD in <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>; and PTP in bPTP webserver <https://species.h-its.org/ptp/>. Species delimitation using the PTP method was done using an input tree generated by IQ-Tree built from the 629-bp COI sequences of the 39 *D. nigra* Philippine specimens and those in Hookabe et al. (2019). *Dushia wijnhoffae* Schwartz & Norenburg in Hookabe et al. (2019) was used as the outgroup.

A TCS haplotype network was created in PopART (Leigh & Bryant 2015) to visualize the relationships between the Philippine *D. nigra* haplotypes and publicly available sequences of the species complex.

Maximum likelihood analyses

A COI-based ML analysis was performed separately for the heteronemerteans (i.e., *D. nigra*, Lineidae sp. PH23-01, and Lineidae sp. PH23-02) and monostiliferans (i.e., Monostilifera sp. PH23, *Prosadenoporus spectaculus* (Yamaoka, 1940a), *Prosadenoporus* sp. PH23-01, and *Prosadenoporus* sp. PH23-02) that were documented in the study. The COI nucleotide sequences used in the analyses included those generated in this study and downloaded from GenBank (Table 1).

In selecting the operational taxonomic units (OTUs) for the Heteronemertea ML analysis, the COI sequences of Lineidae sp. PH23-01 and Lineidae sp. PH23-02 were MegaBLAST searched to see the most closely related or best-hit ribbon-worm GenBank entry. Then, the lineage defined within the family Lineidae (Kajihara et al. 2022; Chernyshev & Polyakova 2022, 2023) to which the best-hit entry for each search belonged was identified. The OTUs within the identified lineage were included in the ML analysis. Three *D. nigra* COI sequences from the Philippines and three from Hookabe et al. (2019), and *D. wijnhoffae* sequence were also included in the analysis. *Baseodiscus*

mexicanus (Bürger, 1893) and *Baseodiscus unicolor* Stiasny-Wijnhoff, 1925 were used as the outgroup. The OTUs selection for the Monostilifera ML analysis was based on the 15 nemertean GenBank entries that showed the highest max/total scores as a result of a MegaBLAST search of the Monostilifera sp. PH23 COI sequence and *Prosadenoporus* congeners whose COI was sequenced and deposited in GenBank (Maslakova & Norenburg 2008) including *P. olympiae* Kajihara, 2020 (Sundberg et al. 2016). *Reptantia* sp. KB2hop8 (Chernyshev & Polyakova 2019) was used as the outgroup.

The Heteronemertea and Monostilifera ML analyses were executed in IQ-Tree ver. 2.1.3 (Minh et al. 2020) using the best-fit models TIM + F + I + G4 and TIM3 + F + I + G4, respectively. Branch support values were derived from 1000 ultrafast bootstrap (UFBoot) (Minh et al. 2013).

Table 1. List of the ribbon worm species included in the maximum-likelihood analyses, specimens, localities, cytochrome c oxidase subunit I (COI) GenBank accession numbers, and references.

Species	Specimen	Locality	COI GenBank accession number	Reference
<i>Apatronemertes albimaculosa</i> Wilfert and Gibson, 1974	MCZ DNA106130	Madrid, Spain	HQ848584	Andrade et al. (2012)
<i>Baseodis unicolor</i> Stiasny-Wijnhoff, 1925	MCZ IZ135323	Panama	KF935505	Kvist et al. (2014)
<i>Baseodiscus mexicanus</i> (Bürger, 1893)	MCZ IZ135321	Mexico	KF935503	Kvist et al. (2014)
<i>Diplomma serpentinum</i> (Stimpson, 1855)	Hoplo_CB019_711_061802_2	Carrie Bow Cay, reef crest, Belize	MW021800	Maslakova et al. (2022)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8665	Initao, Misamis Oriental, Philippines	PP237395	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8666	Initao, Misamis Oriental, Philippines	PP237397	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8667	Medina, Misamis Oriental, Philippines	PP275106	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8668	Medina, Misamis Oriental, Philippines	PP237486	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8669	Medina, Misamis Oriental, Philippines	PP237488	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8670	Medina, Misamis Oriental, Philippines	PP237490	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8671	Medina, Misamis Oriental, Philippines	PP237493	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8672	Medina, Misamis Oriental, Philippines	PP237495	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8673	Carmen, Agusan del Norte, Philippines	PP237765	present study

<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8674	Carmen, Agusan del Norte, Philippines	PP237771	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8675	Carmen, Agusan del Norte, Philippines	PP237776	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8676	Carmen, Agusan del Norte, Philippines	PP237777	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8677	Carmen, Agusan del Norte, Philippines	PP237778	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8678	Carmen, Agusan del Norte, Philippines	PP237818	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8679	Carmen, Agusan del Norte, Philippines	PP237821	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8680	Sagay, Camiguin Island, Philippines	PP237823	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8681	Sagay, Camiguin Island, Philippines	PP238069	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8682	Sagay, Camiguin Island, Philippines	PP238074	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8683	Sagay, Camiguin Island, Philippines	PP238090	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8684	Sagay, Camiguin Island, Philippines	PP238099	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8685	Sagay, Camiguin Island, Philippines	PP238185	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8686	Catarman, Camiguin Island, Philippines	PP256688	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8687	Catarman, Camiguin Island, Philippines	PP261303	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8688	Guinsiliban, Camiguin Island, Philippines	PP256871	present study

<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8689	Guinsiliban, Camiguin Island, Philippines	PP256998	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8690	Mambajao, Camiguin Island, Philippines	PP257106	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8691	Mambajao, Camiguin Island, Philippines	PP257109	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8692	Mahinog, Camiguin Island, Philippines	PP257128	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8693	Mahinog, Camiguin Island, Philippines	PP257261	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8694	Mahinog, Camiguin Island, Philippines	PP257277	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8695	Lianga, Surigao del Sur, Philippines	PP275105	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8696	Lianga, Surigao del Sur, Philippines	PP257644	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8697	Lianga, Surigao del Sur, Philippines	PP261304	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8698	Buruanga, Aklan, Philippines	PP264287	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8699	Buruanga, Aklan, Philippines	PP264289	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8700	Buruanga, Aklan, Philippines	PP264291	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8701	Buruanga, Aklan, Philippines	PP264293	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8702	San Joaquin, Iloilo, Philippines	PP264295	present study
<i>Dushia nigra</i> (Stimpson, 1855) species complex	ICHUM 8703	San Joaquin, Iloilo, Philippines	PP264296	present study

<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise07	Bisezaki, Okinawa, Japan	LC389860	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise05	Bisezaki, Okinawa, Japan	LC389859	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise08	Bisezaki, Okinawa, Japan	LC389867	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake09	Kakeroma Island, Kogoshima, Japan	LC389868	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake05	Kakeroma Island, Kogoshima, Japan	LC389864	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise04	Bisezaki, Okinawa, Japan	LC389857	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake03	Kakeroma Island, Kogoshima, Japan	LC389862	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake04	Kakeroma Island, Kogoshima, Japan	LC389863	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise06	Bisezaki, Okinawa, Japan	LC389858	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake07	Kakeroma Island, Kogoshima, Japan	LC389866	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhKN	Kakeroma Island, Kogoshima, Japan	LC389869	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise08	Bisezaki, Okinawa, Japan	LC389861	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhkake06	Kakeroma Island, Kogoshima, Japan	LC389865	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhsesoko	Sesoko, Okinawa, Japan	LC389870	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise01	Bisezaki, Okinawa, Japan	LC389854	Hookabe et al. (2019)

<i>Dushia nigra</i> (Stimpson, 1855) species complex	nhbise02	Bisezaki, Okinawa, Japan	LC389855	Hookabe et al. (2019)
<i>Dushia nigra</i> (Stimpson, 1855) species complex	NemBar0977	Vietnam	KU840276	Sundberg et al. (2016)
<i>Dushina wijnhoffae</i> Schwartz & Norenburg, 2019	USNM 1594812	Anse de Grand Fond, St. Barhélemy, France	EF124967	Schwartz & Norenburg (unpubl.)
<i>Evelineus</i> sp.	Kuril A12	Iturup Island, Russia	OM456684	Chernyshev & Polyakova (2022)
<i>Gurjanovella littoralis</i> Ushakov, 1926	JLN080401E8		MZ216518	Chernyshev et al. (2021)
<i>Gurjanovella</i> sp.	11DS	Northwest Pacific	MF512123	Chernyshev & Polyakova (2018)
<i>Hinumanemertes kikuchii</i> Iwata, 1970	Hinkik	Sea of Japan, Russia	OQ450491	Chernyshev & Polyakova (2023)
<i>Iwatanemertes piperata</i> (Stimpson, 1855)	ICHUM 6192	Kanagawa, Araiama, Japan	LC625622	Kajihara et al. (2022)
<i>Kulikovia alborostrata</i> (Takakura, 1898)	L.albo1	Vostok Bay, Russia	KU821529	Chernyshev et al. (2017)
<i>Kulikovia manchenkoi</i> Chernyshev et al., 2017	LtSkh1	Sakhalin Island, Kholmsk, Russia	KU821523	Chernyshev et al. (2017)
<i>Kulikovia torquata</i> (Coe, 1901)	LtUr1	Urup Island, Russia	KU821511	Chernyshev et al. (2017)
Lineidae sp.	Antarctica 5	Clarence Island, Antarctica	OM456691	Chernyshev & Polyakova (2022)

Lineidae sp.	SJ 5	Chirpoy Island, Russia	OM456693	Chernyshev & Polyakova (2022)
Lineidae sp. PH23-01	ICHUM 8654	Carmen, Agusan del Norte, Philippines	PP271299	present study
Lineidae sp. PH23-01	ICHUM 8655	Carmen, Agusan del Norte, Philippines	PP271300	present study
Lineidae sp. PH23-01	ICHUM 8656	Carmen, Agusan del Norte, Philippines	PP271302	present study
Lineidae sp. PH23-01	ICHUM 8657	Carmen, Agusan del Norte, Philippines	PP279835	present study
Lineidae sp. PH23-01	ICHUM 8658	Buruanga, Aklan, Philippines	PP271301	present study
Lineidae sp. PH23-01	ICHUM 8659	Buruanga, Aklan, Philippines	PP271304	present study
Lineidae sp. PH23-01	ICHUM 8660	Buruanga, Aklan, Philippines	PP279838	present study
Lineidae sp. PH23-01	ICHUM 8661	Buruanga, Aklan, Philippines	PP271303	present study
Lineidae sp. PH23-01	ICHUM 8662	Buruanga, Aklan, Philippines	PP279836	present study
Lineidae sp. PH23-01	ICHUM 8663	Talisayan, Misamis Oriental Philippines	PP279837	present study
Lineidae sp. PH23-02	ICHUM 8664	Liang, Surigao del Sur, Philippines	PP279839	present study
<i>Lineus acutifrons</i> Southern, 1913		Spain	GU590937	Andrade et al. (2012)
<i>Lineus flavescens</i> Coe, 1904	OR_C10	Charleston, Oregon, USA	KP682050	Heibert & Maslakova (2015)

<i>Micrura callima</i> Sundberg & Gibson, 1995	KB2het2	Guam, USA	MN205496	Chernyshev & Polyakova (2022)
<i>Micrura verrilli</i> Coe, 1901	SK41	USA	KF935508	Kvist et al. (2014)
<i>Monostilifera</i> sp.	Hoplo CB171	Bocas del Toro, Panama	OK081421	Maslakova et al. (2022)
<i>Monostilifera</i> sp.	MCZ IZ 133023	Panama	KF935533	Kvist et al. (2014)
<i>Monostilifera</i> sp.	Hoplo CB058	Bocas Del Toro, Panama	OK081392	Maslakova et al. (2022)
<i>Monostilifera</i> sp. PH23	ICHUM 8704	Liang, Surigao del Sur, Philippines	PP275145	present study
<i>Nipponnemertes incainca</i> Gonzales-Cueto et al., 2017	Hoplo CB063	Bocas Del Toro, Panama	OK081430	Maslakova et al. 2022
<i>Nipponomicrura</i> sp.	Kuril 7	Iturup Island, Russia	OM456695	Chernyshev & Polyakova (2022)
<i>Nipponomicrura</i> sp.	Kuril A08	Urup Island, Russia	OM456694	Chernyshev & Polyakova (2022)
<i>Nipponomicrura uchidai</i> (Yamaoka, 1940b)	Nuch	Peter the Great Bay, Sea of Japan	KY561815	Chernyshev et al. (2017)
<i>Oerstedtia fuscosparsa</i> Abato et al., 2023	ICHUM 8406	Chiba, Japan	OP265742	Abato et al. (2023)
<i>Oerstedtia phoresiae</i> (Kukilova, 1987)	KB2hop17	Sea of Japan, Russia	MN205513	Chernyshev & Polyakova (2019)

<i>Oerstedtia</i> sp.	SJ 64	Sea of Japan, Russia	OM456733	Chernyshev & Polyakova (2022)
<i>Prosadenoporus californiensis</i> (Gibson et al., 1982)	#4-15-92	Tomales Bay, CA, USA	EF157597	Maslakova & Norenburg (2008)
<i>Prosadenoporus floridensis</i> Maslakova & Norenburg, 2008	#2-2298-2	Link Port, FL, USA	EF157596	Maslakova & Norenburg (2008)
<i>Prosadenoporus mooreae</i> (Gibson, 1982)	#778-03-23-03-1,2,3	Picnic Bay, Magnetic Island, Australia	EF157595	Maslakova & Norenburg (2008)
<i>Prosadenoporus mortoni</i> (Gibson, 1990)	#910913	Fujian Province, China	EF157593	Maslakova & Norenburg (2008)
<i>Prosadenoporus olympiae</i> Kajihara, 2020	NemBar0833	Cebu, Philippines	KU840160	Sundberg et al. (2016)
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8705	Liang, Surigao del Sur, Philippines	PP271291	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8706	Lawigan, Iloilo, Philippines	PP271293	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8707	Catarman, Camiguin Island, Philippines	PP271292	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8708	Catarman, Camiguin Island, Philippines	PP271294	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8709	Catarman, Camiguin Island, Philippines	PP271295	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8710	Catarman, Camiguin Island, Philippines	PP271297	present study
<i>Prosadenoporus</i> sp. PH23-02	ICHUM 8711	Talisayan, Misamis Oriental, Philippines	PP271298	present study

<i>Prosadenoporus spectaculus</i> (Yamaoka, 1940a)		Shenzhen, Guangdong, China	KC710981	Sun & Sun (2014)
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8712	Lianga, Surigao del Sur, Philippines	PP264547	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8713	Lianga, Surigao del Sur, Philippines	PP264611	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8714	Lianga, Surigao del Sur, Philippines	PP271296	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8715	Lianga, Surigao del Sur, Philippines	PP264619	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8716	Lawigan, Iloilo, Philippines	PP264623	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8717	Guinsiliban, Camiguin Island, Philippines	PP264624	present study
<i>Prosadenoporus</i> sp. PH23-01	ICHUM 8718	Guinsiliban, Camiguin Island, Philippines	PP264626	present study
<i>Prosadenoporus winsori</i> (Moore & Gibson, 1981)	#777-03-18-03S-5	Ross River, Townsville, Australia	EF157594	Maslakova & Norenburg (2008)
<i>Pseudotetrastemma</i> sp.	KB2hop25	Sea of Japan, Russia	MN205520	Chernyshev & Polyakova (2019)
<i>Quasilineus sinicus</i> Gibson, 1990		China	MZ274345	Shen et al. (2022)
Reptant nemertean	481	Okinawa, Japan	AJ436929	Thollesson & Norenburg (2003)
Reptantia sp.	KB2hop8	Kuril-Kamchatka Trench, Russia	MN205507	Chernyshev & Polyakova (2019)

<i>Siphonenteron nakanoi</i> Kajihara et al., 2022	ICHUM 6223	Kanagawa, Japan	LC625646	Kajihara et al. (2022)
<i>Tetranemertes bifrost</i> et al., 2023	CBdT0063	Bocas del Toro, Panama	MT578874	Cherneva et al. (2023)
<i>Tetrastemma bilineatum</i> Coe, 1904	BOIMB 0456	Oregon, USA	OK081638	Maslakova et al. (2022)
<i>Tetrastemma bilineatum</i> Coe, 1904	E2F3	Charleston, Oregon, USA	KU197635	Heibert & Maslakova (2015)
<i>Vulcanonemertes rangitotoensis</i> Gibson & Strand, 2002	H71b	Takapuna, New Zealand	AB505828	Kajihara et al. (2011)
<i>Yininemertes pratensis</i> (Sun & Lu, 1998)	Hn_CO1_8	Han River Estuary, Korea	KY274010	Kvist et al. (2014)
<i>Zygonemertes simonae</i> Corrêa, 1961		Fort Pierce, Florida, USA	AJ436922	Thollessen & Norenburg (2003)

Results and systematic remarks

Intertidal ribbon worm fauna

A total of 232 nemertean specimens (Fig. 2) were collected in the survey based on the morphology examination of the collected specimens and on the COI MegaBLAST searches executed in the NCBI website. The collection was composed of eight species: four in Heteronemertea—two members of the *D. nigra* species complex, Lineidae sp. PH23-01, and Lineidae sp. PH23-02 (Fig. 3), and four in Monostilifera—*P. spectaculus*, *Prosadenoporus* sp. PH23-01, *Prosadenoporus* sp. PH23-02, and Monostilifera sp. PH23 (Figs. 4, 5). The members of *D. nigra* species complex and *P. spectaculus* are recorded and reported for the first time in the Philippines while the rest could represent species that are potentially new to science and are to be established as new species in future taxonomic works.

Taxonomy

Class Pilidiophora Thollessen & Norenburg, 2003

Order Heteronemertea Coe, 1905

Family Lineidae McIntosh, 1874

Genus *Dushia* Corrêa, 1963

Dushia nigra (Stimpson, 1855) species complex (Fig. 3a–c).

Material examined. Sequenced specimens, 39 individuals, collected intertidally in shorelines of fine and coarse sand materials and in rocky intertidal zones, extracted genomic DNA, ICHUM 8665, 8666, collected by Jamael Abato and Jerric Delfin in Brgy. Tubigan, Initao, Misamis Oriental (8.5438°N, 124.3202°E) on March 19, 2023; ICHUM 8667–8672, collected by Millanie Abatay, Merimee Dahilog-Caralde, and Jigger Caralde in Brgy. Duka, Medina, Misamis Oriental (8.9254°N, 125.0096°E) on March 22, 2023; ICHUM 8673–8675, 8679, collected by Jamael Abato, Archie Along, and Laurence Calagui in Brgy. Tagcatong, Carmen, Agusan del Norte (9.0141°N, 125.2569°E) on April 3, 2023; ICHUM 8676–8678, collected by Meljan Demetillo and Julius Anthony Leones in Brgy. San Agustin, Carmen, Agusan del Norte (9.0472°N, 125.2338°E) on April 3, 2023; ICHUM 8680–8685, collected by Jamael Abato, Warren Caneos, and Marjun Dela Cerna

in Brgy. Bugang, Sagay, Camiguin Island (9.1102°N, 124.7008°E) on March 30, 2023; ICHUM 8686, 8687, collected by Richard Caneos and Joselito Sacupayo Jr. in Brgy. Catibac, Catarman, Camiguin Island (9.1738°N, 124.6355°E) on March 29, 2023; ICHUM 8688, 8689, collected by Jamael Abato and Warren Caneos in Brgy. Liong, Guinsiliban, Camiguin Island (9.0788°N, 124.7663°E) on March 30, 2023; ICHUM 8690, 8691, collected by Richard Caneos and Joselito Sacupayo Jr. in Brgy. Naasag, Mambajao, Camiguin Island (9.2350°N, 124.6480°E) on March 29, 2023; ICHUM 8692–8694, collected by Jamael Abato, Warren Caneos, and Richard Caneos in Brgy. Hubangon, Mahinog, Camiguin Island (9.1724°N, 124.7884°E) on March 30, 2023; ICHUM 8695, 8697, collected by Jamael Abato and Archie Along in Brgy. Payasan, Lianga, Surigao del Sur (8.6175°N, 126.0875°E) on April 5, 2023; ICHUM 8696, collected by Anthony Leones in Brgy. Anibongan, Lianga, Surigao del Sur (8.5936°N, 126.1083°E) on April 5, 2023; ICHUM 8698–8701, collected by Segrid Manderico and James Erol de Castro in Brgy. Panilongan, Buruanga, Aklan, Panay (11.8086°N, 121.4277°E) on April 13, 2023; and ICHUM 8702, 8703, collected by Mellescent Jean Sobrevega and George Amenin in Brgy. Lawigan, Iloilo, Panay (10.4991°N, 122.0580°E) on April 14, 2023.

Description. *External features.* Living worm contractile, highly mobile; length in anaesthetized state 4.6–14.5 cm (n = 30) width 0.1–0.2 cm (n = 30); entire body uniformly black without any markings (Fig. 3a); other specimens have brown or orange body coloration (Fig. 3b, c), dorsal side always darker than ventral; head not demarcated from the rest of the body, slightly narrower than anterior body end (sometimes as wide as the anterior body), generally oblong, anterior end pointed, triangular (i.e., acute), generally darker in color on both sides than rest of the body, anterolateral edges and tip colorless; deep, lateral, horizontal cephalic furrows extend on both sides of the head up to the anterior end of the mouth opening; cerebral ganglia visible as a reddish hue at the widest portion of the head on both sides; proboscis pore and mouth very notable at ventral view; posterior body end tapers, paler to colorless in some specimens, with caudal cirrus.

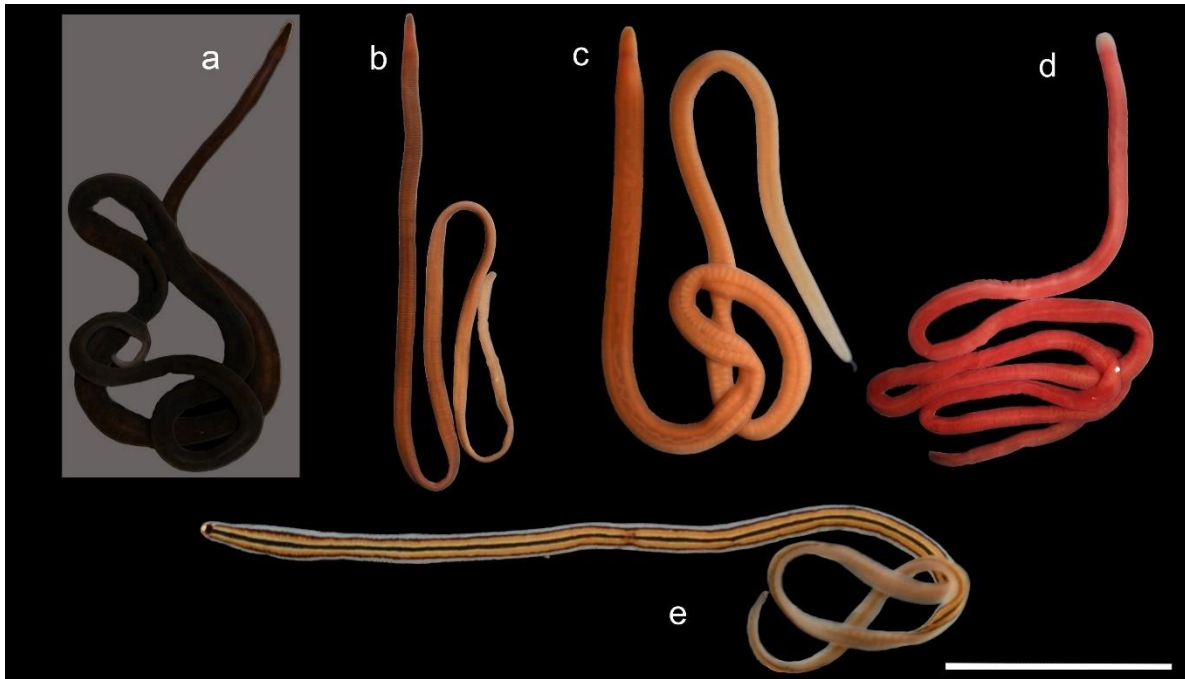


Figure 3. Representatives of heteronemerteans collected in the 2023 faunal survey. Members of one of the species in the *Dushia nigra* complex showing the (a), black form, ICHUM 8667 from Medina, Misamis Oriental; (b), brown form, ICHUM 8696 from Lianga, Surigao del Sur; and (c), orange form, ICHUM 8681 from Sagay, Camiguin Island; (d), Lineidae sp. PH23-01, ICHUM 8654 from Carmen, Agusan del Norte; (e), Lineidae sp. PH23-02, ICHUM 8664 from Lianga, Surigao del Sur. Scale bar: 1 cm.

Taxonomic remarks. The molecular species delimitation analysis on *D. nigra* suggested the presence of two putative species, with ICHUM 8666, a black form, different from the rest of the collected Philippine specimens. However, there is no sufficient external characteristic(s) that can be used to morphologically separate this species from the rest, hence, I consider *D. nigra* as a species complex as in Hookabe et al. (2019).

Distribution. Previously known only in China (Stimpson 1855), Japan (Iwata 1952; Kajihara 2017; Hookabe et al. 2019), Taiwan (Yamaoka 1939), and Vietnam (Chernyshev 2016). New distribution, the Philippines (present study).

Genus incertae sedis

Lineidae sp. PH23-01 (Fig. 3d).

Material examined. Ten individuals, collected intertidally in shorelines of fine and coarse sand materials and in rocky intertidal zones with signs of freshwater inputs at low tide, extracted genomic DNA, ICHUM 8654–8657, collected by Jamael Abato and Archie Along in Brgy. Tagcatong, Carmen, Agusan del Norte (9.0141°N, 125.2569°E) on April 3, 2023; ICHUM 8658–8661, collected by Kristi Rose Jorge and Dennis Espartero in Brgy. Panilongan, Buruanga, Aklan, Panay (11.8086°N, 121.4277°E) on April 13, 2023; ICHUM 8662, collected by Jigger Caralde in Brgy. Pampangon, Talisayan, Misamis Oriental (8.9702°N, 124.9302°E) on March 20, 2023; and ICHUM 8663, collected by Jamael Abato in Brgy. Anibongan, Lianga, Surigao del Sur (8.5936°N, 126.1083°E) on April 5, 2023.

Description. *External features.* Living worm highly mobile and contractile, entire body pale pink to deep pink in color (Fig. 3d) without any mottles, bands, or stripes, ventral side always lighter than dorsal, body uniform in width but tapers on posterior end, length in anaesthetized state 6.0–14.8 cm (n = 7), width 0.1–0.2 cm (n = 7); head not demarcated from the rest of the body, round in shape, appears thin and flat when worm contracts at living state, as wide as the body but width expands when worm is active, anterolateral edges non-pigmented or opaque white on both sides; junction between head and body narrower; cerebral ganglia visible as reddish hues on both sides of the junction; cephalic furrows not observed at dorsal view, at ventral view, a pair of cephalic furrow runs from the lateroposterior head towards and connects at mid anterior region; mouth situated slightly far below the head-body junction; intestine visible at ventral view; rhynchocoel runs the entire body length; proboscis not branched; no caudal cirrus.

Taxonomic remarks. According to the result of the phylogenetic analysis (see Fig. 6) Lineidae sp. PH23-01 is closely related to *Yininemertes pratensis* (Sun & Lu, 1998) and *Apatronemertes albimaculosa* Wilfert & Gibson, 1974. The three species have pinkish background color. Based on external features, Lineidae sp. PH23-01 can be differentiated from *Y. pratensis* by unpigmented anterolateral edge of the head (i.e., appears opaque white on both sides); entire body uniformly deep pink or paler; and absence of narrow transverse rings on the body (Sun & Lu 1998; Park et al. 2019). Likewise, it can be differentiated from *A. albimaculosa* by the absence of the rice-grain-

shaped white dots on its body surface although both sometimes share similarities in background body coloration and head being unpigmented on anterolateral edges (Kajihara et al. 2016).

Genus *incertae sedis*

Lineidae sp. PH23-02 (Fig. 3e).

Material examined. One individual, extracted genomic DNA, ICHUM 8664, collected intertidally in a slightly silty and rocky shoreline by Julius Anthony Leones in Brgy. Payasan, Lianga, Surigao del Sur (8.6175°N, 126.0875°E) on April 5, 2023.

Description. *External features.* Thin and long nemertean, width uniform throughout the body except tapering posterior region, length in anaesthetized state 5.56 cm, width 0.1 cm (but much longer and thinner in the non-anaesthetized state), dorsal body entirely decorated with vertical black, orange, and yellow bands against an opaque white background (Fig. 3e), ventral side not pigmented instead opaque white in color; head not demarcated from the rest of the body, tip colorless, anterior head patched with the same color combination as the body, with notable bright orange, round pigmentation on the anterolateral sides, and a hexagon-like, black patch on mid posterior end; colored patches in head continuous with the bands on the dorsal; intestine visible ventrally; no caudal cirrus.

Taxonomic remarks. Lineidae sp. PH23-02 shares the same dorsal body color combination with *Iwatanemertes piperata* (Stimpson, 1855), *Quasilineus sinicus* Gibson, 1990, and *Quasilineus pulcherrimus* Gibson, 1981 (i.e., the four species have dorsal bodies with black, yellow, and orange colorations and with bright orange pigments on the tip of the head). However, based on its general external body features, Lineidae sp. PH23-02 resembles more *Q. sinicus* and *Q. pulcherrimus* by having vertical bands of black, yellow, and orange running the entire dorsal body than *I. piperata* which has no vertical bands of the same color, rather, black blotches sprinkled on the dorsal body against a very pale-yellow background (Gibson 1981, 1990). Lineidae sp. PH23-02

morphologically differs from *Q. sinicus* by the orange dorsal bands obviously originating and posteriorly extending from the antero-lateral orange cephalic patches and with the two outermost black dorsal bands not reaching the hexagon-like black pigmentation in the head. Moreover, it differs with *Q. pulcherrimus* on the arrangement of the dorsal bands, being black the outermost bands in the former and orange on the latter; and on the shape and location of the cephalic patch, being two nearly circular patches on the lateral sides of the head in the former and a single crescent-shaped patch facing downward near the tip of the head on the latter. The morphological features of Lineidae sp. PH23-02 do not match any of the described congeners of the genus *Quasilineus* Gibson, 1981, indicating that the present species is likely an undescribed *Quasilineus*, however, a more detailed morphological and molecular phylogenetic study are needed to ascertain this claim.

Class Hoplonemertea Coe, 1905

Order Monostilifera Brinkmann, 1917

Family Prosorhochmidae Bürger, 1895

Genus *Prosadenoporus* Bürger, 1890

Prosadenoporus spectaculus (Yamaoka, 1940a) (Fig. 4a–o).

Material examined. Seven individuals, collected intertidally in shorelines with fine and coarse sand materials, from slightly silty, and rocky intertidal zones, extracted genomic DNA, ICHUM 8712–8715, collected by Archie Along, Laurence Calagui, and Julius Anthony Leones in Brgy. Payasan, Lianga, Surigao del Sur (8.6175°N, 126.0875°E) on April 5 and 6, 2023; ICHUM 8716, collected by Mellescent Jean Sobrevega and George Amenin in Brgy. Lawigan, Iloilo, Panay (10.4991°N, 122.0580°E) on April 14, 2023; and ICHUM 8717, 8718, collected by Warren Caneos and Richard Caneos in Brgy. Liong, Guinsiliban, Camiguin Island (9.0788°N, 124.7663°E) on March 30, 2023.

Description. *External and internal features.* Living worm, contractile, less mobile; body length at anaesthetized state 5.6–9.2 cm (n = 7), width 0.1–0.3 cm (n = 7); body uniformly brownish (Fig. 4a, m) at dorsal view, pale brown (Fig. 4b, d, h, n) ventrally, sometimes

worm appears creamy white (Fig. 4k, l) at posterior region due to intestinal structures, dorsal body with minute brown dots at higher magnification, less prominent at the posterior region, transition of dorsoventral coloration indistinct; head round, as wide as the anterior body, notched at the mid anterior edge, with dark-brown to black cephalic patch (i.e., the spectacle or helmet-shaped pigmentation in the head, darker than the rest of the body) at dorsal view (Fig. 4c, g), anterolateral edges and mid posterior of the patched head notably lighter; anterior horizontal cephalic furrow ('prosorchochmid smile') (Fig. 4e, i) present, posterior cephalic furrow encircling head, mid-dorsally 'V' shape pointing backward (Fig. 4c, g); ocelli four in number (Fig. 4o), black, anterior pair larger and prominent at dorsal view of the head, situated on the lighter anterolateral edges of the cephalic patch, posterior pair smaller, usually not obviously visible at dorsal and ventral views (Fig. 4c, d, g, h) of the head but notable at the lateral view (Fig. 4e, i); rhynchocoel visible dorsally (Fig. 4k, l, m, n), extends to the entire body length; reflection of the brain, stomach (Fig. 4d, h), and intestine visible at the ventral side of the body (Fig. 4b, l, n); tail blunt.

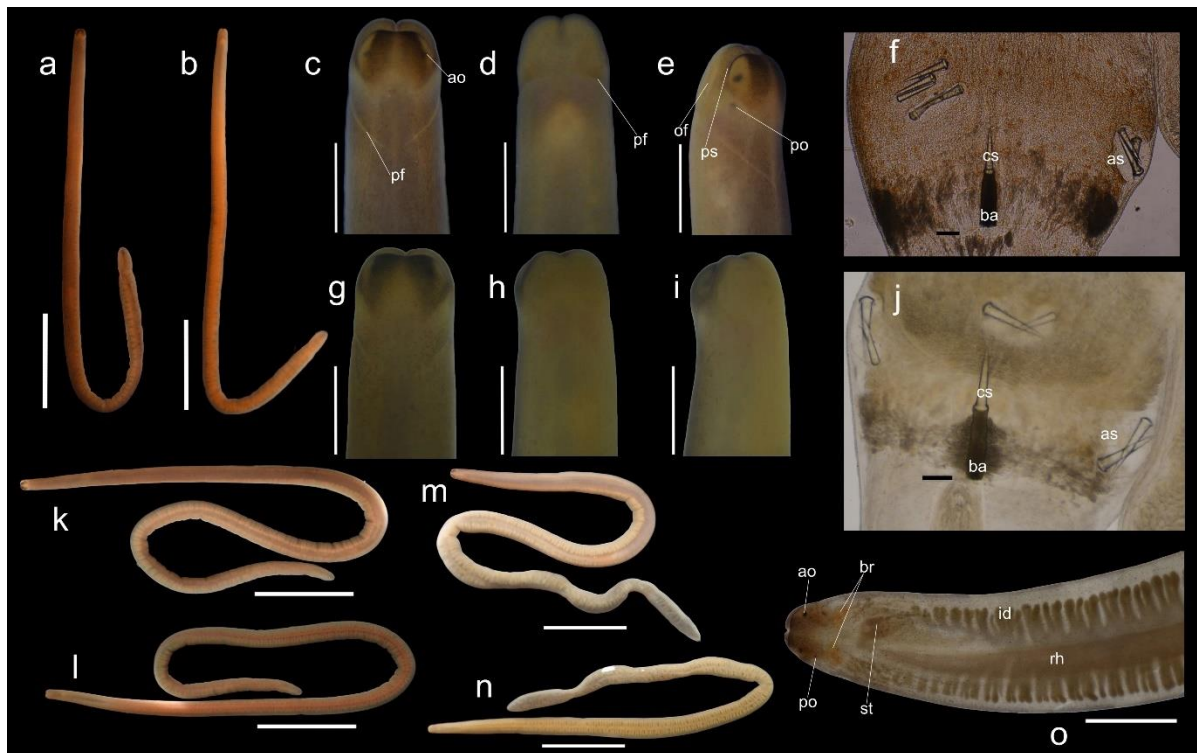


Figure 4. *Prosadenoporus spectaculus* from the Philippines. (a–f), ICHUM 8713, from Brgy. Payasan, Lianga Surigao del Sur; (g–l), ICHUM 8716, from Brgy. Lawigan, San Joaquin, Iloilo; (m, n), ICHUM 8717; and (o), ICHUM 8718, from Brgy. Liang, Guinsiliban, Camiguin Island. Abbreviations: ao, anterior ocellus; as, accessory stylet; ba, basis; br,

brain; cs, central stylet; id, intestinal diverticulum; of, cerebral organ furrow; pf, posterior cephalic furrow; po, posterior ocellus; ps, prosorhochmid smile; rh, rhynchocoel; st, stomach. Scale bar: a, b k, l, m, n = 1 cm; c–e, g–i = 1 mm; f = 200 μ m; j = 100 μ m; o = 2 mm.

Central stylet length 211–381 μ m (n = 7), width 26–46 μ m (n = 7); basis length 234–512 μ m (n = 7), width 39–88 μ m (n = 7), brownish to dark in color, anterior end less dense than posterior, surface fine, shape rectangular or cylindrical, narrower at the anterior; 3 stylet pouches present with 2–3 accessory stylets (Fig. 4f, j).

Taxonomic remarks. The collected *P. spectaculus* in the present study are longer than those reported by Yamaoka (1940a), although body widths are almost the same. The shape of the spectacle also varied—Yamaoka’s illustration shows a well-defined and well-bounded spectacle, with a nearly circular pattern at the center that opens both anteriorly and posteriorly. This circular pattern is not observed in the Philippine specimens, instead, this appears consistently as an inverted ‘V’ (also see Kajihara 2017). Additionally, each of the anterior ocelli of the Philippine representatives are always distanced with one another compared to the nearer anterior ocelli in Yamaoka’s. Also, Yamaoka showed a posterior cephalic furrow situated near the spectacle’s posterior end whereas this is notably distanced in the present species. Lastly, as opposed to the original description of *P. spectaculus*, the faint longitudinal white stripe marking the mid-dorsal line of the body is not apparent in the present specimens.

Distribution. Previously known only in China (Sun & Sun 2014), Hong Kong (Gibson 1990), and Japan (Yamaoka 1940a; Kajihara 2017). New locality distribution, the Philippines (present study).

***Prosadenoporus* sp. PH23-01** (Fig. 5a, b, e).

Material examined. Six individuals, collected intertidally in shorelines of fine and coarse sand materials and in rocky intertidal zones, extracted genomic DNA, ICHUM 8705, collected by Archie Along in Brgy. Payasan, Lianga, Surigao del Sur (8.6175°N, 126.0875°E) on April 6, 2023; ICHUM 8706, collected by Jamael Abato in Brgy. Lawigan,

Iloilo, Panay (10.4991°N, 122.0580°E) on April 14, 2023; and ICHUM 8707–10, collected by Jamael Abato, Warren Caneos, and Richard Caneos in Brgy. Catibac, Catarman, Camiguin Island (9.1738°N, 124.6355°E) on March 29, 2023.

Description. *External and internal features.* Living worm contractile, less mobile; length in anaesthetized condition 9.5–13.5 cm (n = 6), width 0.1–0.2 cm (n = 6); body brownish to light brownish orange (Fig. 5a, b), ventral side lighter than dorsal, no body markings; head not demarcated from the rest of the body, round, narrower than anterior body, notched at the mid anterior edge, with brown cephalic patch of no distinct pattern, posteriorly paler in color above posterior cephalic furrow, lateral and anterior margins lighter in color to opaque white in appearance, with paler longitudinal line at the mid dorsal head extending downwards to the posterior cephalic furrow; with ‘prosorchochmid smile’, posterior cephalic furrow encircling the head; ocelli four in number, black in color. Central stylet (Fig. 5e) shorter than basis; basis nearly rectangular or cylindrical; with 3–4 stylet pouches each with 1–3 accessory stylets.

Taxonomic remarks. *Prosadenoporus* sp. PH23-01 is morphologically distinct from *P. spectaculus* by the absence of the dark-colored spectacle on its dorsal head and not being pigmented on its anterolateral edges (notable at dorsal view); basis being shorter than central stylet; and on the number of stylet pouches and accessory stylets. Although *Prosadenoporus* sp. PH23-01 morphologically resembles *P. olympiae* in terms of the external features, the former can be differentiated from the latter by having black ocelli; lighter dorsal region between the cephalic patch and the posterior cephalic furrow; basis being shorter than the central stylet; and on the number of stylet pouches and accessory stylets.

***Prosadenoporus* sp. PH23-02** (Fig. 5c, f).

Material examined. One individual, extracted genomic DNA, ICHUM 8711, collected by Jerric Delfin from a rocky shoreline in Brgy. Pampangon, Talisayan, Misamis Oriental (8.9702°N, 124.9302°E) on March 20, 2023.

Description. *External and internal features.* Living worm, contractile, less mobile; length in anaesthetized state 5.14 cm; width 0.14 cm; entire worm orange in color, ventral body lighter than dorsal; head not demarcated from rest of the body, round, narrower than anterior body, with dark orange cephalic patch except at the lateral edges downwards; with ‘prosorchochmid smile’; non-connected posterior cephalic furrows only visible at dorsal body; anterior ocelli apparent at dorsal side of the head, posterior ones not visible (Fig. 5c). Central stylet (Fig. 5f) shorter than basis; basis spindle-like; with 2 stylet pouches each with 2–4 accessory stylets.

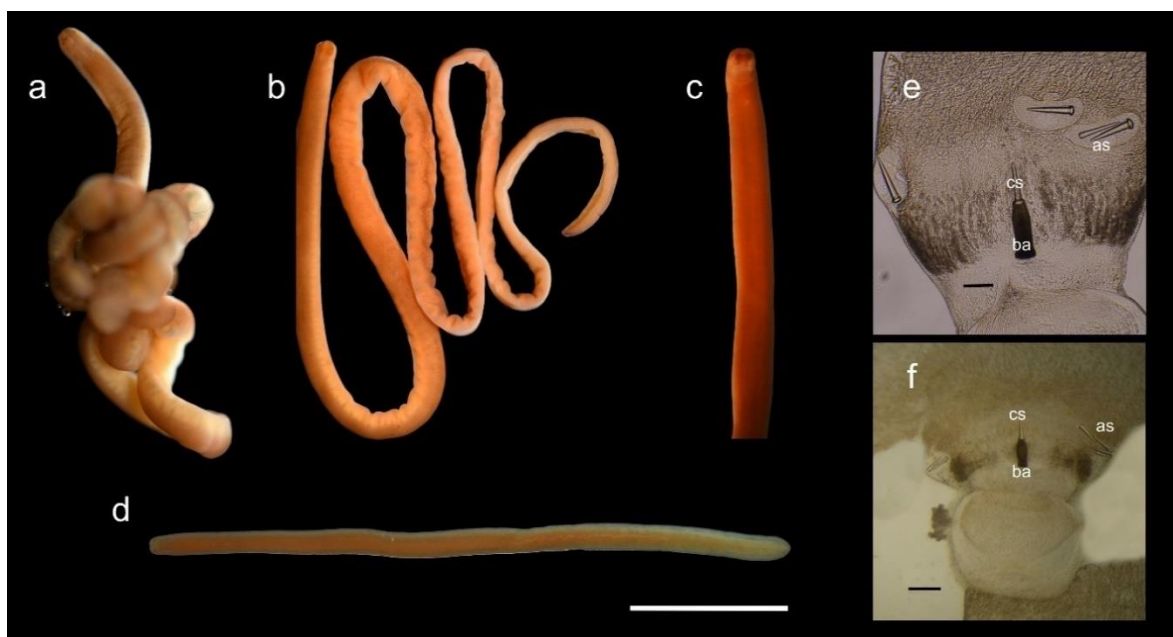


Figure 5. Representatives of monostiliferans collected from the Philippines. (a), *Prosadenoporus* sp. PH23-01, ICHUM 8707 and (b), ICHUM 8708, from Catarman, Camiguin Island; (c), *Prosadenoporus* sp. PH23-02, ICHUM 8711, from Talisayan, Misamis Oriental; (d), *Monostilifera* sp. PH23, ICHUM 8704, from Lianga, Surigao del Sur. Abbreviations: as, accessory stylet; ba, basis; cs, central stylet. Scale bar: 1 cm.

Taxonomic remarks: *Prosadenoporus* sp. PH23-02 can be differentiated from the other *Prosadenoporus* reported from the Philippines by its deep-orange body color, dark-orange cephalic patch, and spindle-like stylet basis. The body of *Prosadenoporus* sp. PH23-01 and *Prosadenoporus* sp. PH23-02 is both orange-appearing in color but they can be differentiated by the presence of distinct dark-orange cephalic patch in the latter. *P. spectaculus* is morphologically different from *Prosadenoporus* sp. PH23-02 by being brownish to creamy-white in appearance, having a dark-brown to black spectacle on

the dorsal head, and by the 'V'-shaped posterior cephalic furrow. Similarly, *P. olympiae* can be differentiated from *Prosadenoporus* sp. PH23-02 through its brownish color, absence of cephalic patch, and its 'V'-shaped posterior cephalic furrow.

Family *incertae sedis*

Monostilifera sp. PH23 (Fig. 5d).

Material examined. One individual, extracted genomic DNA, ICHUM 8704, collected by Jamael Abato from a slightly silty and rocky shoreline in Brgy. Payasan, Lianga, Surigao del Sur (8.6175°N, 126.0875°E) on April 5, 2023.

Description. *External and internal features.* Worm pale yellow in color, ventral side lighter than dorsal, posterior end lighter in color to opaque white (Fig. 5d); length in anaesthetized state 2.13 cm, width 0.1 cm; head not demarcated from the rest of the body, as wide as anterior body, lighter in color; ocelli four in number, anterior ocelli less indented, bigger, and more prominent than more indented and smaller posterior pair; cephalic furrows not evident at dorsal view; at ventral view, a pair of non-connected, anterolateral cephalic furrow notable, posterior cephalic furrow poorly visible. Central stylet longer than basis; basis cylindrical; with 2 stylet pouches each with 3 accessory stylets.

Molecular analyses on Heteronemertea

The ML tree (Fig. 6) for the heteronemerteans documented in the study confirmed the presence of two unidentified lineids (i.e., Lineidae sp. PH23-01 and Lineidae sp. PH23-02) and the occurrence of *D. nigra* from the Philippines. Lineidae sp. PH23-01 and Lineidae sp. PH23-02 were nested within Lineage N+P and Lineage A of the family Lineidae, respectively (Chernyshev & Polyakova 2022; Kajihara et al. 2022). The tree also showed that the Philippines and Japan *D. nigra* formed a fully supported clade together with *D. wijnhoffae*. Additionally, the tree revealed two COI-lineages of *D. nigra* from the Philippines.

Based on the 657-bp partial COI sequences, the genetic variation (p -distance) between the 10 specimens of Lineidae sp. PH23-01 was 0–2.4%, which suggested that they were conspecific. Lineidae sp. PH23-01 from Aklan, Misamis Oriental, Agusan del Norte and Surigao del Sur formed a fully supported clade. This species was sister to *A. albimaculosa* and together formed a highly supported clade with *Y. pratensis* (Fig. 6). Lineidae sp. PH23-01 did not match any morphologically described species in Lineage N+P, indicating that it could be an undescribed member of the lineage. Lineidae sp. PH23-02 was closely related to *I. piperata*, together, they formed a clade with *Q. sinicus* (Fig. 6).

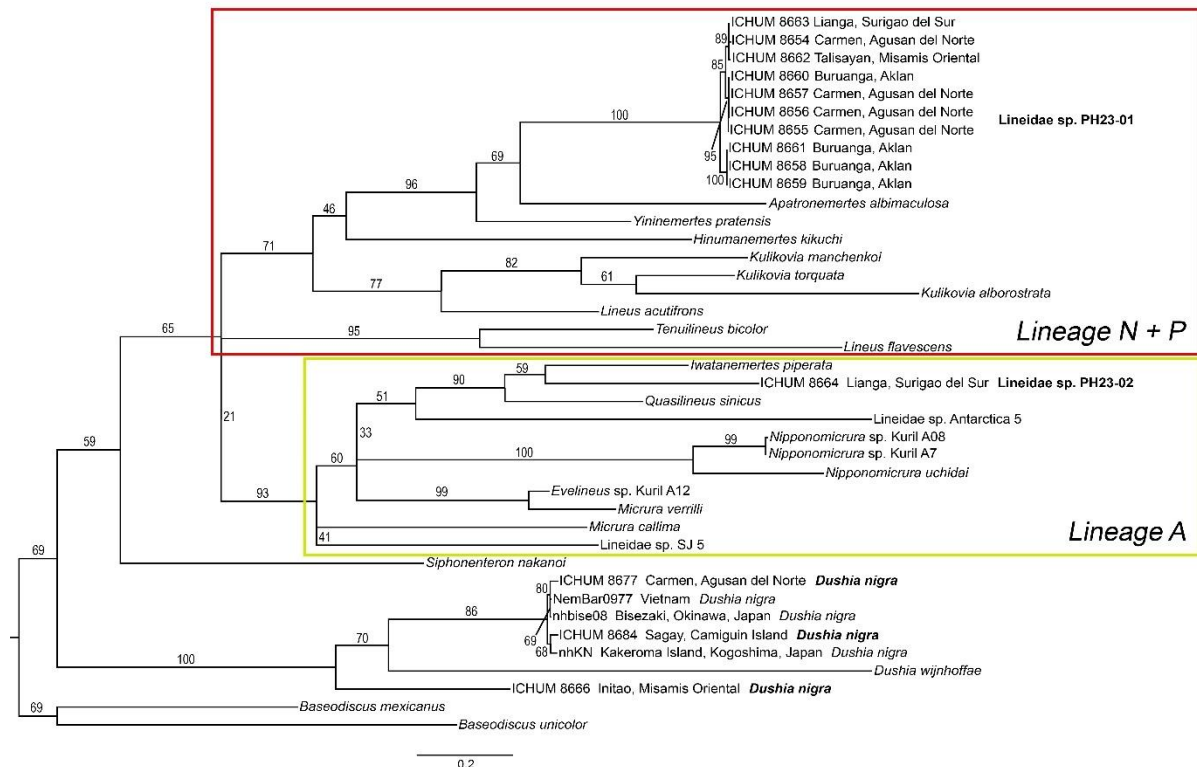


Figure 6. A rooted maximum-likelihood tree for Heteronemertea generated by IQ-Tree based on the best-fit model TIM + F + I + G4 model with 696-bp partial sequence of the COI gene from members of the Lineage A and Lineage N+P of the family Lineidae, and *Dushia* spp. Number at each branch represents branch support derived from 1000 ultrafast bootstrap value. Species in the present study are represented in boldface. *B. mexicanus* and *B. unicolor* were used as outgroup.

From the 207 specimens of the *D. nigra* species complex collected in the survey, partial COI sequence was generated from 39 individuals. These sequences together

with the *D. nigra* COI sequences from public databases were subjected to species delimitation analysis. ABGD, ASAP (ASAP score = 1.00), and PTP best partitioned the generated *D. nigra* COI sequences into two distinct clusters which both contained representatives from the Philippines and Japan (Fig. 7b). Cluster A contained the common member of the species complex (i.e., the member which has a wide distribution and abundant in terms of number) while cluster B appeared to include the rare member (i.e., the member which has a limited distribution and less abundant in terms of number). Cluster B contained only one Philippine representative, namely ICHUM 8666 from Brgy. Tubigan, Initao, Misamis Oriental and two representatives from Japan. Using *p*-distance, the genetic variation between the two groups was 14.3% while the genetic

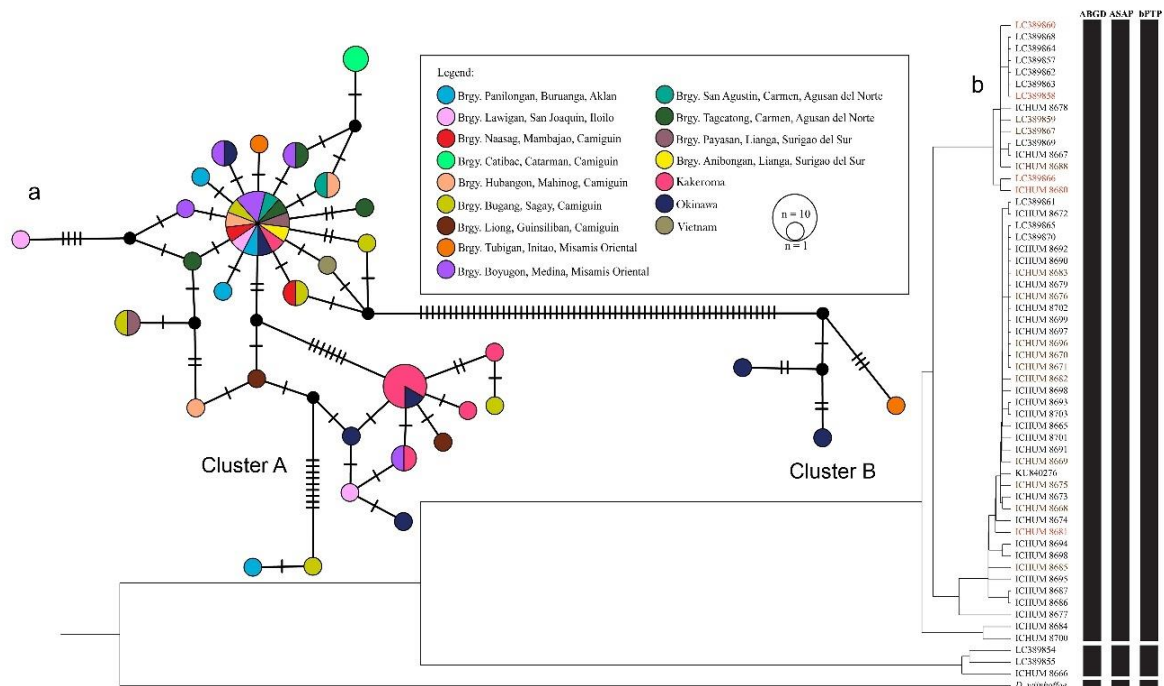


Figure 7. Delimiting the 39 *Dushia nigra* species complex from the Philippines. (a), A TCS haplotype network generated in PopART using partial sequences (629-bp) of the COI showing two clusters of the species complex. The number of black bars corresponds to the number of nucleotide substitution between haplotypes. (b), ASAP COI-based delimitation tree for the Philippine *D. nigra* specimens with *D. wijnhoffae* as outgroup. Vertical bars show the species delimitation result of the ABGD, ASAP, and bPTP method.

variation within each group was 1% (Table 2). Within cluster A, the mean *p*-distance was 1% while the range was 3.91%. For cluster B, these values were 1% and 0.40%,

respectively. Moreover, the TCS haplotype network built using the COI of the 57 *D. nigra* specimens (i.e., 17 from Japan, 39 from the Philippines, and 1 from Vietnam) indicated that *D. nigra* is currently composed of two genetically distinct members and no other COI lineage was detected using the Philippine specimens (Fig. 7). Both the species delimitation and haplotype network analyses revealed that *D. nigra* complex was composed of two distinct lineages whose members were distributed in waters around Japan, the Philippines, and Vietnam with one, less dominant and less common member, represented by the black form of the species, so far distributed only in the Philippines and Japan.

Table 2. Genetic distance (%) summary based on *p*-distance within and between the two clusters of *D. nigra* species complex.

Comparison level	Sample	Minimum distance	Mean distance	Maximum distance	Standard deviation
Within cluster A	54	0.00	1.00	3.91	1.02
Within cluster B	3	1.00	1.00	1.40	0.20
Between clusters	57	0.00	14.30	15.50	4.02

Molecular analyses on Monostilifera

The ML phylogenetic tree for monostiliferans (Fig. 8) confirmed the occurrence of *P. spectaculus* and two undescribed congeners namely *Prosadenoporus* sp. PH23-01 and *Prosadenoporus* sp. PH23-02 from the Philippines. The tree supported the genus placement of the undescribed *Prosadenoporus* spp. being both nested within the clade formed by *Prosadenoporus* members. Monostilifera sp. PH23 was sister to *Vulcanonemertes* Gibson & Strand, 2002.

ICHUM 8712–8718 turned out to be composed of a single species with *p*-distance of 0–1.52% based on the 658-bp partial COI sequences. This species was conspecific with *P. spectaculus* reported from China (0–1.52%). Based on the identification by Sun and Sun (2014), molecular data confirmed the first record of *P. spectaculus* in the Philippines. ICHUM 8705–8710 were conspecific (0–0.15%), herein designated as *Prosadenoporus* sp. PH23-01, and formed a fully supported clade. *Prosadenoporus* sp. PH23-01 morphologically resembled *P. olympiae* in terms of

external features but were genetically different from each other (9.55–9.75%). Both species exhibited a sister-taxon relationship (Fig. 8). Meanwhile, *Prosadenoporus* sp. PH23-02 was genetically different from *P. spectaculus* (5.62%), *P. olympiae* (10.72%), and *Prosadenoporus* sp. PH23-01 (10.49%), the other *Prosadenoporus* currently distributed in the Philippine marine waters, as well as with the non-Philippine congeners available in GenBank (Table 1). *Prosadenoporus* sp. PH23-02 was sister to *P. spectaculus* with high support.

Monostilifera sp. PH23 was genetically different from *Vulcanonemertes rangitotoensis* Gibson & Strand, 2002 (12.5%). Both species exhibited a sister-taxon relationship (Fig. 8).

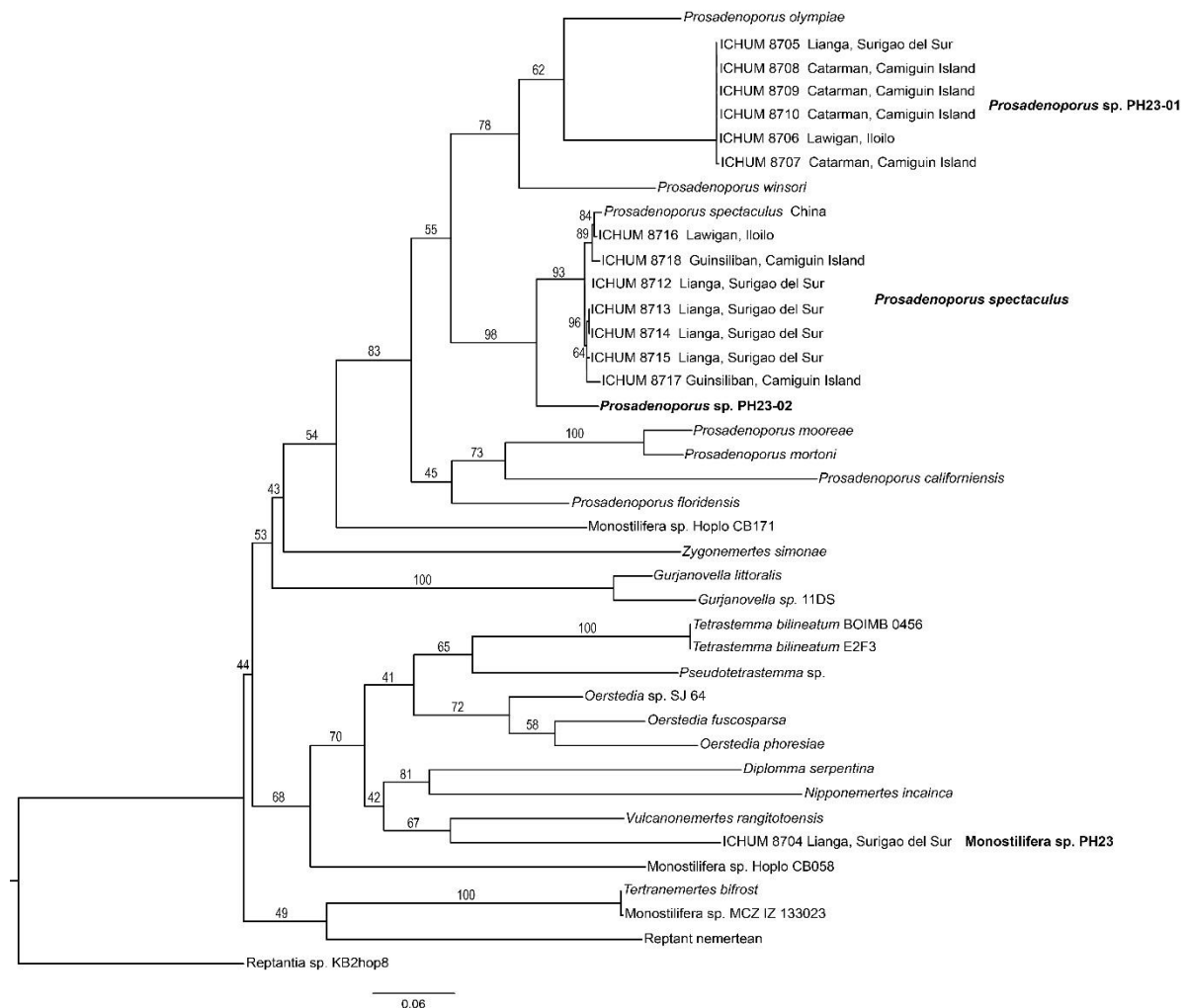


Figure 8. A rooted maximum-likelihood tree for Monostilifera generated by IQ-Tree based on the best-fit model TIM3 + F + I + G4 model with 655-bp partial sequence of the COI gene from 15 GenBank entries that showed the highest max/total scores after the MegaBLAST search of Monostilifera sp. PH23's COI sequence and *Prosadenoporus* COI sequences available in GenBank. Number at each branch represents branch support

derived from 1000 ultrafast bootstrap value. Species in the present study are represented in boldface.

Discussion

The present survey revealed that the most abundant species from the sites sampled was the *D. nigra* species complex (207 specimens) followed by Lineidae sp. PH23-01 (10), *P. spectaculus* (7), and *Prosadenoporus* sp. PH23-01 (6), while each of the other taxa was represented by a single specimen, namely Lineidae sp. PH23-02, Monostilifera sp. PH23, and *Prosadenoporus* sp. PH23-02.

This survey indicated the dominance of *D. nigra* species complex, occurring in all the 16 collection sites (Fig. 2). It was especially abundant in Brgy. Naasag, where 50 specimens were collected. As observed during the survey, this species (or, species complex) often occurred with other marine invertebrates such as polychaetes, sea cucumbers, and ophiuroids and was often found under rocks embedded in a semi-dry fine or coarse sand material, and in a moistened silty sediment. Meanwhile, the species was very limited in Brgy. Pampangon, Misamis Oriental (Fig. 1d) as the intertidal area in this collection site was made up of smooth rock layers with no sufficient sediment or sandy material underneath.

Previously, *D. nigra* complex was documented in Japan (in Okinawa and Kogoshima), Taiwan (in Su'ao), China, Vietnam (in Van Phong Bay, Nha Trang Bay, and in Hon Cau Island), and Australia (in Pelarous Island, Queensland, based only in morphology) (Stimpson 1855; Yamaoka 1939; Chernyshev 2016; Kajihara 2017; Hookabe et al. 2019) which surround and connect to the Philippine archipelago by the water currents along these localities. The geographic location of the Philippines in the Southeast Asia, apart from being included in the so-called Coral Triangle (CT), makes it a 'sink' for waters coming from the Western Pacific Ocean (WPO) through the Surigao Strait and San Bernardino Strait in the eastern parts of the archipelago; waters from the West Philippine Sea (WPS) through the Mindoro-Panay Strait complex, Balabac Strait, Karimata Strait, and Makassar Strait. Water currents from the WPO and WPS (Qu et al. 2006, 2009; Sprintall et al. 2012) could transport nemertean larvae and adults into the Philippine waters. The CT serves as a 'catch-all' for larvae moving towards the region

(Jokiel & Martinelli 1992; Veron 1995). It is important to note that the sampling sites where *D. nigra* species complex was abundant were influenced by water flow from the WPS and WPO (i.e., in Aklan and Iloilo) and mostly from WPO (i.e., in Camiguin Island, Agusan del Norte, and Surigao del Sur).

Meanwhile, the previous distribution localities of *P. spectaculus* are also neighbors to the Philippines (i.e., Okinawa, China, and Hong Kong). Therefore, finding this species in the Philippines is highly likely. To date, Philippine marine waters harbor four species of *Prosadenoporus*. Other congeners are also reported in the neighboring regions—*P. mortoni* in China, *P. winsori* and *P. mooreae* in Australia. This indicates that in the southwestern Pacific region, *Prosadenoporus* is distributed from Okinawa, Japan going south to the Philippines then to Australia.

In terms of diversity, the municipality of Lianga, Surigao del Sur, which faces the Pacific Ocean, was the most species-rich area (with 5 species) among the sampled municipalities (Fig. 2). The occurrence of nemerteans in the Philippines could be attributed to several factors like the presence and abundance of their food or prey, their ability to produce long-lived planktonic larvae, and the water currents around and towards the Philippines among others. Many intertidal ribbon worms actively chase their prey and track them to their dwellings (McDermott & Roe 1985; Thiel & Kruse 2001); they produce larvae with extended pelagic duration (Maslakova & Hiebert 2014), which have a greater potential for long-distance water current-driven dispersal (Veron 1995).

Conclusions

The present study showed that members of the order Heteronemertea (class Pilidiophora) and the order Monostilifera (class Hoplonemertea) in the phylum Nemertea inhabit silty to rocky shorelines in the intertidal zone along Panay and Mindanao Islands, which, prior to this study, have limited records of ribbon worm species. From the field sampling, the *D. nigra* species complex is the most common and dominant nemerteans in the studied areas. Most of the nemertean species collected in the present study are potentially new to science, suggesting that there could be a plethora of undescribed ribbon-worm species in the Philippines, awaiting discovery and formal taxonomic description. Hence, conducting similar faunal assessments in other areas in the Philippines to document further the ribbon-worm species diversity in the

archipelago is recommended. Furthermore, with extensive sampling efforts, extended sampling periods, and diversification of sample collection (e.g., collection from bottom sediments, silty shorelines, kelps, sargassums, and seagrasses; collection by diving), a more diverse Philippine ribbon-worm fauna could be revealed.

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Chapter 2.2. Occurrence of *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1833) (Pilidiophora: Heteronemertea) in Mindanao, Philippines

Introduction

Members of the genus *Baseodiscus* Diesing, 1850 can be identified by external morphology, mainly based on body color and markings (Kajihara et al. 2022). The heteronemertean ribbon worm *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1833) of the family Valenciniidae Hubrecht, 1879 is a long nemertean easily identifiable by having five brown or black longitudinal lines (hence, “*quinquelineatus*”, for having five lines/stripes) at the dorsal body and two brown or black longitudinal lines at the ventral side. The entire body is grayish-white; the head is small, heart-shaped; and the posterior end of the body is obtuse. The most common combination of these lines/stripes would be three on the dorsal side and two on the ventral side, which is observed in the nominal taxa *Borlasia quinquelineata* Quoy & Gaimard, 1833, *Eupolia trilineata* Staub, 1900, and *Taeniosoma aequale* Stimpson, 1857. However, this combination of body lines/stripes could vary in other nominal taxa; the dorsal stripes on both sides can be doubled (as in *Taeniosoma aequale*) or tripled (as in *Eupolia novemlineata* Bürger, 1893), or anteriorly tripled and posteriorly doubled (as in *Taeniosoma septemlineatum* Stimpson, 1897). Because of their external morphological features, these nominal taxa are considered representatives of the species *B. quinquelineatus* (Quoy & Gaimard, 1833) (Gibson 1979; Kajihara et al. 2022).

Currently, *B. quinquelineatus* is widely distributed in the Indo-West Pacific: India (Venkataraman et al. 2012), Torres Straits (Punnett 1900a), Gulf of Thailand (Putchakarn 2009), Singapore (Bürger 1895; Punnett 1900b), Indonesia (Bürger 1893; Staub 1900), Philippines (Stimpson 1857), Japan (Stimpson 1857; Kajihara 2017; Kajihara et al. 2022), New Guinea (Quoy & Gaimard 1833), Australia (Bürger 1895; Gibson 1979), Solomon Islands (Gibson & Sundberg 2002), and New Caledonia (Punnett 1900c).

In the Philippines, the first published record of *B. quinquelineatus* was in Gaspar Island in Gasan, Marinduque province in Luzon (Stimpson 1857). The same species was then reported in Sogod, Cebu, based on an image made available by Poppe and Poppe (2009) at SeaLifeBase. Here, I report the first sighting of this heteronemertean in

Carmen, Agusan del Norte in Mindanao, the third record of the species in the Philippines, hence updating its geographic distribution in the country (Fig. 9).

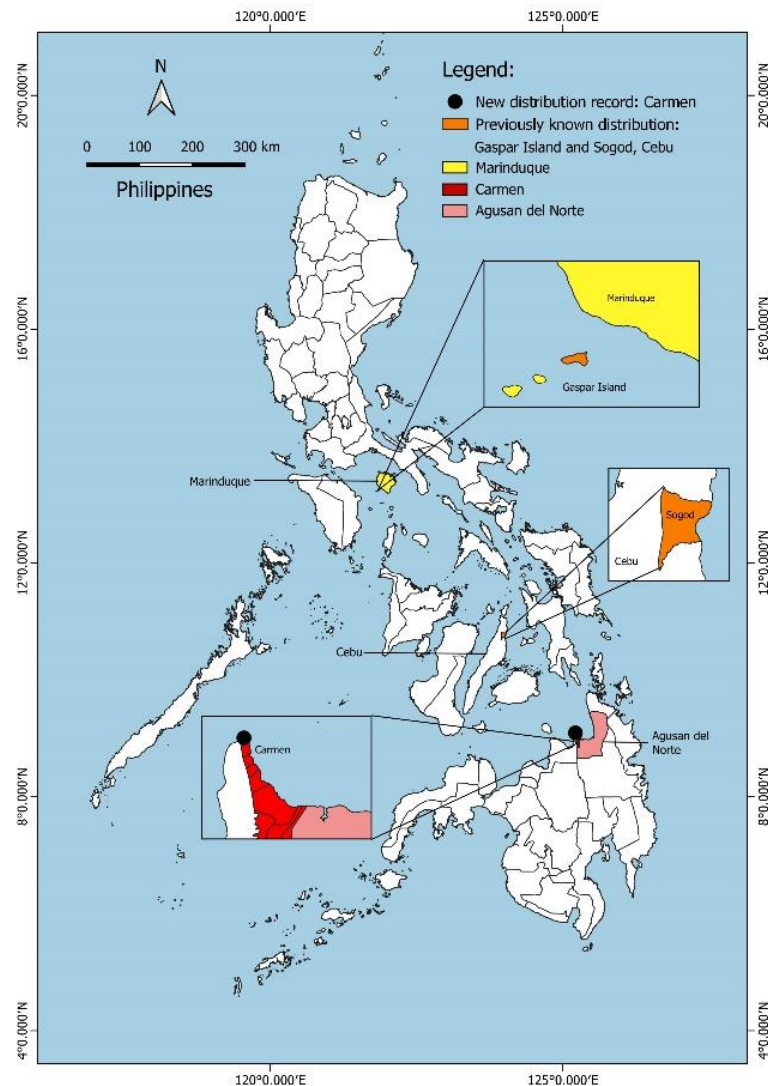


Figure 9. Updated distribution of *B. quinquelineatus* in the Philippines. Gaspar Island, Marinduque (Stimpson 1857), Sogod, Cebu (Poppe & Poppe 2009) and Brgy. Vinapor, Carmen, Agusan del Norte (present study).

Materials and methods

A single specimen of *B. quinquelineatus* was collected from a seagrass bed [dominated by *Cymodocea rotundata* Asch. and Schweinf. with some *Thalassia hemprichii* (Ehrenberg) Ascherson, 1871, *Halodule* sp. and *Halophila* sp.] (Fig. 10a) in the littoral zone of Brgy. Vinapor, Carmen, Agusan del Norte (Fig. 10b) (9.094°N, 125.211°E) on 13 May 2023. The specimen was broken anteriorly while being extracted from the seagrass bed and the cut part was not recovered, rendering the specimen

decapitated. After extraction, the live specimen was placed in a plastic bottle with saline water and brought to the Department of Biology, Caraga State University and photographed using a Canon EOS 800D camera then the entire specimen was preserved in 99% ethanol. The voucher specimen was deposited in the Department of Biology, Caraga State University, Butuan City.

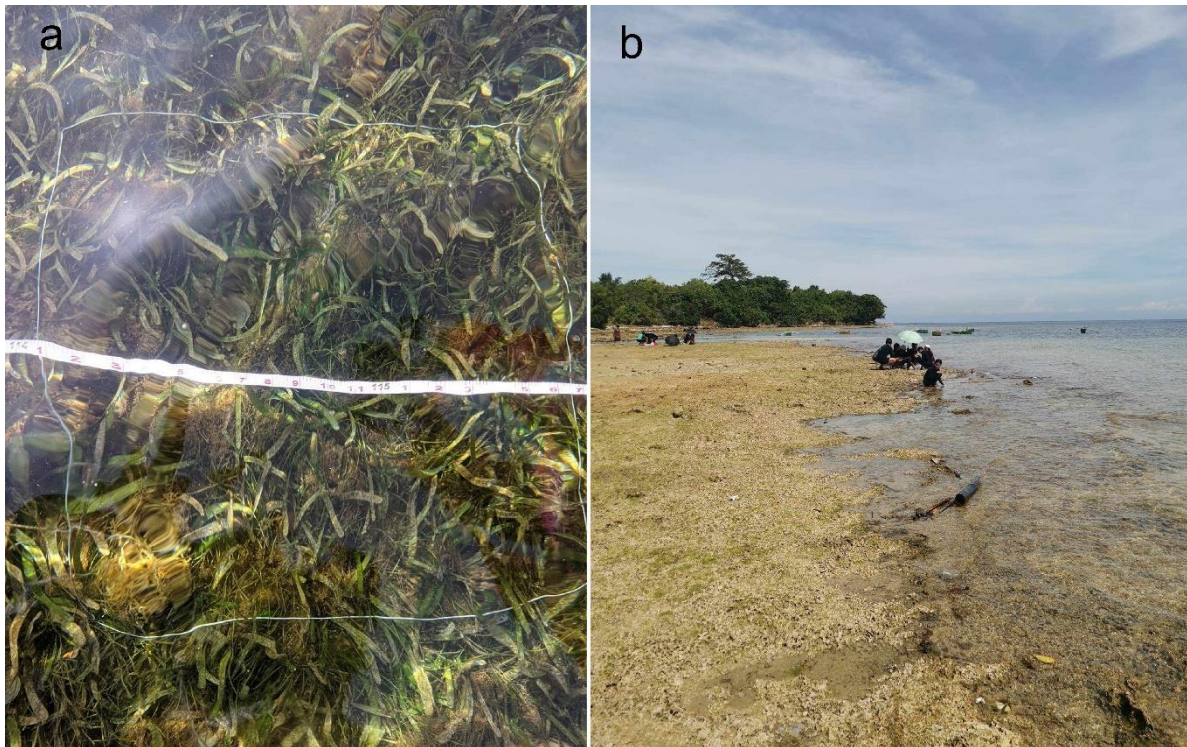


Figure 10. Collection site of *B. quinquelineatus*. (a), the seagrass bed from which the species was extracted; (b), the intertidal zone collection site in Brgy. Vinapor, Carmen, Agusan del Norte.

Results and systematic remarks

The length of the decapitated specimen (Fig. 11) is approximately 40 cm, anterior body diameter is 0.8 cm, posterior body diameter near tail end is 0.4 cm, making the anterior wider than the posterior. The ground color is grayish-white on both dorsal and ventral sides; however, the ventral body is mostly pale brown in color within which the two longitudinal stripes are embedded, with grayish-white coloration only on the margin. The dorsal body is decorated with five longitudinal, continuous black stripes from anterior to posterior end (Fig. 11a), only two of these continuous black longitudinal stripes lined the ventral body (Fig. 11b). Though the collected specimen was cut from

the anterior end and beheaded, its identification as *B. quinquelineatus* was still possible because it fits the external diagnostic characteristics for this species. The junior synonyms of this species, which are considered invalid, are comprehensively summarized by Kajihara et al. (2022).

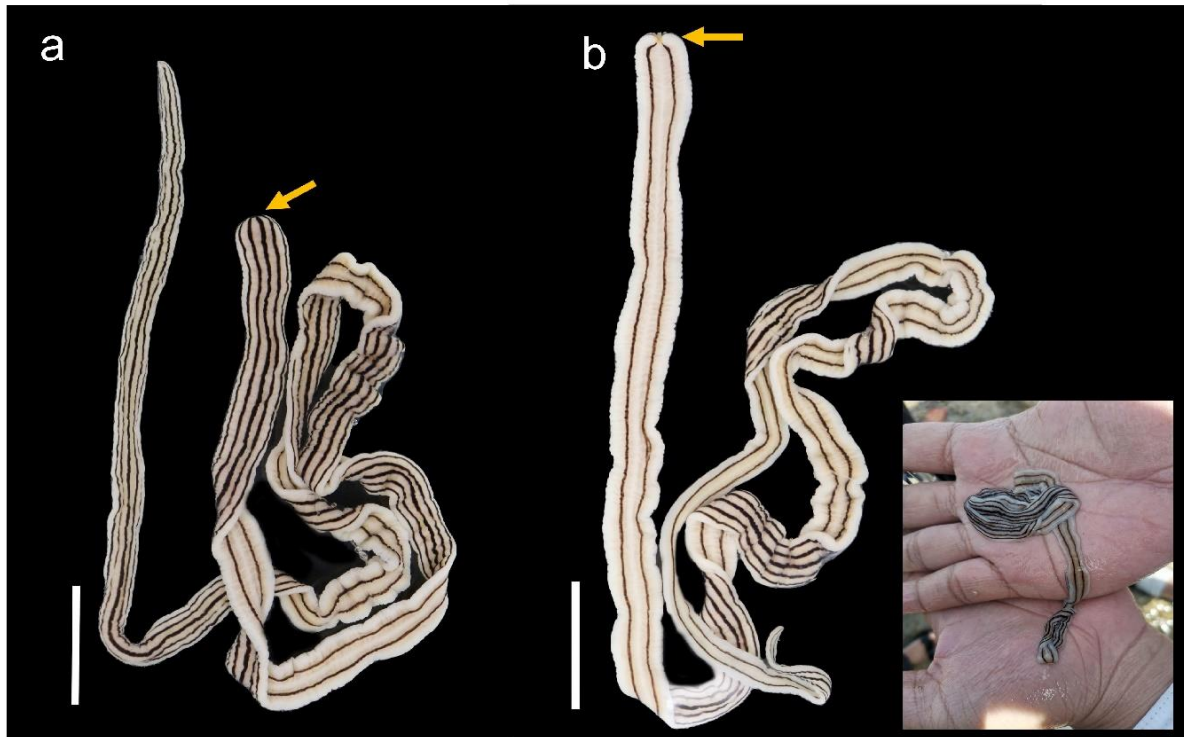


Figure 11. *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1833), BIOCSU: 0001 from Carmen, Agusan del Norte. (a), decapitated specimen showing most of the dorsal body and (b), most of the ventral body. Arrow pointed at the decapitated anterior end of the worm. Inset is a photograph of the specimen taken on site after being extracted from its habitat. Scale bar: 2 cm.

Conclusions

Ribbon worm taxonomic studies are very limited in the Philippines mainly due to the lack of researchers specializing in this taxon. Despite this limitation, ribbon worms have been recorded (i.e., sighted, collected but not published in scientific journals) and reported (i.e., published in scientific journals) in the country, mainly from Luzon and Visayas regions by foreign researchers (Humes 1942; Collin & Arneson 1995; Gibson & Moore 1998; Kajihara 2007, 2020; Kajihara et al. 2007, 2011). To explore further the occurrence, diversity, and geographic distribution of nemerteans in the country, most especially in the Mindanao region, which has no official record yet of ribbon worm

sightings (except this first report paper), Mindanao ribbon worm fauna has been surveyed from March to May 2023. Further ribbon worm reports and species descriptions of potentially new species collected from this survey will be published separately in the future. This work represents the second report for *B. quinquelineatus* in the Philippines and the first report of a nemertean from Mindanao.

Citation: This work has been published in the Philippine Journal of Science. See and read the published article for use of any information in this chapter.

Abato, J., Kajihara, H., Calagui, L., Along, A., Leones, J.A., Calagui, S.I., Salo, I.I. 2023. Occurrence of *Baseodiscus quinquelineatus* (Quoy and Gaimard, 1833) (Pilidiophora: Heteronemertea) in Mindanao, Philippines. *Philippine Journal of Science*. 152(6), 2413–2418.

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Chapter 2.3. Diversity of pilidiophorans from the intertidal zones of selected sites in Mindanao, Philippines

Introduction

Nemerteans (phylum Nemertea) are among those neglected marine invertebrate animals in the Philippines mainly due to the absence of local experts or researchers working on this group. Because the taxonomy of the phylum Nemertea has never been the focus of Philippine research, Filipinos are not familiar with these marine worms and researchers have not considered studying their biodiversity and biology. An exploration of the Philippine nemertean fauna was performed in 2004–2006 in selected islands of the Visayas. This one-decade-old work resulted in the discovery and establishment of three nemertean species from the country (Kajihara 2007, 2020; Kajihara et al. 2011). The most recent attempt to discover the diversity of nemerteans in the Philippines was conducted in 2023 (Abato et al. 2025). This intertidal ribbon worm survey in the Visayas and Mindanao Islands led to the discovery of eight nemertean species, four each in Hoplonemertea and Pilidiophora (Abato et al. 2025). Considering all the Philippine nemerteans (i.e., named and unidentified) reported in scientific papers, to date, 15 species of the phylum were reported from the country, one terrestrial and 14 marine species. These are i) *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1933); ii) *Balionemertes* cf. *australiensis* Sundberg, Gibson & Olsson, 2003; iii) *Callinera emiliae* Kajihara, 2007; iv) *Carcinonemertes mitsukurii* Takakura, 1910; v) *Coella gloriae* Kajihara, 2020; vi) *Diplomma serpentinum* (Stimpson, 1855); vii) *Dushia nigra* (Stimpson, 1855) species complex; viii) *Geonemertes philippinensis* Gibson & Moore, 1998; ix) Lineidae sp. PH23-01; x) Lineidae sp. PH23-02; xi) Monostilifera sp. PH23; xii) *Prosadenoporus olympiae* Kajihara, 2020; xiii) *Prosadenoporus spectaculus* (Yamaoka, 1940); xiv) *Prosadenoporus* sp. PH23-01; and xv) *Prosadenoporus* sp. PH23-02 (Stimpson 1857; Humes 1942; Gibson & Moore 1998; Kajihara 2007, 2020; Kajihara et al. 2011; Abato et al. 2023, 2025).

To date, only four piliophorans are reported from the Philippine waters, these are: i) *Baseodiscus quinquelineatus*; ii) *Dushia nigra* species complex; iii) Lineidae sp. PH23-01; and iv) Lineidae sp. PH23-02. To further explore the occurrence of this nemertean group from the Mindanao Island, members of Pilidiophora that are newly reported from

the island (i.e., not documented and published in scientific papers) are herein presented. The collected ribbon worms were identified based on their external morphology and comparing their cytochrome c oxidase subunit I (COI) with those deposited in GenBank.

Materials and methods

Nemerteans were collected along slightly silty to sandy and rocky shores in the intertidal zone at the following 14 sites: *i*) Brgy. Naasag, Mambajao in Camiguin; *ii*) Brgy. Catibac, Catarman in Camiguin; *iii*) Brgy. Liong, Guinsiliban in Camiguin *iv*) Brgy. Poblacion, Naawan in Misamis Oriental; *v*) Brgy. Pontacon, Initao in Misamis Oriental; *vi*) Brgy. Poblacion, Naawan in Misamis Oriental, *vii*) Brgy. San Agustin, Carmen in Agusan del Norte, *viii*) Brgy. Vinapor, Carmen in Agusan del Norte, *ix*) Brgy. Baucawe, Lianga in Surigao del Sur, *x*) Brgy. Payasan, Lianga in Surigao del Sur, *xi*) Brgy. Poblacion, Lianga in Surigao del Sur, *xii*) Brgy. Campa, Hinatuan in Surigao del Sur, *xiii*) Brgy. Gamut, Barobo, Surigao del Sur, and *xiv*) Brgy. Unidad, Barobo, Surigao del Sur.

Ribbon worms were collected intertidally, processed, and photographed following Abato et al. (2025). Representative specimens of the collected species were used for DNA extraction. The procedures for DNA extraction, TaKaRa Ex Taq PCR amplification of the COI also adopted Abato et al. (2025).

Results and systematic remarks

From the recent nemertean faunal survey conducted in Mindanao, Philippines, seven morphologically distinct pilidiophorans, recorded for the first time in the country, were documented. Three of the seven represent known species while the rest are left unidentified and potentially new species.

Taxonomy

Class Pilidiophora Thollessen & Norenburg, 2003

Order Heteronemertea Coe, 1905

Family Lineidae McIntosh, 1874

Genus *Eousia* Gibson, 1990

Eousia verticivaria Gibson, 1990 (Fig. 12a–e).

Material examined. One specimen collected in Brgy. Poblacion, Naawan in Misamis Oriental, among *Sargassum* on April 25, 2024, one specimen of the same morphology was observed in Kawayan Reef, Initao also in Misamis Oriental from a sandy substrate on April 27, 2024.

Description. *External features.* Body in anaesthetized state is 15 mm in length, 1.5 mm in width; translucent, light yellow in color at dorsal view (Fig. 12a), anterior end darker than the rest of the body, ventral side lighter (Fig. 12b), more transparent than dorsal side but anterior end noticeably darker; head demarcated from the body (Fig. 12b), pointed, triangular (i.e., acute), dotted with brownish to black granules which extend as smaller and more numerous ones to the anterodorsal body (Fig. 12d), ventral side lacking the speckled granules (Fig. 12e), lateral edges to tip colorless; with deep horizontal cephalic slits (Fig. 12c); brain visible as red mass at the rear side of the head, visible at dorsal and ventral views (Fig. 12a–e); rhynchocoel extends to the posterior end of the body, noticeable at ventral view; intestinal diverticula visible at ventral view; with short caudal cirrus (Fig. 12a, b).

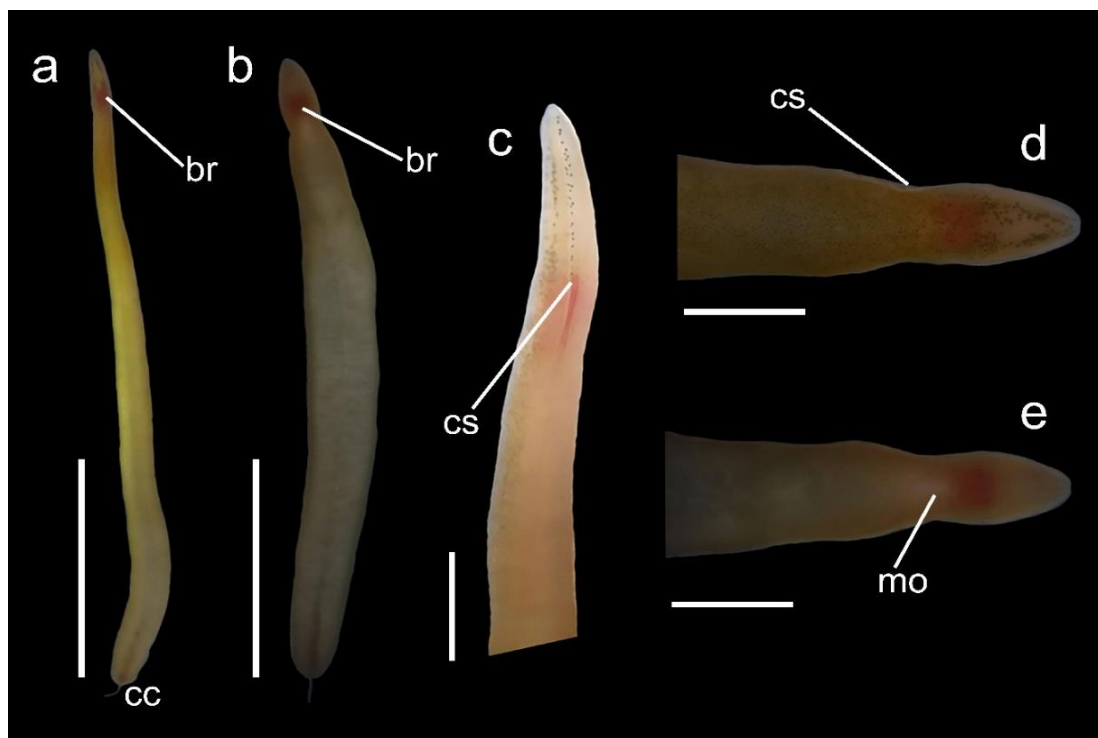


Figure 12. *Eousia verticivaria* collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), dorsal, (b), ventral, and (c), ventrolateral views, (d), magnification of the head at dorsal and (e), ventral views. Abbreviations: br, brain, cc, caudal cirrus, cs, cephalic slit. Scale bar: a, b = 5 mm; c–e = 1 mm.

Taxonomic remarks. It is no doubt that the specimen examined belongs to *E. verticivaria* as its external morphology fits Gibson’s descriptions of the species (Gibson 1990, fig. 14A–D, pp. 99–101). Also, this specimen is morphologically similar to the one identified as *E. verticivaria* from Vietnam (Chernyshev 2016, fig. 5i).

Distribution. The species was first reported from Starfish Bay, Hong Kong (Gibson 1990), later reported in Van Phong Bay, Nam Du Island, and Cu Lao Cham Islands all in Vietnam (Chernyshev 2016). New distribution record in Brgy, Poblacion, Naawan, Misamis Oriental, Philippines (present study).

Genus *Notospermus* Huschke, 1830

***Notospermus* sp. PH24-03** (Figs. 13–19).

Material examined. Two specimens collected in Brgy. Poblacion, Naawan, Misamis Oriental on March 4 and March 8, 2024; one specimen collected in Brgy. Vinapor, Carmen, Agusan del Norte; three specimens collected in Brgy. Poblacion, Lianga, Surigao del Sur on March 17, 2024. All specimens were collected under intertidal rocks that are kept wet/submerged in water.

Description. *External features.* All the specimens have dark green body color, with ventral side slightly lighter than dorsal, one specimen collected in Brgy, Poblacion, Naawan, herein designated as *Notospermus* sp. PH24-03a is 22 mm in length (part of posterior body was lost), 1.3 mm in width; with 17 white rings completely encircling the greenish body resulting to an alternating thick green transverse bands and thin white ones (Fig. 13a); head not obviously demarcated from the body, rectangular on dorsal view (i.e., typical head shape for the genus) (Fig. 13a–c), a thick, white transverse ring

completely encircles the head, just slightly above the origin of the deep horizontal lateral cephalic slit and above the brain region (Fig. 13b–d), a thick, greenish transverse band completely encircles the head anteriorly, dorsally this band follows the shape of the head without reaching its anterior edges, split at the center by a white horizontal line except at the mid anterior tip of the band (i.e., incomplete bisection) (Fig. 13a, b), ventrally, this band is rectangular, does not reach the proboscis pore, making the ventral tip of the head colorless, completely divided/bisected by a less colored, light green vertical mid line (Fig. 13c); brain is noticeable as red mass in the first, thick green band posterior to the white cephalic ring and above the anterior tip of the mouth in ventral view (Fig. 13b, c); numerous black ocelli are distributed on the dorso-lateral edges of the head, from the white cephalic ring towards the colorless tip of the head (Fig. 13a, d), very close to the margin of the green, anterior cephalic patch visible in dorsal and lateral views, 12–17 in number on each side of the head.

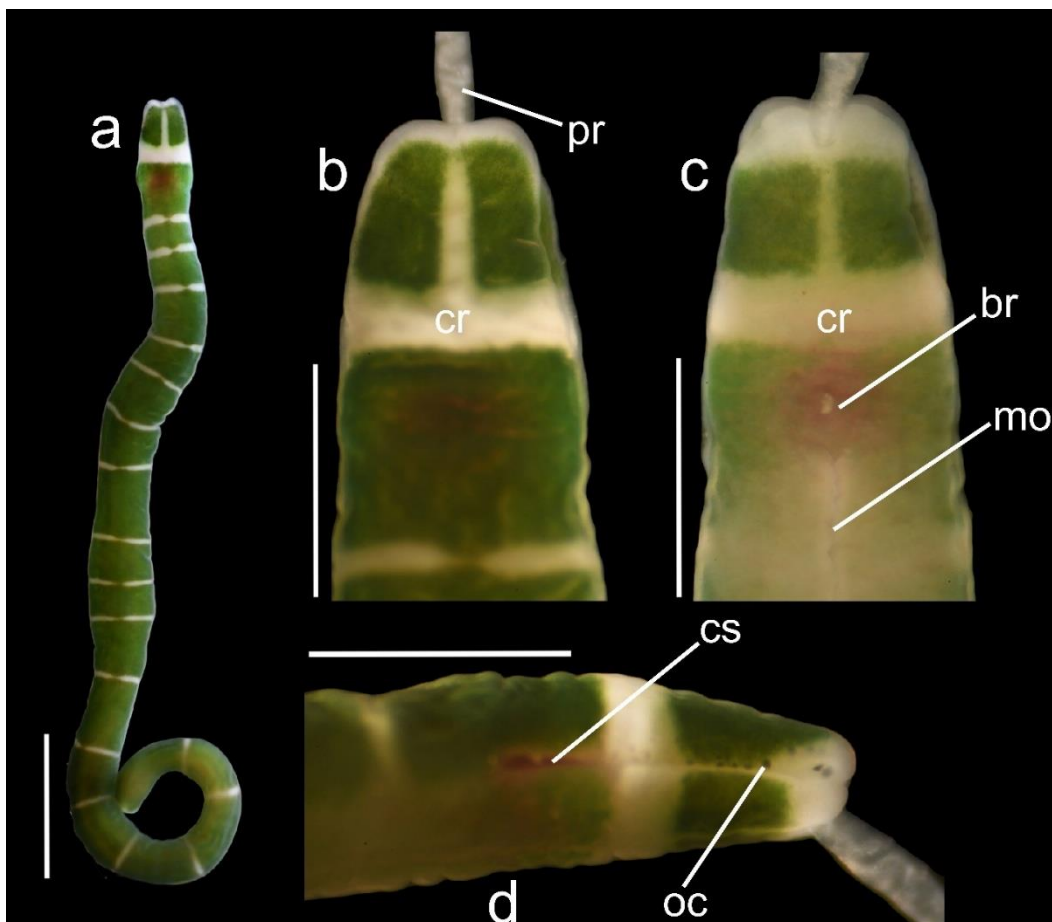


Figure 13. *Notospermus* sp. PH24-03a, collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), entire body at dorsal view, (b),

magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli; pr, proboscis. Scale bar: a= 5 mm; b–d = 1 mm.

The other specimen herein designated as *Notospermus* sp. PH24-03b (Fig. 14a, b) was also from Brgy. Poblacion, Naawan. This specimen is intact, 100 mm in length and

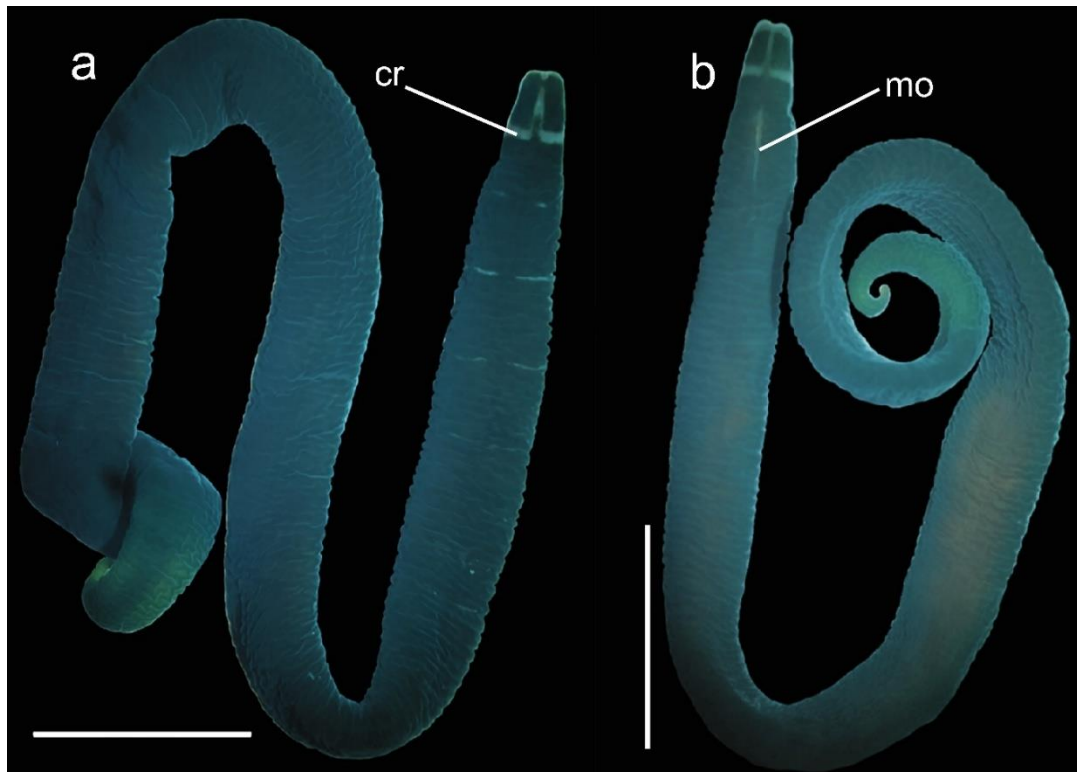


Figure 14. *Notospermus* sp. PH24-03b, collected from Brgy. Poblacion, Naawan, Misamis Oriental at anaesthetized condition. (a), entire body at dorsal view, (b), entire body at ventral view. Scale bar: a, b= 10 mm.

4 mm in width; body is dark green dorsally, ventrally lighter and with shades of brown, posterior end lighter in color than the rest of the body; head obviously demarcated from the body, characteristics of the head similar with *Notospermus* sp. PH24-03a except that the thick, white cephalic ring is bisected by patches of green vertical band extending and inserting anteriorly to the non-pigmented division of the green anterior cephalic patch without completely reaching the tip (Fig. 14a), this vertical band originates from the green transverse band at the posterior end of the head; this feature

is similarly observed at the ventral side of the head with the vertical band lighter in color; four (Fig. 14b), thin, white transverse bands are present on dorsal body, these bands are not completely transversely continuous and not seen ventrally (Fig. 14a, b); brain more prominent ventrally just above the anterior edge of the mouth, ocelli arranged in the same manner as in *Notospermus* sp. PH24-03a, 12 in number on each side of the head; with short caudal cirrus.

Another specimen from Brgy. Vinapor, Carmen, designated as *Notospermus* sp. PH24-03c (Fig. 15a–d).

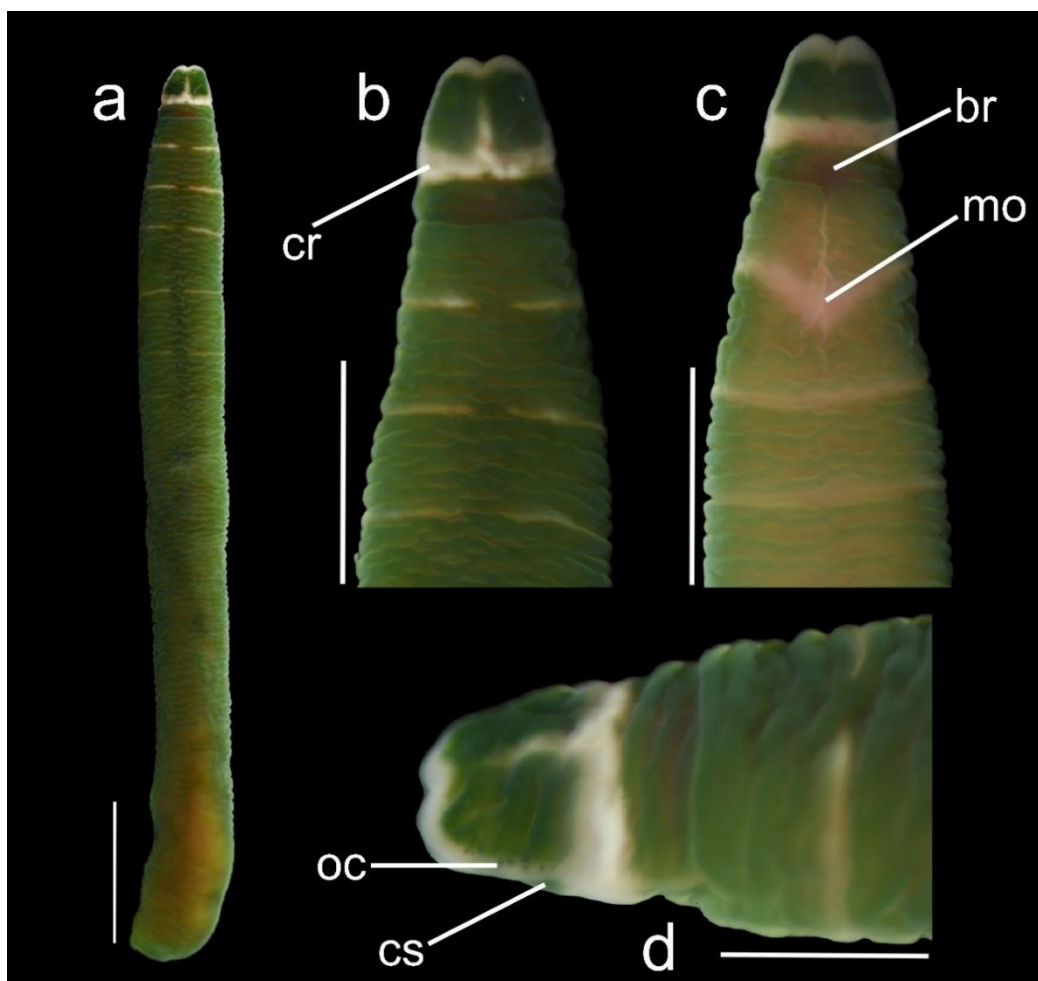


Figure 15. *Notospermus* sp. PH24-03c, collected from Brgy. Vinapor, Carmen, Agusan del Norte at anaesthetized condition. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at dorso-lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 3 mm.

The specimen is 34 mm in length (part of the posterior body was cut) and 3 mm in width; also greenish in color, ventrally lighter and with shades of brown; head features (Fig. 15a–d) exactly similar with *Notospermus* sp. PH24-03a; dorsally, five white, thin, transverse bands (Fig. 15a) constructed in the same manner as in *Notospermus* sp. PH24-03b are present, continuous at the ventral side of the body (Fig. 15c), the first one appears as a wide ‘V’ while the rest are completely horizontal (Fig. 15c); numerous black ocelli were observed but not counted (Fig. 15d).

Three specimens, designated *Notospermus* sp. PH24-03d, *Notospermus* sp. PH24-03e, and *Notospermus* sp. PH24-03f, all from Brgy. Poblacion, Lianga.

Notospermus sp. PH24-03d (Fig. 16a–d) is also greenish in color at dorsal view, lighter and with shades of brown at ventral view. It is 56 mm in length, 3 mm in width; pigmentation pattern in the head is slightly different from the previous specimens by

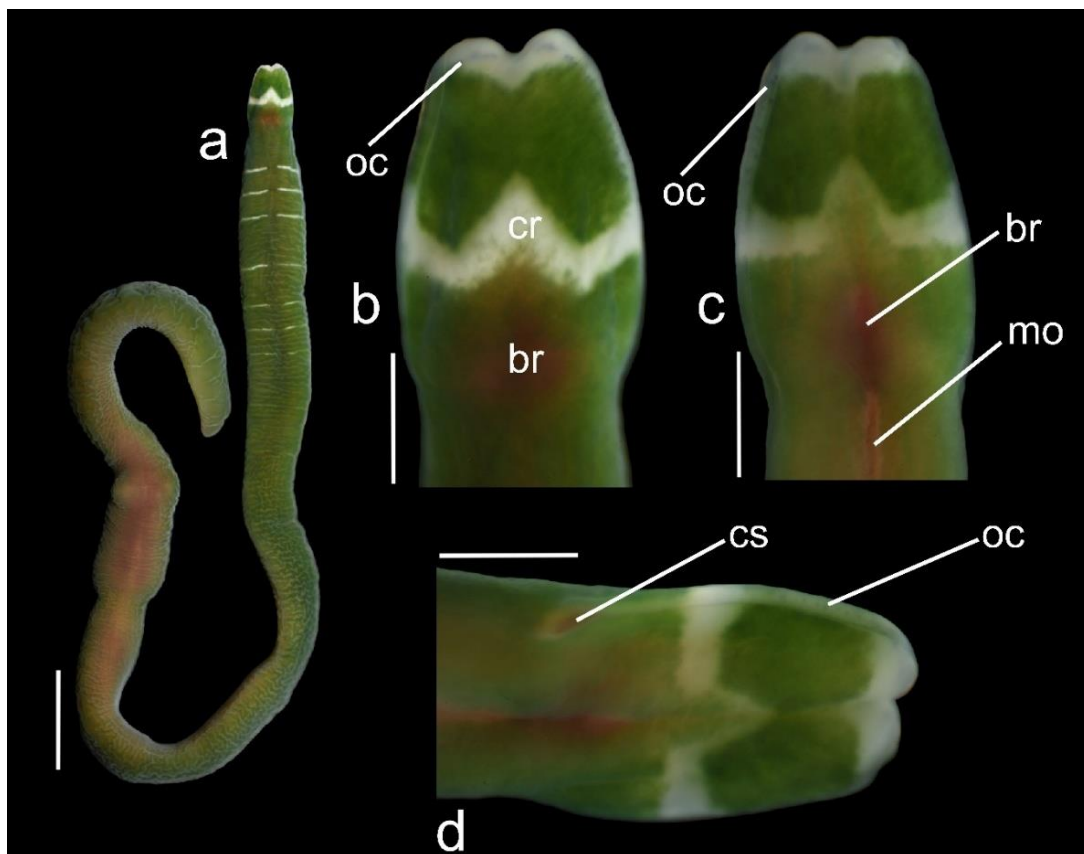


Figure 16. *Notospermus* sp. PH24-03d, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized condition. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at ventro-lateral view. Abbreviations: br, brain; cr, cephalic ring; cs, cephalic slit; mo, mouth; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 1 mm.

having the mid portion of the thick cephalic ring extended anteriorly and inserting to the base of the green cephalic patch as 'Λ' without reaching the tip (Fig. 16b), the same pattern is observed at ventral side except that a light green patches originating from the posterior greenish cephalic band extends anteriorly and inserts to the 'Λ' shaped white cephalic ring (Fig. 16c); nine thin, white transverse bands (the last two posterior ones being not completely evident) are present at dorsal body (Fig. 16a), these bands are constructed similarly as in *Notospermus* sp. PH24-03c; numerous black ocelli, 20–24 in number on each side of the head are present (Fig. 16b–d), oriented in the same manner as in *Notospermus* sp. PH24-03a, except that the ocelli are not distributed on the lateral edges of the white cephalic ring (Fig. 16d); with short caudal cirrus.

Notospermus sp. PH24-03e was collected together and with *Notospermus* sp. PH24-03d, the former's morphological features are the same with the latter (except for its undetermined length and width), not photographed, and directly preserved in 99% ethanol.

Notospermus sp. PH24-03f (Fig. 17a, b), an intact specimen, color of the entire body same as other specimens, 100 mm in length, 3.2 mm in width; pigmentation pattern in the head (Fig. 17b) similar with *Notospermus* sp. PH24-03d; the white trans-

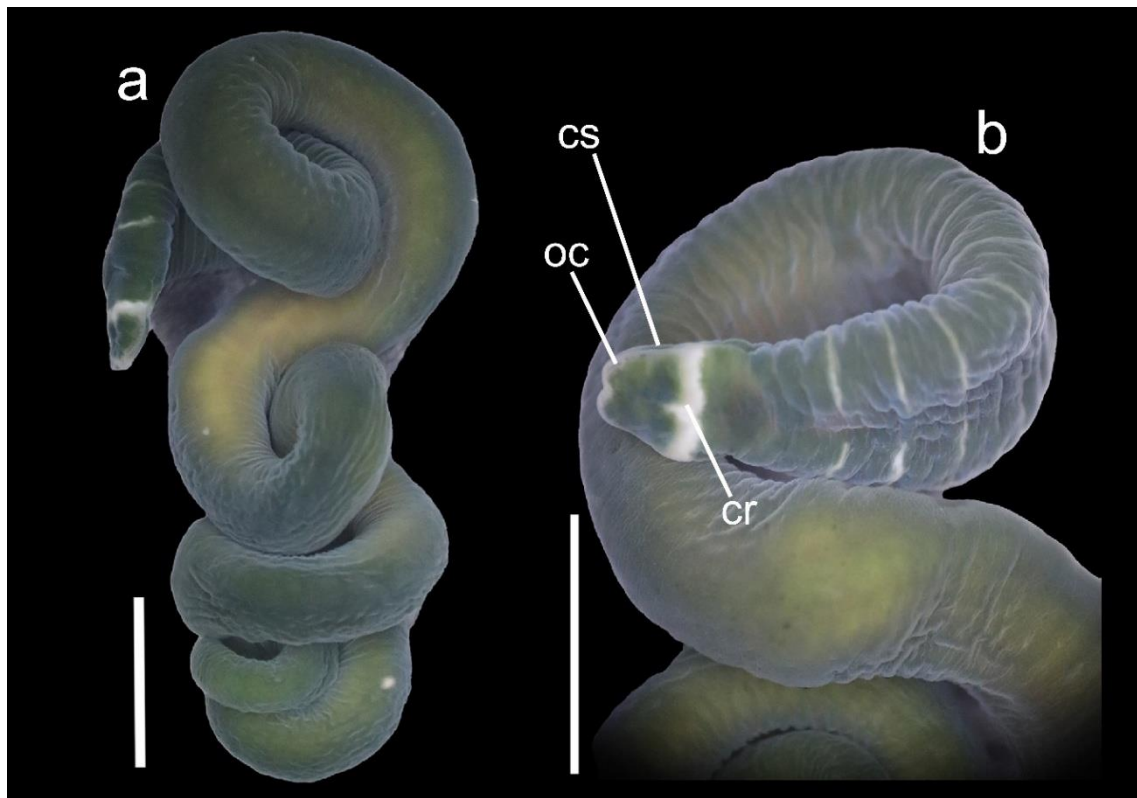


Figure 17. *Notospermus* sp. PH24-03f, collected from Brgy. Poblacion, Lianga, Surigao del Sur at living state. (a), entire body at dorsal view, (b), magnification of the head at dorsal view, (c), magnification of the head at ventral view, (d), magnification of the head at ventro-lateral view. Abbreviations: cr, cephalic ring; cs, cephalic slit; oc, ocelli. Scale bar: a, = 5 mm; b–d, = 1 mm.

verse band (Fig. 17b) is seven in number, constructed in the same manner as in *Notospermus* sp. PH24-03d; numerous ocelli situated on the lateral edges of the head, number not determined; with short caudal cirrus.

Two other specimens of this greenish *Notospermus* also designated as *Notospermus* sp. PH24-03g and PH24-03h were collected in Kawayan Reef in Initao, Misamis Oriental.

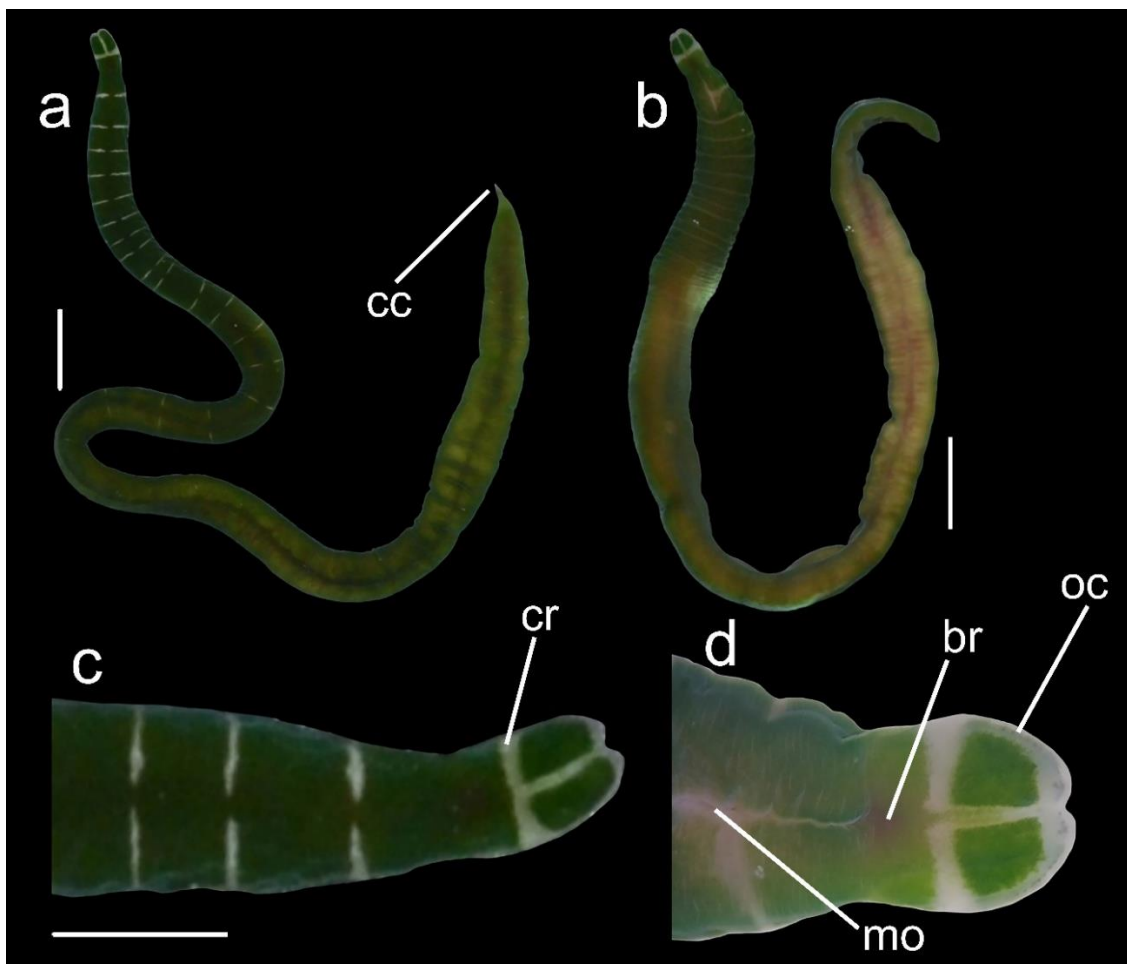


Figure 18. *Notospermus* sp. PH24-03g, collected from Kawayan Reef, Initao, Misamis Oriental at anaesthetized state. (a), entire body at dorsal view, (b), entire body at ventral view, (c), magnification of the head at dorsal view, (d), magnification of the head at ventral view. Scale bar: a, b = 10 mm; c, = 5 mm; not available for d.

Notospermus sp. PH24-03g (Fig. 18a–d) is 230 mm in length, 2 mm in width; has its cephalic pigmentation pattern similar to *Notospermus* sp. PH24-03a but with the green cephalic patch completely divided by the vertical white cephalic line on both sides on the head (Fig. 18c, d), the ventral side shows traces of thin, vertical light green patches originating from the posterior cephalic patch extending to the colorless gap of the anterior cephalic patch without reaching the tip; with 21 white, non-completely transverse bands on the dorsal body (the last two posterior ones not completely discernible) (Fig. 18a), continuous ventrally as horizontal bands (Fig. 18b), 12 in number; numerous ocelli, 40–50 is seen distributed on the dorso-lateral edges of the head, noticeable at the ventral view (Fig. 18d); beginning from the colorless edges of the head next to the green cephalic patch extending anteriorly up to the tip of the head; with short caudal cirrus (Fig. 18a).

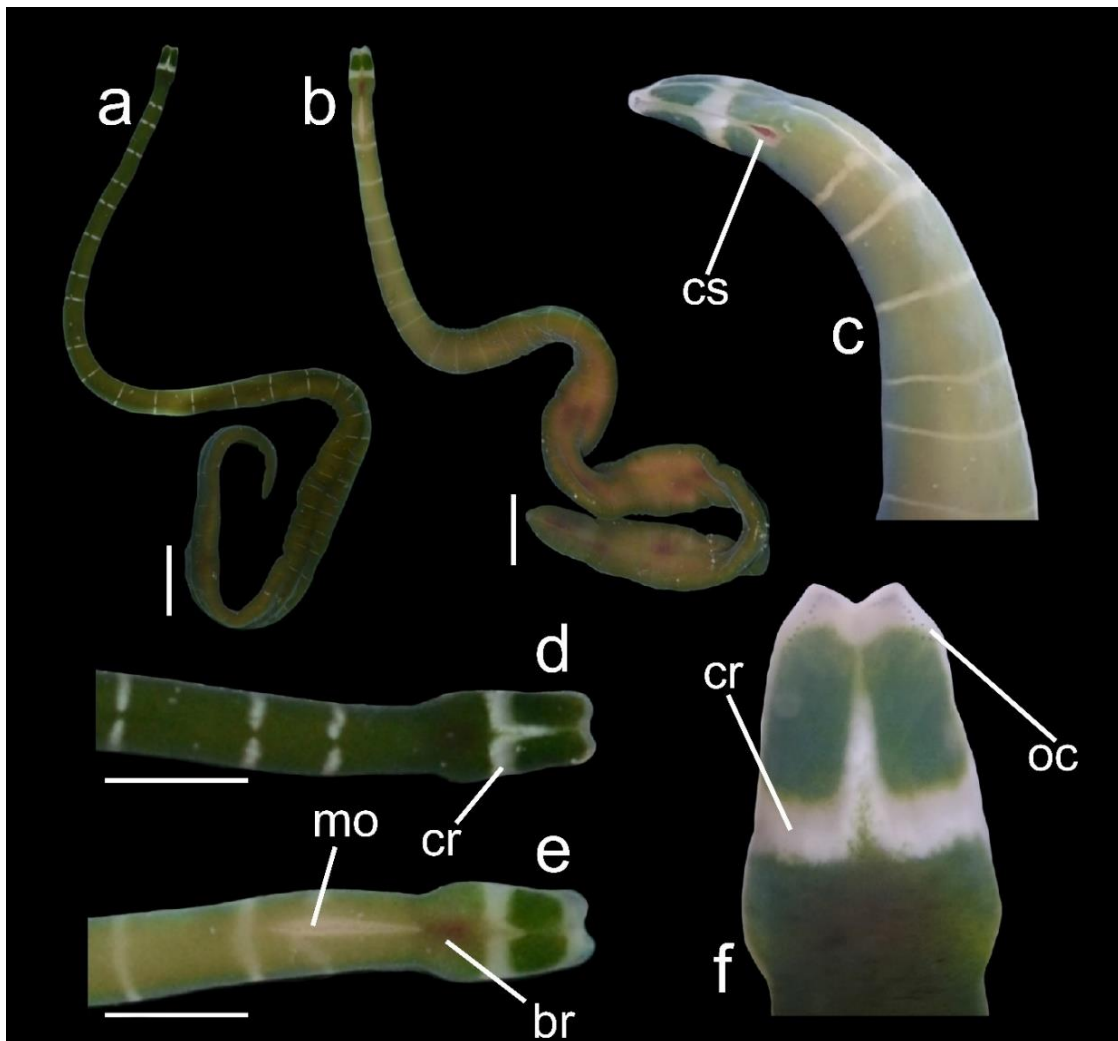


Figure 19. *Notospermus* sp. PH24-03h, collected from Kawayan Reef, Initao, Misamis Oriental at anaesthetized state. (a), entire body at dorsal view, (b), entire body at ventral view, (c), magnification of the anterior end at lateral view, (d), magnification of the anterior end at dorsal view, (e), magnification of the anterior end at ventral view, (f), magnification of the head at dorsal view. Scale bar: a, b = 10 mm; d, e = 5 mm; not available for c and f.

Notospermus sp. PH24-03h (Fig. 19a–f) is 180 mm in length, 4 mm in width; dorsal body is green, lighter ventrally; pigmentation features of the head similar with *Notospermus* sp. PH24-03d; with 37 white, non-completely transverse bands on the dorsal body (Fig. 19a), continuous ventrally as horizontal bands (Fig. 19b), 24 in number; numerous ocelli (Fig. 19c, f) are distributed on the dorso-lateral edges of the head in the same manner as with *Notospermus* sp. PH24-03g, 20–30 in number on each side of the head.

Taxonomic remarks. The specimens PH24-03a–PH24-03f reported in this chapter—despite differences in the cephalic pigmentation pattern, construction and number of the white body rings, and the position and the number of the ocelli—all belong to a single species based on DNA data (COI *p*-distance = 0–2.3%). Although *Notospermus* sp. PH24-03g and *Notospermus* sp. PH24-03h were not sequenced, it is highly likely that they are the same species as the others based on their morphology. From the currently named congeners, *Notospermus* sp. PH24-03 is morphologically similar to *N. annulatus* (Grube, 1840)—in terms of the over-all appearance. However, their conspecificity could not be verified molecularly as the MCZ IZ-132741 specimen of *N. annulatus* (Kajihara et al. 2022, fig. 3o) has no available COI sequence. However, the 16S *p*-distance between the two species was 10.5–11.6%. Since 16S is a slow-evolving mitochondrial gene compared to COI, comparison of the latter gene marker between the two species, when available, would result to a higher *p*-distance, supporting the distinction between the two species, both in terms of 16S and COI. *Notospermus* sp. PH24-03 is morphologically different from the greenish *N. fuscoviridis* (Takakura, 1898) by the absence of the white cephalic ring and transverse bands in the body of the latter (Kajihara et al. 2022, fig. 3l). Although *N. geniculatus* (Delle Chiaje, 1822) and *N. mitellatus* (Takakura, 1898) have the ‘Λ’-shaped cephalic ring as the present species,

their body color differs (Kajihara et al. 2022, fig. 3m–q). Although *N. albovittatus* (Stimpson, 1855) can be pale green aside from being brownish green in color and has a white cephalic ring encircling the head, it lacks the white transverse bands in the body (Chernyshev 2016, fig. 5h) unlike *Notospermus* sp. PH24-03 which has these bands in varying numbers.

Genus *Quasilineus* Gibson, 1981

Quasilineus pulcherrimus Gibson, 1981 (Fig. 20a–d).

Material examined. Two specimens, collected from Brgy, Poblacion, Naawan Misamis Oriental on March 18, 2024; one specimen from Initao, Misamis Oriental on March 27, 2024; two specimens collected from Brgy. Vinapor, Carmen, Agusan del Norte on March 12, 2024; three specimens collected from Brgy. Baucawe, Lianga, Surigao del Sur on March 16 and 18, 2024; one specimen collected from Brgy. Poblacion, Lianga, Surigao del Sur on March 17, 2024. All specimens were found crawling on rock cervices in the intertidal zone, also found on rocks above slightly silty substrates and on rocks decorated with small clam shells.

Description. *External features.* Colorful worm (Fig. 20), 55.5–89.0 mm in length (n = 6), 1.2–1.7 mm in width (n = 6), ground body cream white or opaque white, dorsally decorated with seven longitudinal bands, orange, yellow, and black in color (Fig. 20a); the central band, a thick black band, begins as a nearly hexagonal band in the cephalic region which extends down to the posterior end of the body as central longitudinal band; on each side of the central black band are thick longitudinal yellow bands, originates from the lateroposterior edges of the black cephalic band, extends down to the posterior end of the body; thin, non-solid, black longitudinal band lines the outer edges of each yellow band, also extends posteriorly; each of the thin, black, longitudinal band is lined by a bright orange band on its outer edges, the orange bands also extend posteriorly; the dorsal body margin is similar with the ground color (Fig. 20a); a bright orange cephalic patch, thick crescent, facing downward in shape is present (Fig. 20a, b, d), the most anterior tip of the head is colorless (Fig. 20b, c), in

between the orange cephalic and black patches are opaque, cloudy cephalic patches (Fig. 20a, b, d); ventral body generally colorless, sometimes pale cream at the posterior region, bright orange cephalic patch and the opaque white patches are visible from the ventral view of the head (Fig. 20c); with a deep, horizontal lateral cephalic slit which begins from the mouth region, extends anteriorly to the tip of the head (Fig. 20c, d); no caudal cirrus.

Taxonomic remarks. The identification of this species was mainly based on the external features of the worm provided by Gibson (1981).

Distribution. This species was originally established from Picnic Bay, Magnetic Island in Australia (Gibson 1981). New distribution record in Misamis Oriental, Agusan del Norte, and Surigao del Sur, Philippines (present study).

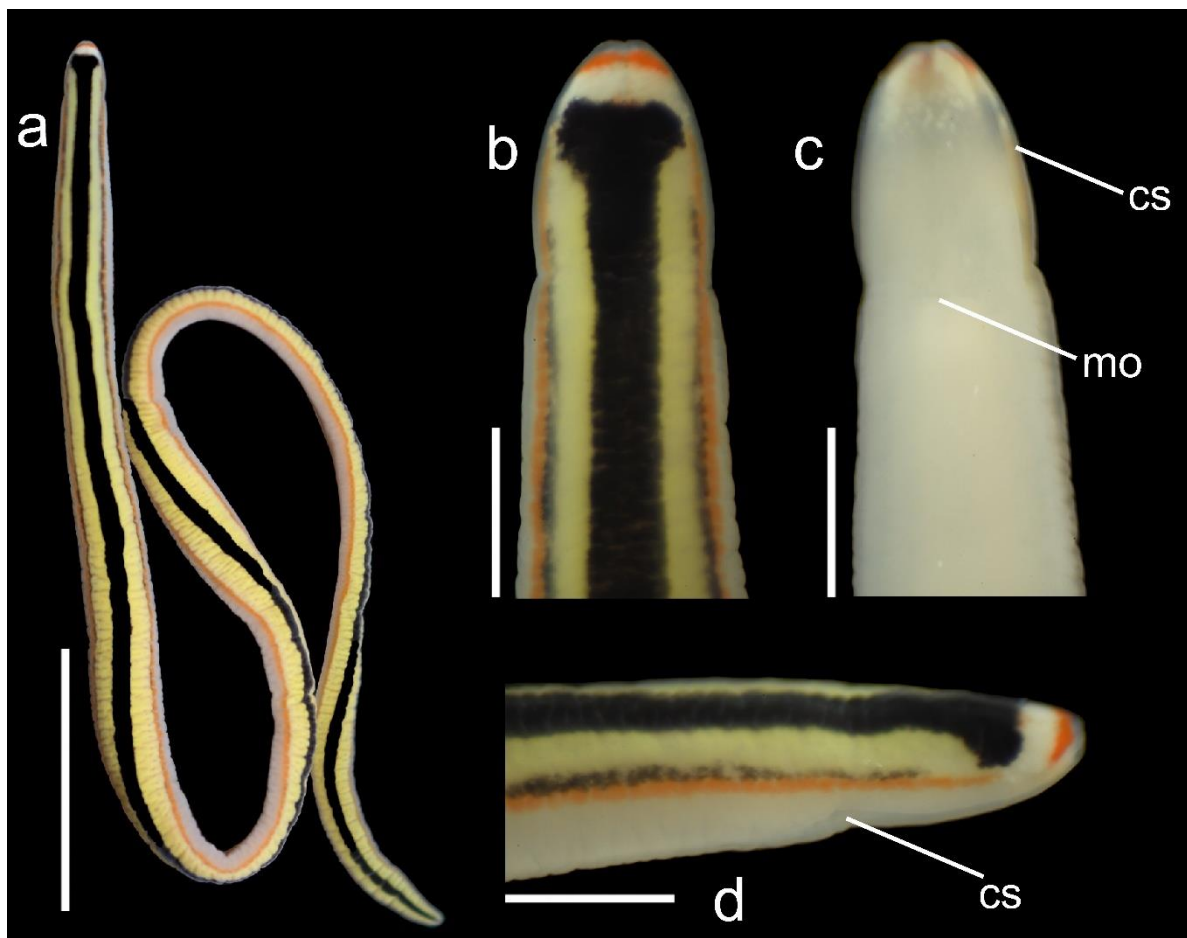


Figure 20. *Quasilineus pulcherrimus*, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the

anterior end at dorsal view, (c), magnification of the anterior end at ventral view, (d), magnification of the anterior end at lateral view. Abbreviations: cs, cephalic slit; mo, mouth. Scale bar: a = 10 mm; b–d = 1 mm.

Genus *incertae sedis*

Lineidae sp. PH24-01 (Fig. 21a–c).

Material Examined. One specimen collected from Brgy. Poblacion, Lianga, Surigao del Sur on March 17, 2024, obtained under rock crevices in the intertidal zone.

Description. *External features.* Worm about 48.5 mm in length, 2.5 mm in width; entire body opaque, uniformly dark red in color (Fig. 21a), no mottles, dorso-ventrally flat, posterior most portion cylindrical; no caudal cirrus; head not demarcated from the rest of the body (Fig. 21b, c), acute, no distinct patches, dorsally, posterior region of the head with folds or ridges (Fig. 21b) making this region more elevated than the anterior region of the head, ventrally, the same ridge is located immediately anterior to the

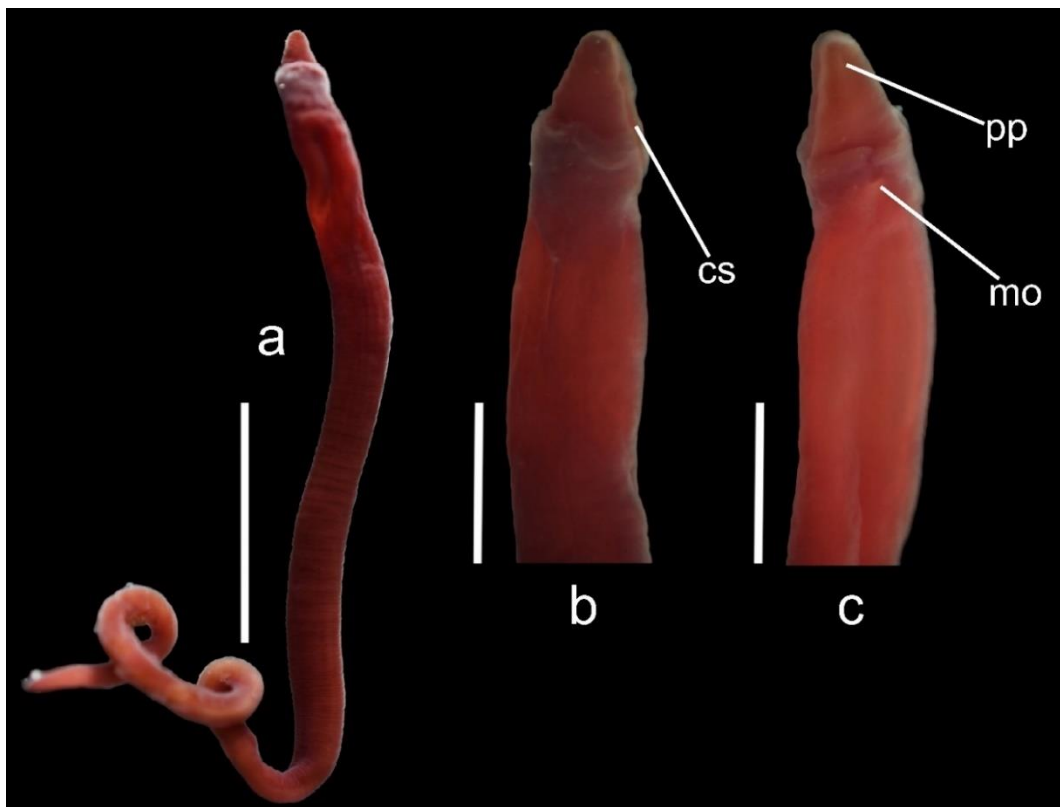


Figure 21. Lineidae sp. PH24-01, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cs, cephalic slit; mo, mouth; pp, proboscis pore. Scale bar: a = 10 mm; b, c = 2.5 mm.

mouth (Fig. 21c); a deep, horizontal lateral cephalic slit originating from the level of the mouth region runs anteriorly to the tip of the head on both sides (Fig. 21b, c).

Taxonomic remarks. Lineidae sp. PH24-01 is morphologically distinct from the lineids previously documented from the Philippines. The cephalic ridges are distinct features of this unidentified species.

Lineidae sp. PH24-02 (Fig. 22a–d).

Material Examined. One specimen collected from Brgy. Poblacion, Lianga, Surigao del Sur on March 17, 2024, obtained under rock crevices in the intertidal zone.

Description. *External features.* Body dorso-ventrally flat, anterior body narrower than rest, posterior end pointed, body color orange, no patches, mottles, intensity of color lightens from anterior to posterior body, posterior end pale orange to creamy in color, same (Fig. 22a); rhynchocoel runs to the entire body length; head not demarcated from the body (Fig. 22a), acute, pointed; brain visible on both sides of the head as reddish mass (Fig. 22a–e), ventrally, this reddish mass is found anterior to the short mouth (Fig. 22d), with deep horizontal lateral cephalic slit originating from the brain region extending anteriorly to the tip of the head (Fig. 22c–e), on both sides of the head, the posterior ends of the slit protrudes outward (Fig. 22c–d); with short caudal cirrus. The worm was noticeably sensitive to the magnesium chloride solution as its body constricts then breaks into several fragments a few minutes after exposure (Fig. 22b).

Taxonomic remarks. Lineidae sp. PH24-02 is morphologically distinct from the lineids previously documented from the Philippines. It may closely resemble in form and color

the orange *Dushia nigra* (Stimpson, 1855) species complex but several morphological features between the two can be observed. Both differ in the head shape, the construction of the deep cephalic slit, the size and the position of the mouth relative to the brain. Additionally, based on observation, members of *D. nigra* species complex do not constrict and fragment after few-minute exposure to magnesium chloride solution unlike Lineidae sp. PH24-02.

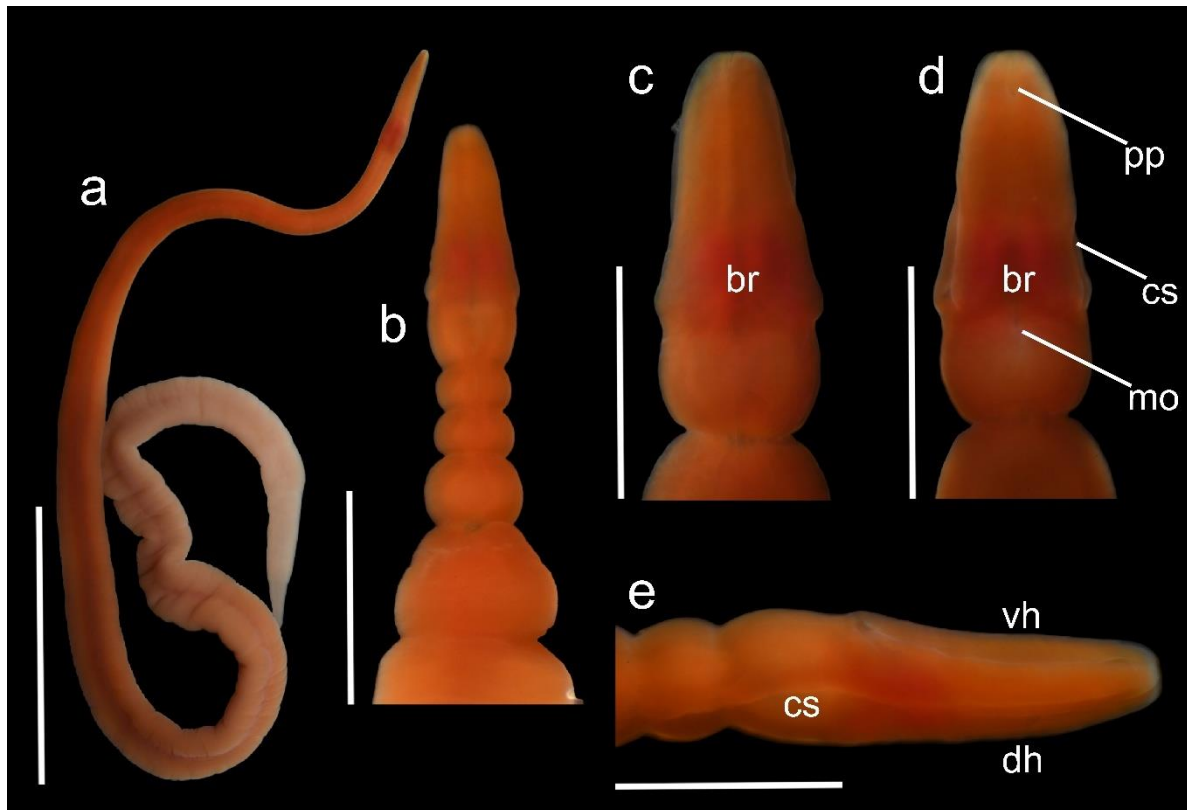


Figure 22. Lineidae sp. PH24-02, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end after worm begins fragmentation; (c), magnification of the head at dorsal view, (d), magnification of the head at ventral view, (e), magnification of the head at lateral view. Abbreviations: br, brain, cs, cephalic slit; dh, dorsal head, mo, mouth; pp, proboscis pore, vh, ventral head Scale bar: a = 10 mm; b = 3 mm; c–e = 2 mm.

Class Pilidiophora Thollessen & Norenburg, 2003

Order Heteronemertea Coe, 1905

Family Valenciniidae Hubrecht, 1879

Genus *Baseodiscus* Diesing, 1850

Baseodiscus jonasii Strand, Hjelmgren & Sundberg, 2005 (Fig. 23a–c).

Materia examined. One specimen collected from Brgy. Poblacion, Lianga Surigao del Sur, collected subtidally in the intertidal zone, barrowed into sediment. Some specimens obviously floating over seagrass beds on March 17, 2024. A similar species was collected from Brgy. Gamut and Brgy. Unidad, Surigao del Sur on September 23, 2024; and Brgy. Poblacion, Naawan, Misamis Oriental on April 25, 2024.

Descriptions. *External features.* Long, cylindrical worm, ground color reddish-brown in color with numerous yellowish longitudinal beige stripes extending to the full length of its body both dorsally and ventrally (Fig. 23a–c); head is round (Fig. 23a–c), demarcated from the body; no ocelli on the head surface; thin transverse cephalic furrow noticeable at the ventral view of the head; mouth obviously visible ventrally; no cephalic slit, no caudal cirrus.

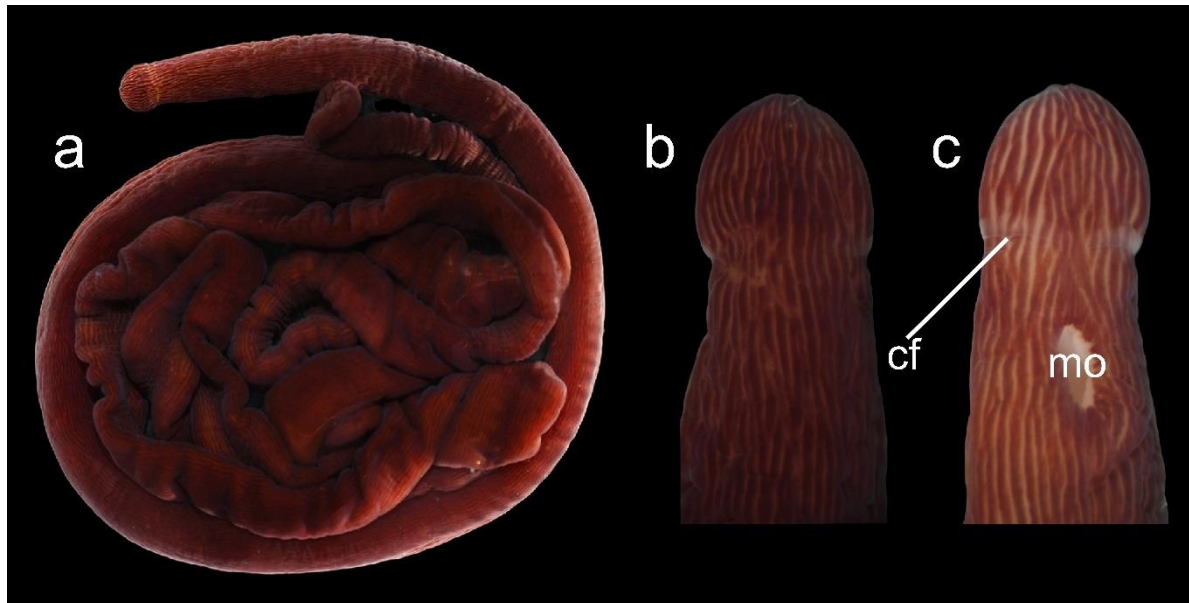


Figure 23. *Baseodiscus jonasii*, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cf, cephalic furrow; mo, mouth.

Taxonomic remarks. *Baseodiscus jonasii* was identified based on the description of the species by Strand et al. (2005) and on the identification by Chernyshev (2016).

Distribution. This species was originally established from Guadalcanal in Solomon Islands (Strand et al. 2005). Later reported from Cu Lao Thu Island in Vietnam (Chernyshev 2016, fig. 4a). New distribution record in Misamis Oriental and Surigao del Sur, Philippines (present study).

***Baseodiscus* sp. PH24-01** (Fig. 24a–c).

Material examined. One specimen collected from Brgy. Poblacion, Lianga Surigao del Sur, obtained on the surface of an intertidal rock, on March 17, 2024.

Description. *External features.* Long worm, body opaque, ground body color cream or pale white; dorsally, with numerous pale brownish longitudinal lines from the head down to posterior end of the body giving the dorsal body a wrinkled impression (Fig. 24a, b), ventrally these lines are absent (Fig. 24a, c); head demarcated from the body, round, anterior edges colorless, mid region decorated by brownish longitudinal lines creating two non-pigmented and non-wrinkled looking cephalic regions (Fig. 24a, b); numerous brownish dots suspected to be ocelli scattered on the non-pigmented regions of the head (Fig. 24b); dark brownish pigmentation apparent in the ‘neck’ region (Fig. 24b) ventrally, entire body non-pigmented, cream or pale white in color; antero-laterally located short cephalic furrow present on each side of the head, runs towards the anterior edge without reaching and converging into the proboscis pore; a thin transverse cephalic furrow in the ‘neck’ region is notable; mouth circular, slightly below the ‘neck’ region (Fig. 24c).

Taxonomic remarks. *Baseodiscus* sp. PH24-01 is unique from the currently known congeners reported from the Philippines namely *B. quinquelineatus* and *B. jonasii*. Morphologically, it does not accord to any of the currently recognized congeners. This could be a potentially new species.

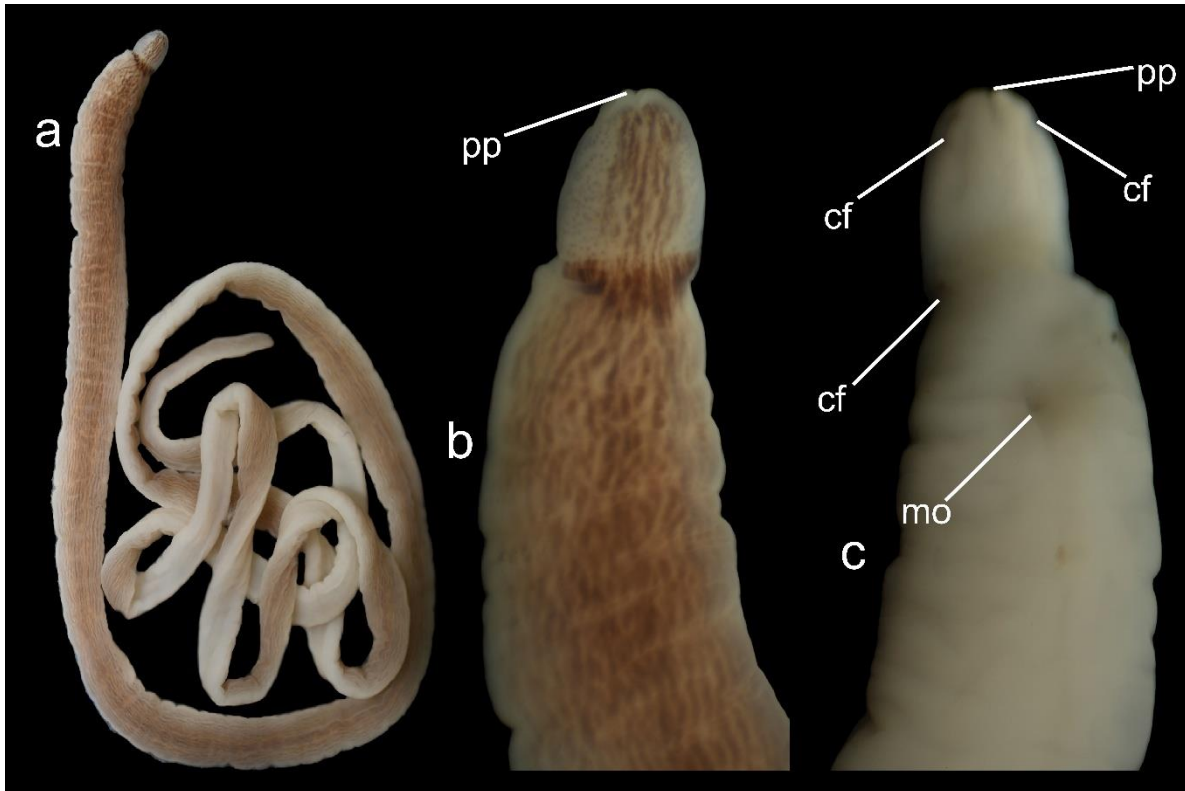


Figure 24. *Baseodiscus* sp. PH24-01, collected from Brgy. Poblacion, Lianga, Surigao del Sur at anaesthetized state. (a), entire body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), magnification of the anterior end at ventral view. Abbreviations: cf, cephalic furrow; mo, mouth; pp, proboscis pore.

Results and systematic remarks

This latest nemertean faunal survey in the Philippines resulted in the discovery of seven Pilidiophora species, three of which are named species, namely *i)* *Baseodiscus jonasii*, *ii)* *Eousia verticivaria*, and *iii)* *Quasilineus pulcherrimus*—all recorded for the first time in the Philippines. Four unidentified species were also documented, namely *i)* *Baseodiscus* sp. PH24-01; *ii)* *Notospermus* sp. PH24-03, *iii)* Linedidae sp. PH24-01, and *iv)* Lineidae sp. PH24-02—all could be species new to science.

Notospermus sp. PH24-03a–PH23f were sequenced in terms of their cytochrome c oxidase subunit I (COI). DNA analysis showed that despite variations in external features, all of them belong to the same species by having an uncorrected COI genetic distance of 0 to 2.3% (mean group *p*-distance is 1%). The remaining specimens *Notospermus* sp. PH24-03g and *Notospermus* sp. PH24-03h, although not sequenced,

could represent the same species based on external morphology. Therefore, *Notospermus* sp. PH24-03 is regarded as another nemertean species that exhibits external polymorphism.

Six specimens of *Q. pulcherrimus* was sequenced in terms of their COI. Their uncorrected COI *p*-distance varied from 0 to 5.6% (mean group *p*-distance is 2%). One specimen, collected from Brgy. Vinapor, Carmen, Agusan del Norte was genetically distinct from the rest. Despite being molecularly distinct, this specimen can't be differentiated from the rest based on external morphology. This molecular finding indicates that *Q. pulcherrimus* population in Mindanao is currently composed of two molecularly distinct members, hence, a species complex.

A MegaBLAST search was made using the COI sequence of Lineidae sp. PH24-01. This inquiry resulted to a closest hit GenBank entry *Cerebratulus* sp. WA01, GenBank accession number OQ322814, 100% coverage and 87.10% identity from the USA (Virginia, Accomack County, Coastal VA, Wachapreague Inlet, Clubhouse Point).

The same MEGABLAST search was executed using the COI of Lineidae sp. PH24-02. Its closest hit GenBank entry is Lineidae sp. ETP014_SMPP0911, accession number OK081540, 99% query coverage and 85.08% identity from Panama (Panama Oeste, isla Venao) (Maslakova et al. 2022). Two specimens of the orange form of the *D. nigra* species complex collected in Camiguin Island were sequenced in terms of their COI. Genetic analysis showed that Lineidae sp. PH24-02 is a different species with their *p*-distance varying from 20.03 to 20.24%. This indicated distinctiveness of Lineidae sp. PH24-02 from *D. nigra*, despite close morphological resemblance with the orange form of the species complex.

Baseodiscus sp. PH24-01 is closely related to *Baseodiscus quinquelineatus* from Japan (Okinawa, Irabu-jima Island), accession number LC190955, with 100% query coverage and 93.47% identity based on the MegaBLAST search of its COI, further confirming its genus identification.

This chapter revealed seven species of Pilidiophora that were never documented from previous nemertean survey in the Philippines. This increased the diversity of pilidiophorans in Philippine waters to 11 in total, namely: i) *Baseodiscus hemprichii* from Carmen, Agusan del Norte, ii) *Baseodiscus jonasii*, reported from Naawan, Misamis Oriental and Lianga and Barobo, Surigao del Sur; iii) *Baseodiscus*

quinquelineatus, reported from Carmen, Agusan del Norte; Naawan, Misamis Oriental; and Lianga, Surigao del Sur; *iv*) *Baseodiscus* sp. PH24-01, from Lianga, Surigao del Sur; *v*) *Dushia nigra* species complex, from all the sites surveyed in 2023 (Abato et al., 2025) and in the collection sites in the present study; *vi*) Lineidae sp. PH23-01 from Aklan, Panay; Talisayan, Misamis Oriental; Camiguin Island; Carmen, Agusan del Norte; and Hinatuan, Surigao del Sur; *vii*) Lineidae sp. PH23-02 from Carmen, Agusan del Norte and Hinatuan, Surigao del Sur; *viii*) Lineidae sp. PH24-01 from Lianga, Surigao del Sur; *ix*) Lineidae sp. PH24-02 from Lianga, Surigao del Sur; *x*) *Notospermus* sp. PH24-03 from Naawan, Misamis Oriental; Initao, Misamis Oriental; Carmen, Agusan del Norte; and Lianga, Surigao del Sur; and *xi*) *Quasilineus pulcherrimus* from Naawan, Misamis Oriental; Carmen, Agusan del Norte; and Lianga, Surigao del Sur.

Conclusions

Pilidiophoran diversity in Mindanao Island is worth exploring. In terms of species diversity, Surigao del Sur, a Pacific-facing region, has the highest, being the 10 Philippine pilidiophoran species recorded in the region. This implies that marine waters along the Surigao del Sur region are interesting sites to study nemertean diversity and perhaps other marine invertebrates.

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Chapter 3. Taxonomy and systematics of shallow-water nemerteans from Japan

Ribbon worms in the class Hoplonemertea are classified into Polystilifera and Monostilifera based on the structure of the stylet apparatus; the former has multiple central stylets attached to a basis while the latter has only one. Around 600 species are included in the order Monostilifera, classified into 127 genera (Kajihara 2021) with most of the named congeners obtained from shallow-water habitats.

Up to 2017, 44 species of monostiliferous hoplonemerteans had been recognized in Japanese waters (Kajihara 2017). Since then, 19 new species of monostiliferans (excluding the species reported in this dissertation) have been established from this region and one new species record has been reported (Hookabe & Kajihara 2020; Hookabe et al. 2020, 2021, 2022a, 2022b, 2022c, 2023a, 2023b, 2023c), making the number of species up to 63.

In this chapter, the diversity of the monostiliferous hoplonemerteans in the genera *Oerstedia* Quatrefages, 1846, *Tetrastemma* Ehrenberg, 1831, and *Tetranemertes* Chernyshev, 1992 from the western Pacific region is updated using specimens collected at shallow depths. Two new species in *Tetrastemma* are herein established and one new record of species is reported from Japan: i) *Tetrastemma parallelus* sp. nov., from Chiba; ii) *Tetrastemma luteum* sp. nov., from Hokkaido and Sugashima; and iii) *Tetrastemma phaeobasisae*, from Hokkaido. Additionally, four new species in *Oerstedia* are established as new from Japan, Russia, and Vietnam: i) *Oerstedia fuscosparsa* sp. nov., from Chiba; ii) *Oerstedia pseudoculata* sp. nov., from Hokkaido and Sakhalin Island (Russia); iii) *Oerstedia rugosa* sp. nov., from Misaki and Van Phong Bay (Vietnam); iv) *Oerstedia viridifusca* sp. nov. from Kanagawa. Lastly, the species composition of the genus *Tetranemertes* is increased by describing one new species, *Tetranemertes aurantia* sp. nov. from Mie, reporting two new species of the genus left undescribed, namely, *Tetranemertes* sp. AHK-2024-1 from the Ryukyu Islands and *Tetranemertes* sp. AHK-2024-2, from Kanagawa. *Emplectonema kandai* is transferred to the genus *Tetranemertes* after morphological re-evaluation, resulting in a new combination *Tetranemertes kandai* (Kato, 1930) comb. nov.

This chapter emphasized the necessity of integrative taxonomy in establishing species that are unicolored and lack obvious external body markings. Additionally, this approach is very important when dealing with the polymorphic nemerteans such as the highly polymorphic congeners of the genus *Oerstedia*. Traditional morphology-based taxonomy alone is insufficient and might lead to taxonomic chaos when dealing with species that exhibit polymorphism in external body pigmentation. Lastly, the four longitudinal rows of ocelli, with two on each side of the head and almost on top of one another and the bifurcated stylet basis could be a possible synapomorphy in *Tetranemertes*.

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Chapter 3.1. Histology-free description and phylogenetics of *Tetrastemma parallelus* sp. nov. (Nemertea: Eumonostilifera) from Japan

Introduction

The phylum Nemertea is a group of diverse, soft-bodied, unsegmented, worm-like marine benthic invertebrates with some freshwater (Strand & Sundberg 2015) and terrestrial members (Moore et al. 2001), including about 1300 described species worldwide (Kajihara et al. 2008; Chernyshev 2021; Kajihara 2021a). All nemerteans bear an eversible proboscis contained within a hydrostatic cavity called the rhynchocoel (Döhren 2015). Ribbon worms do not possess any external body appendages, and the external diagnostic characters are often restricted to size, shape, position of mouth and proboscis pore, number and pattern of eyes, and body coloration (Strand et al. 2014; Sundberg et al. 2016). There are about 350 known species of nemerteans from the seas of Northeast and Southeast Asia (Crandall et al. 2002; Kajihara 2007; Chernyshev 2008, 2013, 2014, 2016). In Japanese waters alone a total of 130 species are recognized, while there could be at least 77 more unreported or undescribed species waiting for formal taxonomic descriptions (Kajihara 2017). The phylum Nemertea is currently divided into three classes—Palaeonemertea, Pilidiophora, and Hoplonemertea—with the last comprising the orders Monostilifera and Polystilifera (Strand et al. 2019; Chernyshev 2021). About 600 known monostiliferans are spread in 127 genera which are distributed globally (Kajihara 2021b) and commonly characterised by having an armed proboscis with a stylet apparatus composed of a single central stylet mounted on a basis (Gibson 1994).

The monostiliferous hoplonemertean genus *Tetrastemma* Ehrenberg, 1828 was originally established for *Tetrastemma flavidum* Ehrenberg, 1828, found among tubiporid corals in the Red Sea, near El-Tor, Egypt (Ehrenberg 1828–1831). It contains almost 110 currently accepted species (Gibson 1995; Kajihara et al. 2008; Chernyshev 2016; Chernyshev et al. 2020; Kajihara 2021b; Norenburg et al. 2021). Identification of members of this genus is based mainly on their body color although some morphospecies are, in fact, complexes of externally indistinguishable cryptic species (Strand & Sundberg 2005b; Leasi & Norenburg 2014). Commonly, monostiliferous hoplonemerteans having four eyes and a rhynchocoel extending for nearly the full body

length (Chernyshev et al. 2021), a flattened body, and a well-demarcated head (Strand & Sundberg 2005b) have been attributed to *Tetrastemma*. Over the years, the definition of the genus *Tetrastemma* has grown and become more detailed; however, morphological characteristics pointing to this genus are still limited and sometimes uncertain. As an example, the generic diagnoses of both *Amphiporus* Ehrenberg, 1831 and *Tetrastemma* do not show any synapomorphy/-ies that would identify the taxon as monophyletic with certainty (Strand & Sundberg 2005a). To date, the most comprehensive phylogenetic study on *Tetrastemma* was conducted by Chernyshev et al. (2021), which demonstrated that the genus then recognised was not monophyletic based on an analysis on partial sequences of the 16S rRNA (16S), 18S rRNA (18S), 28S rRNA (28S), cytochrome c oxidase subunit I (COI) and histone H3 (H3) genes from 66 specimens representing 53 species of *Tetrastemma*. Additionally, the major *Tetrastemma* clade was found to be divided into four geographically cohesive subclades: North Pacific, American Atlantic, North Atlantic and Asian–Australian Pacific (Chernyshev et al. 2021). Several *Tetrastemma* species have been recorded and formally described in and near Japanese waters. As reported by Crandall et al. (2002) and Kajihara (2017), about 12 species have been formally recognised, with *Tetrastemma cupido* Hookabe, Kohtsuka & Kajihara, 2021a being very recently added to the list.

Histology-free description and identification of new *Tetrastemma* species have been recently reported (Chernyshev et al. 2020; Hookabe et al. 2021a) and even applied to other nemertean taxa (Strand & Sundberg 2011; Kajihara 2015; Kajihara et al. 2018; Hookabe et al. 2021b). This method is useful for describing *Tetrastemma* species, especially when there are congeners with very similar morphology (Chernyshev et al. 2020). In the present study, I provide a histology-free description of a new *Tetrastemma* species from Japan and infer its phylogenetic position using partial sequences of 16S, 18S, 28S, COI and H3 gene markers.

Materials and methods

Sample collection and processing

A single specimen of *Tetrastemma*, on which this chapter is based, was collected using a biological dredge by the research vessel *Sea Star* of the Marine and

Coastal Research Centre, Ochanomizu University, Japan. Prior to morphological examination, a magnesium chloride (MgCl₂) solution was prepared by dissolving MgCl₂ granules in tap water with salinity adjusted to 3.5% using an IS/Mill-E refractometer (AS ONE, Japan). The live specimen was placed in a transparent dish and then exposed slowly to the MgCl₂ solution isotonic to seawater for anaesthetisation. The worm was observed under a Nikon SMZ 1500 stereo microscope, with an attached Nikon D5600 digital camera and external strobe lighting provided by a pair of Morris Hikaru Komachi Di flash units for photomicrography. It was then mounted onto a glass slide under a coverslip (squeezed specimen) for further external and internal morphological examination using an Olympus BX51 compound light microscope and was photographed again with the Nikon D5600 digital camera. After the morphological observations, the holotype specimen was preserved in 99% ethanol until used in DNA extraction.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from the ethanol-preserved specimen using a DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol. Five gene markers were partially amplified using the primer pairs ar-L/br-H (Palumbi et al. 1991) for 16S, 1F/9R (Giribet et al. 1996) for 18S, LSU5/LSU3 (Littlewood 1994) for 28S, LCO1490/HCO2198 (Folmer et al. 1994) for COI, and aF/aR (Colgan et al. 1998) for H3. A 10-μL volume of polymerase chain reaction (PCR) solution was prepared in a tube by combining 1 μL of extracted total DNA with 9 μL of pre-mix; the latter was prepared by combining 68.5 μL of deionised water, 10 μL of 10× Ex *Taq* buffer (TaKaRa, Japan), 8 μL of deoxynucleotidetriphosphates (dNTPs), and 3 μL each of 10 pmol/μL forward and reverse primers.

The 16S and COI genes were amplified in an Applied Biosystems 2720 thermal cycler with the following PCR conditions: 1 min at 98°C, 35 cycles of [10 sec at 98°C, 30 sec at 50°C, 40 sec at 72°C], and 7 min at 72°C. A touch-down PCR using a TaKaRa Thermal Cycler Dice Gradient was performed for amplification of 18S, 28S and H3. Conditions for 18S and 28S amplification were as follows: 2 min at 94°C, 10 cycles of [1 min at 94°C, 30 sec at 55°C (decreasing by 1°C each time), 90 sec at 72°C], and 25 cycles of [1 min at 94°C, 30 sec at 45°C, 90 sec at 72°C], followed by 7 min at 72°C. For

the H3 gene: 1 min at 98°C, 10 cycles of [10 sec at 98°C, 30 sec at 50°C (decreasing by 1°C each time), 40 sec at 72°C], and 25 cycles of [10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C], followed by 7 min at 72°C. Each amplified product was mixed with a loading buffer with SYBR green dye (TaKaRa, Japan) and loaded in a solidified 1% agarose gel for electrophoresis to visualise the quality of PCR. The PCR products were then purified using 28 mU of Exonuclease I (TaKaRa, Japan) and 6 mU of Shrimp Alkaline Phosphatase (TaKaRa, Japan) in an 18-µL reaction volume for 30 min at 37°C, followed by deactivation of the enzymes for 15 min at 80°C.

The purified products were then subjected to a sequencing reaction using the BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, USA) with a reaction volume of 5 µL (1 µL of 0.8 pmol/µL of each primer, 2 µL of the purified product, and 2 µL of BigDye). For BigDye reaction, additional internal primers were used for 18S and 28S. Internal primers used for 18S were 5R and 3F (Giribet et al. 1996), and 18Sbi and S2.0 (Whiting et al. 1997), while for 28S, D2F (Littlewood 1994), 28Z (Hillis & Dixon 1991) and Sa (Whiting et al. 1997) were used. BigDye reaction was performed in the following conditions: 25 cycles of 10 sec at 96°C, 0.5 sec at 50°C, and 4 min at 60°C. Purification and BigDye Terminator reactions were performed in a 2720 thermal cycler (Applied Biosystems). BigDye reaction products were then added with sodium acetate and 75% ethanol and centrifuged to precipitate the DNA into pellets. The DNA pellets were then dissolved with 14 µL of formamide prior to gene sequencing. The amplified gene markers were sequenced using a 3130 Genetic Analyser (Applied Biosystems, USA) (3130KB_POP7_V3). Base calling was performed using GeneStudio Professional Edition. BLAST searches (Altschul et al. 1997), as available on the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>), were performed to check for possible contamination; these searches also indicated that the species in question was closely related to members of the Asian–Australian Pacific *Tetrastemma* subclade of Chernyshev et al. (2021).

Maximum-likelihood analysis and Bayesian inference

To infer the phylogenetic position of the new *Tetrastemma* species within the Asian–Australian Pacific subclade, available partial sequences of the selected five gene markers from this subclade and two more Australian species as outgroup (Chernyshev

et al. 2021) were downloaded from GenBank (Table 3). The COI and H3 sequences were aligned using the MUSCLE algorithm (Edgar 2004) implemented in MEGA ver. 11 (Tamura et al. 2021) using default settings, while MAFFT ver. 7, online version (Kato et al. 2019), was used for aligning 16S, 18S and 28S, applying the E-INS-i iterative refinement method in the advanced settings. Ambiguously aligned sites were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al. 2020). Trimmed sequences were then concatenated in MEGA ver. 11 (Tamura et al. 2021). The best-fit partition and model for maximum likelihood (ML) analyses and Bayesian inference (BI) were determined with PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) using the greedy algorithm (Lanfear et al. 2012). According to the best-suggested scheme, the concatenated sequences were divided into six partitions and models: GTR + G for 16S (subset 1); TRNET + I for 18S and H3 (2nd codon) (subset 2); TRN + I + G for COI (1st codon), 28S, H3 (1st codon) (subset 3); F81 + I for COI (2nd codon) (subset 4); HKY + G for COI (3rd codon) (subset 5); and, lastly, TVM + G for H3 (3rd codon) (subset 6). ML analyses were performed with IQ-Tree ver. 2.1.3 (Minh et al. 2020) and RAxML ver. 8.2.3 (Stamatakis 2015). The best-suggested models were used for ML analysis in IQTree ver. 2.1.3 (Minh et al. 2020) with nodal support values derived from 1000 ultrafast bootstrap (UFBoot) (Minh et al. 2013). For ML analysis in RAxML ver. 8.2.3 (Stamatakis 2015), GTR + G was applied to all subsets; nodal support values were calculated from 1000 bootstrap pseudoreplicates. BI was performed using MrBayes ver. 3.2.6 × 64 (parallel version) (Ronquist & Huelsenbeck 2003; Altekari et al. 2004) employing GTR + G (subset 1), GTR + I (subset 2), GTR + I + G (subset 3), F81 + I (subset 4), HKY + G (subset 5) and GTR + G (subset 6) models, launching two parallel runs with four Markov chains in each run (three cold and one hot) for 5×10^7 generations, sampling every 100 generations from the chain. Run convergence was evaluated by standard deviation of split frequencies (0.000756), estimated sample sizes for all parameters (> 200), and potential scale reduction factors for all parameters (1.000). Results obtained from the ML analyses and BI were used to visualise the best phylogenetic tree using FigTree ver. 1.4.4 (Rambaut 2018).

Voucher material has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan. Nucleotide sequences newly generated in this study have been registered in GenBank with accession numbers ON112235–ON107495 (Table 3).

Table 3. List of *Tetrastemma* species included in the phylogenetic analysis, GenBank accession numbers for 16S, 18S, 28S rRNA, cytochrome c oxidase subunit I (COI), and histone H3 gene markers, and their references.

Species	GenBank accession number					Reference
	16S	18S	28S	COI	H3	
<i>Tetrastemma albomaculatum</i>	–	MZ231215	MZ231305	MZ216537	MZ216607	Chernyshev et al. (2021)
<i>Tetrastemma olgarum</i>	NC024670	–	–	NC024670	–	Sun et al. (2016)
<i>Tetrastemma</i> cf. <i>olgarum</i>	–	MZ231233	MZ231323	MZ216552	MZ216624	Chernyshev et al. (2021)
<i>Tetrastemma parallelus</i> sp. nov.	ON112235	ON112234	ON112277	ON098244	ON107495	present study
<i>Tetrastemma pimaculatum</i>	–	MZ231235	MZ231325	MZ216554	MZ216626	Chernyshev et al. (2021)
<i>Tetrastemma pimaculatum</i>	MZ231166	MZ231236	MZ231326	–	MZ216627	Chernyshev et al. (2021)
<i>Tetrastemma pimaculatum</i>	MZ231167	MZ231237	MZ231327	MZ216555	MZ216628	Chernyshev et al. (2021)
<i>Tetrastemma phaeobasisae</i>	MZ231165	MZ231234	MZ231324	MZ216553	MZ216625	Chernyshev et al. (2021)
<i>Tetrastemma pseudocoronatum</i>	MN211502	MN211408	MN211458	MN205521	MN205478	Chernyshev & Polyakova (2019)
<i>Tetrastemma roseocephalum</i>	MZ231169	MZ231239	MZ231329	MZ216557	MZ216630	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. GA Guam	MZ231174	MZ231247	MZ231337	MZ216565	MZ216638	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. GU Guam	MZ231178	MZ231251	MZ231341	MZ216566	MZ216642	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. VE Vietnam	MZ231188	MZ231267	MZ231357	MZ216580	MZ216658	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. VI Vietnam	MZ231189	MZ231268	MZ231358	MZ216581	MZ216659	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. VT Vietnam	–	MZ231269	MZ231359	MZ216582	MZ216660	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. 1G4 Australia	MZ231170	MZ231240	MZ231330	MZ216558	MZ216631	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. 2B5 Australia	–	MZ231241	MZ231331	MZ216559	MZ216632	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. 3A5 Australia	MZ231171	MZ231242	MZ231332	MZ216560	MZ216633	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. 4E5 Australia	–	MZ231243	MZ231333	MZ216561	MZ216634	Chernyshev et al. (2021)
Outgroup						
<i>Tetrastemma</i> sp. 5D5 Australia	MZ231172	MZ231244	MZ231334	MZ216562	MZ216635	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp. 6C5 Australia	–	MZ231245	MZ231335	MZ216563	MZ216636	Chernyshev et al. (2021)

Results and systematic remarks

Taxonomy

Tetrastemma parallelus sp. nov. Abato, Yoshida & Kajihara (Fig. 26a–d).

urn:lsid:zoobank.org:act:1C3F647F-EA58-44F3-9F4F-868D3B25B868.

Material examined. Holotype, ICHUM 8300, total DNA extracted from a single specimen dredged from a depth between 11 and 18 m on December 2, 2021, off Kouyatsu, Tateyama, Chiba, Japan (between 34.988°N, 139.809°E and 34.984°N, 139.803°E).

Diagnosis. A *Tetrastemma* with a reddish cephalic patch and two orange, parallel dorsal stripes on a semi-transparent/translucent body.

Etymology. The new specific name is a noun in apposition (in the nominative case), derived from the Greek παράλληλος (*parallelus*, ‘parallel’). The specific name pertains to the orange-pigmented parallel lines on the dorsal body, which are one of the diagnostic features of the new species.

Type locality, distribution, and habitat. At present, the species is only known from its type locality, off Kouyatsu, Tateyama, Chiba Japan, 11–18 m depth, mudstone and sandy bottom (Fig. 25); the *in-situ* water temperature was 18–20°C when the holotype was collected.

Sequences. Sequences from *T. parallelus* sp. nov. were deposited in GenBank with accession numbers shown in Table 3.

Description. *External features.* The holotype specimen (Fig. 26) in the anaesthetised state is 2.6 mm long and 0.52, 0.65 and 0.60 mm wide at the anterior, mid and posterior body, respectively. Generally, the body is nearly flat, colorless except for some pigmented regions and marginally round at both ends. Dorsally, the head is spatulate in shape, narrower, and not completely demarcated from the body. The cephalic lobe is

dotted with a quadrangular reddish patch somewhat restricted and concentrated at the centre of the cephalic lobe, in the anterior-eye region, leaving a transparent lobe margin. Two pairs of distinct, oblique lateral cephalic furrows, anterior and posterior, are present.



Figure 25. Conditions of the bottom surface where *Tetrastemma parallelus* sp. nov. was collected.

The cephalic furrows in each pair do not join with one another. Two large, dark red anterior eyes are prominently visible within the pigmented cephalic region (above the anterior cephalic furrow) (Fig. 26a). These anterior eyes appear to be almost black at higher magnification (Fig. 26c). The two posterior eyes are situated near the posterior cephalic furrows, smaller than the anterior ones, and visible only ventrally (Fig. 26d) or in squeezing preparation (Fig. 26c). Dorsally, the body is characterised by the presence of two orange-pigmented parallel lines, which originate near the posterior cephalic furrows and are transversely connected to each other at their anterior ends. These orange lines run through the entire body: anteriorly, the stripes appear thinner;

posteriorly, the stripes are thicker and begin to converge near the anus but never connect to each other (Fig. 26a). Ventrally, the body is semi-transparent/translucent (Fig. 26).

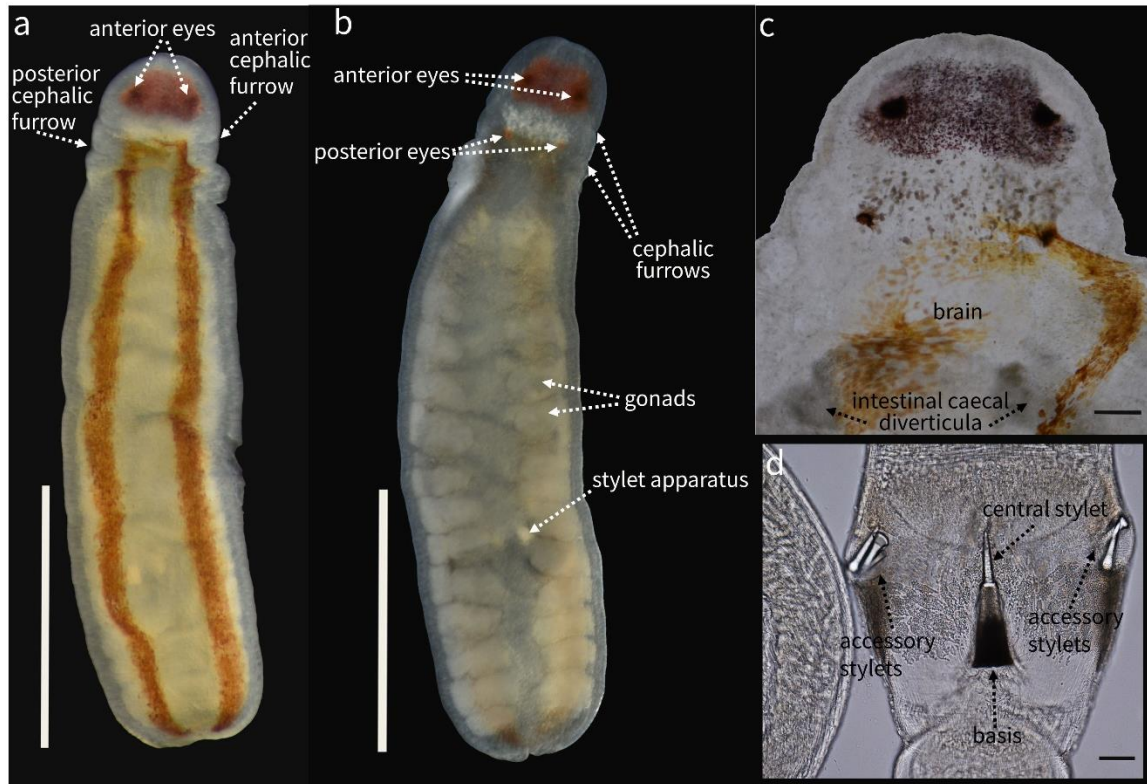


Figure 26. *Tetrastemma parallelus* sp. nov., holotype ICHUM 8300. (a), anaesthetised specimen, dorsal view; (b), anaesthetised specimen, ventral view; (c), magnification of the head, squeezed specimen, dorsal view; (d), magnified stylet apparatus. Scale bars: a, b = 1 mm; c, d = 50 μ m.

Internal features. Dorsally, portions of the digestive system are visible through the transparent body as a wide, creamy to pale yellow region running along the body from below the neck area down to the posterior end (Fig. 26a). Ventrally, an opaque-white region is notable between the cephalic patch and the posterior eyes. The rhynchocoel is visible and as long as the body. Lateral diverticula of the intestine are apparent; the anterior-most intestinal caecal diverticula extend behind the brain (Fig. 26c). Gonads are very apparent (Fig. 26b). Central stylet is 67 μ m long. Stylet basis is 93 μ m long and 55 μ m wide at the most posterior part. It is nearly triangular, with darker,

truncated posterior region and lighter, nearly acute anterior region. Two accessory-stylet pouches are visible, each containing 2–3 accessory stylets (Fig. 26).

Molecular phylogeny

The phylogenetic analyses confirmed that *Tetrastemma parallelus* sp. nov. belongs to the Asian–Australian Pacific *Tetrastemma* subclade. My species was more closely related to those *Tetrastemma* species labelled as VE Vietnam, VI Vietnam and 1G4 Australia (in Chernyshev et al. 2021) than to the remaining species included in the analyses (Fig. 27).

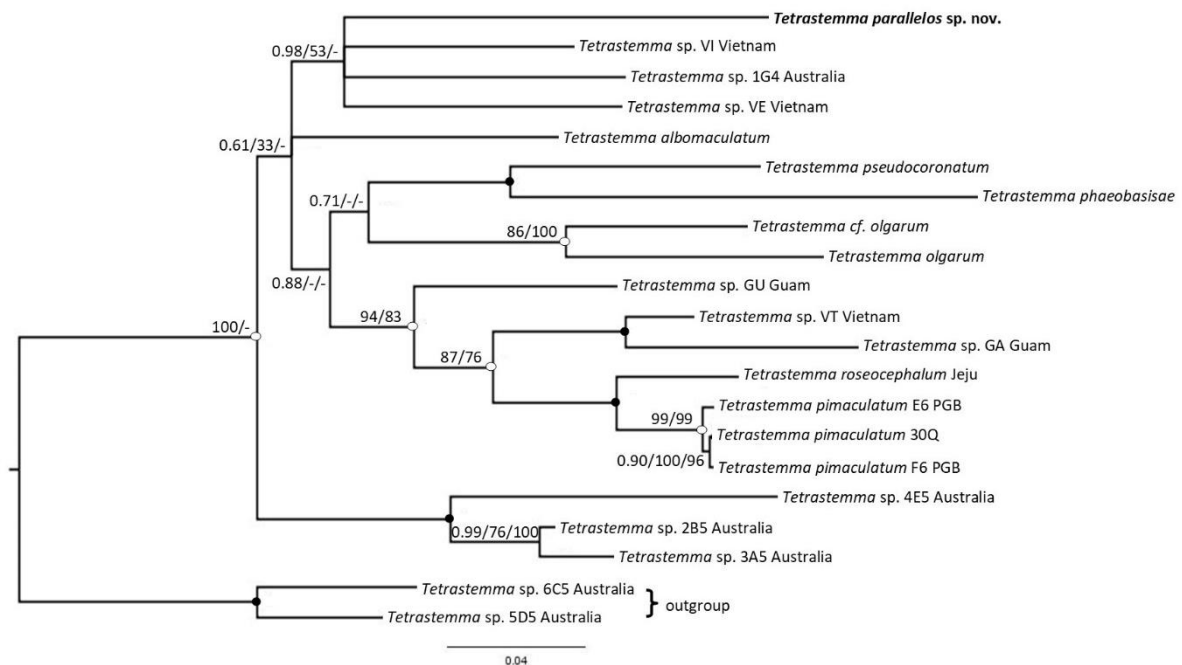


Figure 27. Rooted Bayesian phylogenetic tree showing the position of *Tetrastemma parallelus* sp. nov. within the *Tetrastemma* Asian–Australian Pacific subclade (Chernyshev et al. 2021) based on concatenated sequences of five gene markers (16S, 18S, 28S, COI and H3). Numbers near each node indicate Bayesian posterior probability (PP)/RAxML maximum-likelihood bootstrap value (BS)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot). Solid circles indicate full support (1.00 PP/ 100% BS/100% UFBoot). Open circles indicate 1.00 PP.

Taxonomic remarks. Several *Tetrastemma* species are known to have one or more longitudinal dorsal stripes. In having two parallel dorsal stripes, *Tetrastemma parallelus* sp. nov. resembles some congeners such as *Tetrastemma bilineatum* Coe, 1904,

Tetrastemma bistriatum (Timofeev, 1911), *Tetrastemma herthae* Corrêa, 1963, *Tetrastemma nigrolineatum* Wheeler, 1934 and *Tetrastemma scutelliferum* Bürger, 1895, as well as some varieties of *Tetrastemma nigrifrons* Coe, 1904 including *bilineatum* (Iwata 1954), *bimaculatum* (Chernyshev 1998) (see also Zaslavskaya et al. 2010) and *trimaculatum* (Chernyshev 1998).

In *T. bilineatum*, the dorsal twin stripes are reddish brown to deep chocolate in coloration. Anteriorly, the stripes terminate somewhat in front of the ocelli but sometimes reach the very tip of the snout. Posteriorly, they extend nearly, though not quite, to the posterior extremity of the body and are sharp and conspicuous throughout. Anteriorly, these stripes are not transversely connected with one another but converge at the tail region (Coe 1904). Additionally, this species lacks a cephalic patch.

Tetrastemma bistriatum has two dark brown stripes running dorsally that are separated by a wide area darker than the rest of the body. A dark-brown half-ring is present behind the posterior eyes, from which the two dorsal stripes extend (Timofeev 1911).

Dorsally, *T. herthae* has two lateral longitudinal stripes strongly conspicuous for their deep brown color. Sometimes they are not continuous but appear as rows of brown irregular spots placed closely together. The stripes do not join at the ends (Corrêa 1963).

Tetrastemma nigrolineatum is characterised by two parallel black lines passing dorsally from the tip of the snout to the tip of the tail. At the head, these lines are thinner than they are in the body (Wheeler 1934).

Tetrastemma scutelliferum has two wide, dark brown longitudinal dorsal stripes, which begin behind the cephalic region and almost reach the hind end of the body. Also, the two dark brown stripes are separated by a broad yellow stripe which similarly extends near the posterior end. It also has a dark brown, lobular cephalic patch, which is divided into four lobules: two large lobes at the front and two smaller ones at the sides (Bürger 1895).

The three varieties of *Tetrastemma nigrifrons* commonly have brown or dark brown dorsal stripes, with varied color and shape of the cephalic patch (dark brown, non-split cephalic patch in var. *bilineatum*; two dark brown, split cephalic patches in

var. *bimaculatum*; and three dark brown, split cephalic patches in var. *trimaculatum*) (Iwata 1954; Chernyshev 1998; Zaslavskaya et al. 2010).

Tetrastemma parallelus sp. nov. can be differentiated from these *Tetrastemma* species with two dorsal stripes by its orange-pigmented parallel lines transversely connected anteriorly and by its reddish cephalic patch. Although *T. parallelus* sp. nov. is smaller, the general body shape and the dorsal body feature of my species likely resemble those of *T. bistratum* and *T. scutelliferum*. My species is similar to *T. bistratum* as the latter's dorsal stripes appear to be connected anteriorly by way of its dark-brown half-ring. Internally, the morphology of the stylet basis differs between the two. The basis in *T. bistratum* is rounded at the posterior end, unlike the truncated one in *T. parallelus* sp. nov. Aside from the slight dorsal-body feature resemblance, my species, like *T. scutelliferum*, has two accessory-stylet pouches. However, *T. parallelus* sp. nov. has only 2–3 accessory stylets in each pouch, compared with four accessory stylets per pouch in *T. scutelliferum*.

In the resulting trees, *T. parallelus* sp. nov. was more closely related to the three undescribed forms in Chernyshev et al. (2021)—*Tetrastemma* sp. VE Vietnam, *Tetrastemma* sp. VI Vietnam, and *Tetrastemma* sp. 1G4 Australia than to other congeners (Fig. 27). However, *T. parallelus* sp. nov. can be distinguished from these three by the presence of the reddish cephalic patch and dorsal stripes. In addition, my species is shorter in body size than *Tetrastemma* sp. VI Vietnam (8–12 mm long) and VE Vietnam (8 mm long); and it has a semi-transparent/translucent body compared to the pale yellowish-orange and pale-yellow body coloration of *Tetrastemma* sp. VI Vietnam and *Tetrastemma* sp. VE Vietnam, respectively.

In *Tetrastemma*, body coloration and patterns have been considered effective morphological characters to delimit species (Strand & Sundberg 2005b). Meanwhile, two or more closely related, similar-looking species of eumonostiliferous hoplonemertean can be distinguished by subtle differences such as the epidermal texture (Abato pers. obs.), which would have not been noticed and verbalised without the use of a high-quality digital camera. This indicates that external features of all the *Tetrastemma* species in living state —no matter if the species is already described or not—should be photographed at high resolution, so that future histology-free descriptions continue to be effective.

Conclusions

In this study, the histology-free approach was successful because *i)* the assessment of genus affiliation was facilitated by molecular phylogenetics and *ii)* the new species obviously differed from all congeners in external appearance. If the new species had nested in a clade to which no generic name was reliably applied, its generic affiliation would have had to be assessed by traditional histology. My enumeration indicates that there are ~40 eumonostiliferous hoplonemertean genera that are not yet represented by any molecular data; until all these generic names are molecularly tagged, histological examination will continue to be indispensable in eumonostiliferous hoplonemertean systematics.

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Chapter 3.2. Taxonomy and molecular phylogeny of *Tetrastemma luteum* sp. nov. (Nemertea: Eumonostilifera: Tetrastemmatidae) and first record of *Tetrastemma phaeobasisae* Kulikova, 1987 from Japan

Introduction

Nemerteans, also known as ribbon worms, are unsegmented, soft-bodied, vermiform invertebrates with an eversible proboscis contained in a rhynchocoel, and they inhabit marine, brackish water, freshwater, and terrestrial environments (McDermott & Roe 1985; Thiel & Kruse 2001; Döhren 2015; Chernyshev 2021). The phylum Nemertea comprises around 1300 valid species distributed globally (Chernyshev 2021; Kajihara 2021a), of which around 600 species are included in the order Monostilifera Brinkmann, 1917, classified into 127 genera (Kajihara 2021b). The genus *Tetrastemma* Ehrenberg, 1828 is one of the largest nemertean genera, along with *Cerebratulus* Renier, 1804; *Amphiporus* Ehrenberg, 1931; and *Lineus* Sowerby, 1805. It contains 110 accepted species worldwide (Gibson 1995; Kajihara et al. 2008; Chernyshev 2016; Chernyshev et al. 2020; Hookabe et al. 2021, 2023a, 2023b; Kajihara 2021a; Abato et al. 2022; Sagorny et al. 2022). A multi-locus phylogenetic analysis of the genus *Tetrastemma* revealed that true congeners are nested in a clade designated as *Tetrastemma*, which is further divided into five geographically cohesive subclades: North Pacific, American Atlantic, North Atlantic, Asian–Australian Pacific, and Australian subclades (Chernyshev et al. 2021).

To date, 17 *Tetrastemma* species have been recorded and formally described from Japanese waters: *Tetrastemma album* Hookabe, Kohtsuka, Fujiwara, Tsuchida & Ueshima, 2023a; *Tetrastemma candidum* sensu Iwata (1954); *Tetrastemma carneum* Hookabe, Oya, Tsuchida, Fujiwara & Ueshima, 2023b; *Tetrastemma coronatum* sensu Iwata (1954); *Tetrastemma cupido* Hookabe, Kohtsuka & Kajihara, 2021; *Tetrastemma insolens* Iwata, 1952; *Tetrastemma melanocephalum* sensu Yamaoka (2005); *Tetrastemma nigrifrons* Coe, 1904; *Tetrastemma parallelus* Abato, Yoshida & Kajihara, 2022; *Tetrastemma persona* Hookabe, Kohtsuka, Fujiwara, Tsuchida & Ueshima, 2023a; *Tetrastemma pimaculatum* Chernyshev, 1998; *Tetrastemma pinnatum* Iwata, 1954; *Tetrastemma pseudocoronatum* Chernyshev, 1998; *Tetrastemma roseocephalum* (Yamaoka, 1947); *Tetrastemma stimpsoni* Chernyshev, 1992; *Tetrastemma verinigrum*

Iwata, 1954; and *Tetrastemma shohoense* Hookabe, Kohtsuka, Fujiwara, Tsuchida & Ueshima, 2023a (Iwata 1952, 1954; Crandall et al. 2002; Yamaoka 2005; Kajihara 2007, 2017; Hookabe et al. 2021, 2023a, 2023b; Abato et al. 2022).

The identification of *Tetrastemma* congeners, like many other nemerteans, is mainly based on body color (Strand & Sundberg 2005b; Zaslavskaya et al. 2010). However, intraspecific color variation in ribbon worms is common and should therefore be considered and correctly interpreted in a taxonomic sense. Some of the *Tetrastemma* species that inhabit Japanese waters exhibiting color and pigmentation-pattern variations include: i) *T. nigrifrons*, now with nine (previously 11) color varieties (i.e., *bicolor* Manchenko & Kulikova, 1996; *bilineatum* Iwata, 1954; *bimaculatum* Chernyshev, 1998; *pallidum* Manchenko & Kulikova, 1996; *punctata* Manchenko & Kulikova, 1996; *punctatum* Iwata, 1954; *purpureum* Manchenko & Kulikova, 1996; *spadix* Iwata, 1954; and *trimaculatum* Chernyshev, 1998); ii) *T. stimpsoni* with two color varieties (i.e., with and without pigments); and iii) *T. verinigrum* with two varieties (i.e., *verinigrum* s.str. and var. *meridianum* Iwata, 1954). Molecular methods have ascertained the true identities of some *T. nigrifrons* color varieties: i) the previously considered var. *albino* Manchenko & Kulikova, 1996 was reclassified as a color variety of *T. stimpsoni* based on alloenzyme analysis (Zaslavskaya et al. 2010), and ii) var. *aequicolor* Chernyshev, 1998 was revealed as a distinct *Tetrastemma* species based on alloenzyme and multi-locus phylogenetic analyses (Zaslavskaya et al. 2010, Chernyshev et al. 2021).

In this chapter, I report two species of *Tetrastemma*. One is a polymorphic, new species collected in Oshoro, Otsuchi, and Sugashima, Japan, and the other is an already-known species previously found only in Peter the Great Bay, Russia, but reported here for the first time in Oshoro, Japan. An integrative taxonomy approach is employed to describe and identify the new and known species, respectively. The phylogenetic positions of the two *Tetrastemma* congeners within Clade C of the genus are inferred using the 16S, 18S, and 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene sequences generated *de novo*.

Materials and methods

Specimen collection and morphological observation

Fifteen individuals of *Tetrastemma luteum* sp. nov. reported in this paper were collected in Oshoro (Hokkaido), Otsuchi (Iwate), and Sugashima (Mie), mostly by washing brown algae and filtering the washings using a conical plankton net. Ten specimens were obtained in Oshoro in three collection periods, March 3, 2001, May 10, 2022, and August 30, 2022; two were collected in Otsuchi on September 26–27, 2000; and three specimens in Sugashima on July 27, 2022. A single specimen of *Tetrastemma phaeobasisae* was collected in Oshoro among brown algae on August 30, 2022.

Before morphological examination, a solution was prepared by dissolving MgCl_2 granules in tap water so that it had a salinity of 3.5 permille measured with an IS/Mill-E refractometre (AS ONE, Japan). The specimens were observed and photographed in either alive or in anaesthetised condition. For anaesthetisation, the examined live specimens were placed in a transparent dish, to which the MgCl_2 solution isotonic to seawater was slowly added.

Two of the three *T. luteum* sp. nov. specimens collected in early 2000s were fixed in Bouin's fluid for 24 hrs, dehydrated in an ethanol series, cleared in xylene, embedded in 56–57°C m.p. paraffin wax, sectioned at 8 μm , stained with the Mallory trichrome method, and embedded in Entellan New (Merck); the remaining one has been left unsectioned.

The worms collected in 2022 were observed under a Nikon SMZ 1500 stereo microscope, with an attached Nikon D5600 digital camera and with external strobe lighting provided by a pair of Morris Hikaru Komachi Di flash units for photomicrography. After the morphological observation, the 13 specimens from the 2022 collections were cut into halves. The anterior halves were fixed in Bouin's fluid for 24 hrs then transferred and preserved in 70% ethanol. The posterior halves were preserved in 99% ethanol for genomic DNA extraction.

DNA extraction, PCR amplification, and sequencing

The extraction of total genomic DNA from *T. luteum* sp. nov. and *T. phaeobasisae* specimens collected in 2022, use of primers for the PCR amplification of 16S, 18S, 28S, COI, and H3 genes followed Abato et al. (2022).

The COI gene was amplified in an Applied Biosystems 2720 thermal cycler with the following standard PCR conditions: 1 min at 98°C, 35 cycles of (10 sec at 98°C, 30

sec at 50°C, 40 sec at 72°C) and 7 min of 72°C. When this did not work, a touch-down PCR using a TaKaRa Thermal Cycler Dice Gradient was performed with the following conditions: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 45°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 35°C, 40 sec at 72°C), followed by 7 min at 72°C. Touch-down PCRs were also performed for the amplification of 16S, 18S, 28S, and H3. Conditions for 16S amplification were: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 60°C [decreasing 1°C each time], 50 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 50°C, 40 sec at 72°C), followed by 7 min at 72°C. For 18S and 28S amplification: 2 min at 94°C, 10 cycles of (1 min at 94°C, 30 sec at 55°C [decreasing 1°C each time] at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 45°C, 40 sec at 72°C), followed by 7 min at 72°C. For H3 gene: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 50°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C), followed by 7 min at 72°C.

Downstream procedures (i.e., from gel electrophoresis, PCR products purification, sequencing, and sequence processing) were also adopted from Abato et al. (2022).

Genetic distance and phylogenetic analyses

Uncorrected pairwise genetic distance (p -distance) with respect to successfully amplified COI and 16S was calculated using MEGA ver. 11 (Tamura et al. 2021) to determine the conspecificity among the worms collected in 2022. In this analysis, COI and 16S sequences from a Russian *T. phaeobasisae* was included.

Since a preliminary analysis showed that *T. luteum* sp. nov. and *T. phaeobasisae* are nested within the Clade C of the genus *Tetrastemma*, partial sequences based on the five gene markers from Clade C species (see zsc12511-sup-0002-tables1.doc supplementary material from Chernyshev et al. (2021)) were downloaded from GenBank (Table 4) for the phylogenetic analyses. The accession numbers for COI and H3 sequences of *Tetrastemma* sp. IZ 132742 were interchanged in the supplementary material but fixed in the current study (Table 4). The H3 sequence (MZ216625) for *T. phaeobasisae* from Russia was not included in the phylogenetic analysis since preliminary analysis indicated that this is a contaminated or mislabelled sequence. For *T. luteum* sp. nov. specimens, sequences obtained from ICHUM 8456, 8458, 8465, and

8466 were included in the analyses. MAFFT ver. 7 online version (Kato et al. 2019) was used to align 16S, 18S, and 28S sequences, applying the E-INS-i iterative refinement method in the advanced settings while MEGA ver. 11 was used to align the COI and H3 sequences employing the MUSCLE algorithm (Edgar 2004). Uncertainly aligned sites were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al. 2020); the remaining alignments (494 bp for 16S, 1 783 for 18S, 2 809 bp for 28S, 647 bp for COI, and 325 bp for H3) were concatenated in MEGA ver. 11. The best-fit partitions and models for maximum likelihood (ML) analyses and Bayesian inference (BI) were determined using PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) applying the greedy algorithm (Lanfear et al. 2012). According to the best-suggested schemes, the concatenated sequences were divided into six subsets and models: TRN + I + G for COI (1st codon) (subset 1); TRNEF + I + G for COI (2nd codon), H3 (2nd codon), and 18S (subset 2); HKY + G for COI (3rd codon) (subset 3); TRN + I + G for 28S, and H3 (1st codon) (subset 4); K81UF + G for H3 (3rd codon) (subset 5); and GTR + G for 16S (subset 6).

All the suggested models were used for ML analysis in IQ-Tree ver. 2.1.3 (Minh et al. 2020) with branch support values derived from 1000 ultrafast bootstrap (UFBoot) (Minh et al. 2013). For ML analysis in RAxML ver. 8.2.10 (Stamatakis 2015), GTR + G model was applied to all the six subsets; branch support values were calculated from 1000 bootstrap pseudoreplicates. BI was performed using MrBayes ver. 3.2.6 x64 (parallel version) (Ronquist & Huelsenbeck 2003; Altekar et al. 2004) employing models GTR + I + G for subsets 1, 2, and 4; HKY + G for subsets 3, 5, and 6, launching two parallel runs with four Markov chains in each run (three cold and one hot) for 5×10^7 generations, sampling every 100 generations from the chain. Standard deviation of split frequencies (0.001380), estimated sample sizes for all parameters (> 4456), and potential scale reduction factors for all parameters (1.000) were used to check run convergence.

Results obtained from the ML analyses and BI were used to visualize the best phylogenetic tree using FigTree ver. 1.4.4 (Rambaut 2018), rooted in the same manner as in Chernyshev et al. (2021).

Voucher materials have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan. Nucleotide sequences newly generated in this study have been registered in GenBank.

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Table 4. List of *Tetrastemma* species included in the phylogenetic analyses, collection location, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, and their references.

Species	Voucher number	GenBank accession number					Reference
		16S	18S	28S	COI	H3	
<i>Tetrastemma albomaculatum</i>	35QTal	—	MZ231215	MZ231305	MZ216537	MZ216607	Chernyshev et al. (2021)
<i>Tetrastemma candidum</i>	G6	MZ231152	MZ231218	MZ231308	MZ216540	MZ216610	Chernyshev et al. (2021)
<i>Tetrastemma candidum</i>	24QWS	MZ231153	MZ231219	MZ231309	MZ216541	MZ216611	Chernyshev et al. (2021)
<i>Tetrastemma cf. olgarum</i>	TcfoJ22	—	MZ231233	MZ231323	MZ216552	MZ216624	Chernyshev et al. (2021)
<i>Tetrastemma coronatum</i>	F4	MZ231155	MZ231221	MZ231311	—	MZ216613	Chernyshev et al. (2021)
<i>Tetrastemma coronatum</i>	B4	MZ231154	MZ231220	MZ231310	MZ216542	MZ216612	Chernyshev et al. (2021)
<i>Tetrastemma elongatum</i>	DNA10636	JF277622	JF293026	HQ856874	HQ848609	JF277702	Andrade et al. (2012)
<i>Tetrastemma flavidum</i>	T3	—	—	—	AY791977	—	Strand and Sundberg (2005a)
<i>Tetrastemma fozense</i>	JLN062601C3	MZ231162	MZ231228	MZ231318	MZ216548	MZ216620	Chernyshev et al. (2021)
<i>Tetrastemma laminariae</i>	T59	—	AY928360	—	AY791980	—	Strand & Sundberg (2005a, b)
<i>Tetrastemma longissimum</i>	E2	—	MZ231230	MZ231320	MZ216549	MZ216621	Chernyshev et al. (2021)
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8456*	OQ132627	OQ168357	OQ168365	OQ120977	OQ183423	present study

<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8457	OQ132635	OQ168356	—	—	OQ183428	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8458*	OQ132637	OQ168360	OQ168368	OQ134395	OQ183429	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8459	OQ132630	OQ168363	—	OQ134396	OQ183430	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8460	OQ132629	—	—	OQ134402	OQ183431	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8461	OQ132628	—	OQ168359	—	OQ183424	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8462	OQ132633	OQ198831	OQ168369	—	OQ183425	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8463	OQ132631	OQ198833	OQ168367	—	OQ183426	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8464	OQ132626	—	OQ168366	—	OQ183427	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8465*	OQ132634	OQ168364	OQ168361	OQ134403	OQ183433	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8466*	OQ132632	OQ168354	OQ168358	OQ134401	OQ183434	present study
<i>Tetrastemma luteum</i> sp. nov.	ICHUM:8467	OQ132636	OQ168355	OQ168362	—	OQ183432	present study
<i>Tetrastemma melanocephalum</i>	T20	—	AY928365	—	AY791983	—	Strand & Sundberg (2005a, b)
<i>Tetrastemma olgarum</i>		NC024670	—	—	NC024670	—	Sun et al. (2016)
<i>Tetrastemma parallelus</i>	ICHUM:8300	ON112235	ON112234	ON112277	ON098244	ON107495	Abato et al. (2022)

<i>Tetrastemma phaeobasisae</i>	ICHUM:8508	OQ319610	OQ318526	OQ319609	OQ318514	OQ326500	present study
<i>Tetrastemma phaeobasisae</i>	23QTpha	MZ231165	MZ231234	MZ231324	MZ216553	—	Chernyshev et al. (2021)
<i>Tetrastemma pimaculatum</i>	30Qpim	—	MZ231235	MZ231325	MZ216554	MZ216626	Chernyshev et al. (2021)
<i>Tetrastemma pimaculatum</i>	E6	MZ231166	MZ231236	MZ231326	—	MZ216627	Chernyshev et al. (2021)
<i>Tetrastemma pimaculatum</i>	F6	MZ231167	MZ231237	MZ231327	MZ216555	MZ216628	Chernyshev et al. (2021)
<i>Tetrastemma pseudocoronatum</i>	KB2hop26	MN211502	MN211408	MN211458	MN205521	MN205478	Chernyshev et al. (2021)
<i>Tetrastemma roseocephalum</i>	TrJ	MZ231169	MZ231239	MZ231329	MZ216557	MZ216630	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	1G4 Australia	MZ231170	MZ231240	MZ231330	MZ216558	MZ216631	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	2B5 Australia	—	MZ231241	MZ231331	MZ216559	MZ216632	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	3A5 Australia	MZ231171	MZ231242	MZ231332	MZ216560	MZ216633	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	4E5 Australia	—	MZ231243	MZ231333	MZ216561	MZ216634	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	5D5 Australia	MZ231172	MZ231244	MZ231334	MZ216562	MZ216635	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	6C5 Australia	—	MZ231245	MZ231335	MZ216563	MZ216636	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	GA Guam	MZ231174	MZ231247	MZ231337	MZ216565	MZ216638	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	GU Guam	MZ231178	MZ231251	MZ231341	MZ216566	MZ216642	Chernyshev et al. (2021)

<i>Tetrastemma</i> sp.	IZ 132742	KF935492	KF935325	KF935381	KF935541	KF935437	Kvist et al. (2014)
<i>Tetrastemma</i> sp.	V Vietnam	—	MZ231266	MZ231356	MZ216579	MZ216657	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	VE Vietnam	MZ231188	MZ231267	MZ231357	MZ216580	MZ216658	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	VI Vietnam	MZ231189	MZ231268	MZ231358	MZ216581	MZ216659	Chernyshev et al. (2021)
<i>Tetrastemma</i> sp.	VT Vietnam	—	MZ231269	MZ231359	MZ216582	MZ216660	Chernyshev et al. (2021)
<i>Tetrastemma vermiculum</i>	A4	MZ231190	MZ231270	MZ231360	MZ216583	MZ216661	Chernyshev et al. (2021)
<i>Tetrastemma vermiculum</i>	C6	MZ231191	MZ231271	MZ231361	MZ216584	MZ216662	Chernyshev et al. (2021)

**Tetrastemma luteum* sp. nov. specimens that were included in the phylogenetic analyses

Results and systematic remarks

Taxonomy

Tetrastemma luteum sp. nov. Abato & Kajihara (Figs. 28–33).

urn:lsid:zoobank.org:act:025EDED0-79E7-45A0-BC6D-4FF8745E8035.

?*Tetrastemma candidum*: Iwata, 1954: 35–36, in part.

Material examined. Holotype, ICHUM 8456, extracted total DNA, collected by Jamael Abato and Hiroshi Kajihara by washing brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo, found in Oshoro (43.2095°N; 140.8582°E), Hokkaido, Japan on August 30, 2022. Paratypes, Extracted total DNA from 11 individuals, serial transverse sections representing two individuals and an unsectioned entire specimen preserved in 70% ethanol: ICHUM 8457–8460 collection data same as the holotype; ICHUM 8461–8464 from the topotype, collected by Hiroshi Kajihara on May 10, 2022; ICHUM 8465–8467, collected by the same method as the holotype in Sugashima (34.4838°N; 136.8753°E), Mie, Japan by Shoki Shiraki and Jamael Abato on July 27, 2022; ICHUM 8559, serial transverse sections, eight slides, collected intertidally in Otsuchi (39.3503°N; 141.9360°E) among algae on rocky shore near Otsuchi Coastal Research Centre, the Atmosphere and Ocean Research Institute, The University of Tokyo, by Hiroshi Kajihara on September 26, 2000; ICHUM 8560, unsectioned entire body preserved in 70% EtOH, collected among serpulid tubes on barnacles in the same place as ICHUM 8559, by Hiroshi Kajihara on September 27, 2000; ICHUM 8561, serial transverse sections, 11 slides, from the topotype, collected by Hiroshi Kajihara on March 3, 2001.

Diagnosis. A *Tetrastemma* with entirely yellowish body and head anteriorly knobbed and medially notched when viewed dorsally; four ocelli, anterior pair more indented, brownish-orange and posterior pair less indented, bright-orange in color; anterior cephalic furrows non-connected, not visible dorsally but ventrolaterally notable; posterior cephalic furrow encircles the body.

Etymology. The new specific name is a neuter adjective in the first/second declension which means ‘saffron yellow’ or ‘sallow’ meaning ‘orange-yellow’ and ‘yellowish’ respectively. The specific name pertains to the yellowish coloration of the entire body of the species.

Type locality, distribution, and habitat. The species appears to be distributed along Japan under the influence of warm-water currents. The occurrence was molecularly confirmed in the type locality, Oshoro (Hokkaido). It is also distributed in Sugashima (Mie). A specimen looking similar to the holotype and thus likely representing the same species was also collected from Kannonzaki (35.2589°N; 139.7430°E), Kanagawa by Shiraki Shoki on March 16, 2021 (personal communication). The new species is associated with the brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo and among serpulid tubes on barnacles.

Sequences. Sequences from *T. luteum* sp. nov. were deposited in GenBank with accession numbers shown in Table 4.

Description of the holotype and paratypes. *External features.* Holotype in non-anaesthetised condition, body nearly flat, shape irregular when active (Fig. 28a–c), 2.6–8.5 mm in length (5.7 mm in anaesthetised state) (Fig. 28h, i) (3.5–11.9 mm, in paratypes, n = 11, same in the followings), 0.5–0.8 mm in width (0.7 mm in anaesthetised state) (0.6–1.3 mm); dorsal body entirely bright-yellow in color (Fig. 28a–c, h, j); margin at posterior end translucent in both sides; head demarcated from succeeding body, shape varies in active state, generally round, posterior region narrower than anterior body, widest at mid region, narrows towards anterior end; most anterior edges with a pair of orange-appearing knobs (Fig. 28a–c, h–k), mid anterior edge narrowly notched at dorsal view (Fig. 28a–c, h, j), anterolateral edges less pigmented at dorsal view (Fig. 28a–c, h, j); ocelli four in number, same in size, anterior pair brownish-orange in color, more identified than bright-orange posterior pair (Fig. 28a–c, h, j); anterior cephalic furrow not obvious, posterior one transverse dorsally (Fig. 28c, h, j), same in paratypes (Figs. 29–31); rhynchocoel visible at dorsal view, extends nearly the entire body length, proboscis

visible. Ventral surface lighter than dorsal (Fig. 28i, k); ocelli less notable than dorsal; ventrolateral anterior cephalic furrows not continuous; posterior cephalic furrow less visible (Fig. 28k) but transverse in one paratype (ICHUM 8458) (Fig. 31d) in non-anaesthetised state. Body and head pigmentation-patterns and head shape vary within species (Figs. 30, 31). Paratypes, ICHUM 8461–64, body coloration and head shape and features like holotype (Fig. 30l–p); ICHUM 8457, 8458, 8460, body less pigmented, color pale yellow-orange to pale yellow, appears translucent, entire body margin translucent; head at posterior, mid, and anterior regions almost uniformly wide (Fig. 30a–d, g, h), orange knobs at most anterior edges of head only apparent for ICHUM 8457 (Fig. 31a),

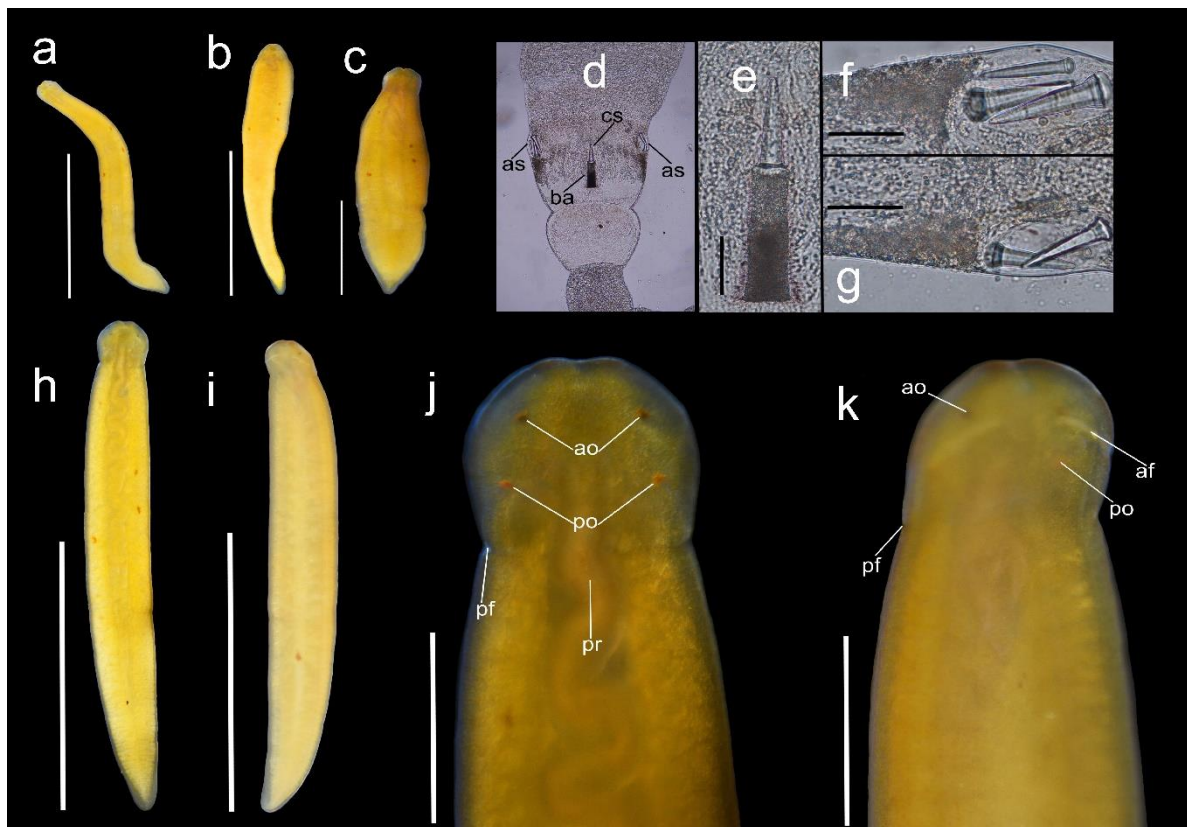


Figure 28. *Tetrastemma luteum* sp. nov., holotype, ICHUM 8456 (a–c), dorsal view at living state and (h, j), dorsal and (i, k), ventral views at anaesthetised condition. (j), magnification of the head at dorsal and (k), ventral views at anaesthetised state; (d, e), structure of the stylet apparatus and (f, g), the accessory stylets. Dorsolateral brownish markings and one at mid ventral body are suspected to be parasites infecting the species. Abbreviations: ao, anterior ocelli; as, accessory stylet; ba, stylet basis; cs, central stylet; pf, posterior cephalic furrow; po, posterior ocelli; pr, proboscis. Scale bars: a, b, h, i = 3 mm; c, j, k = 1 mm; d = 100 μ m; e, f, g = 50 μ m.

head notched but less notable in ICHUM 8460 (Fig. 30g, h), dorsal head less pigmented than succeeding body, ventral side translucent, lighter than succeeding body (Fig. 30a–d, g, h); ICHUM 8459, body color golden-yellow on both sides, appears translucent, entire body margin less pigmented; head shape like holotype, color on both sides same as body, less notched at dorsal view, orange knobs at most anterior edges of head not apparent (Fig. 30e, f); ICHUM 8465–67, body color brown-yellow to pale brown-yellow, pigmentation intensity same on both sides of body, head shape same as holotype except ICHUM 8564 (Fig. 30s) which appears to be as wide as anterior body, notched at dorsal view (Fig. 30q, r, t, u) except ICHUM 8564 (Fig. 30s).

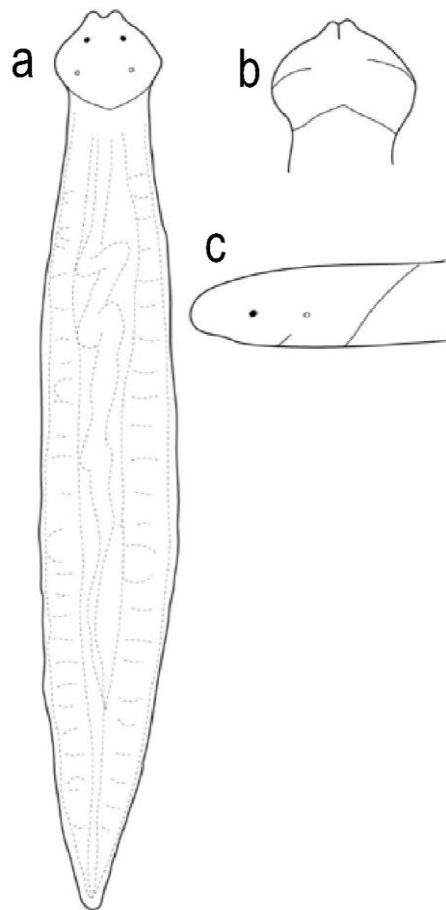


Figure 29. *Tetrastemma luteum* sp. nov., paratype, ICHUM 8559. (a), freehand drawings depicting the general shape of the body in dorsal view, and (b), the anterior tip of the body in ventral and (c), lateral views, showing that the anterior cephalic furrows are invisible in dorsal view.



Figure 30. *Tetrastemma luteum* sp. nov., photographs showing the variations in body color and shape of the anterior end of the body in some paratypes in dorsal and ventral views at anaesthetised conditions. (a, b), ICHUM 8457; (c, d), ICHUM 8458; (e, f), ICHUM 8459; (g, h), ICHUM 8460; (i, j), ICHUM 8461; (k, l), ICHUM 8462; (m, n), ICHUM 8463; (o, p), ICHUM 8464; (s, dorsal only) ICHUM 8465; (t, u), ICHUM 8466; (q, r), ICHUM 8467. Dorsolateral brownish markings on ICHUM 8463, 8467, and 8466 are suspected to be parasites infecting the species. Scale bars: a–p = 3 mm; q–s = 2 mm; t, u = 5 mm.

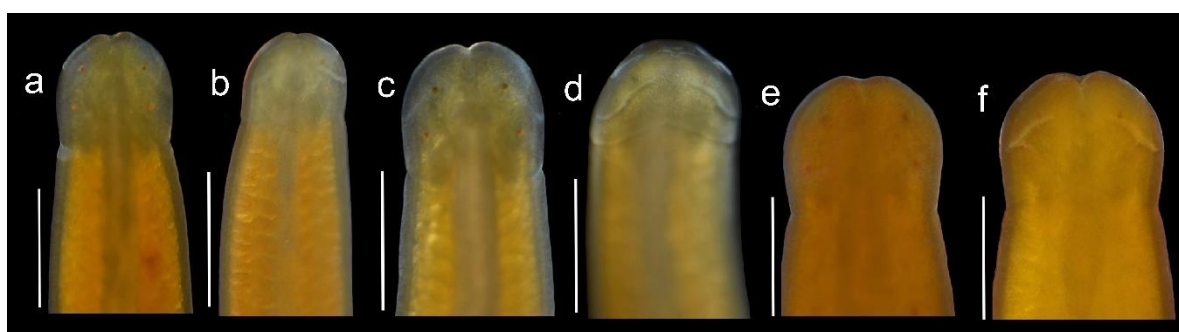


Figure 31. *Tetrastemma luteum* sp. nov., photographs showing the variations in the color and shape of the anterior end of the body in some paratypes in dorsal and ventral views at anaesthetised (a, b, e, f) and non-anaesthetised (c, d) conditions. (a, b), ICHUM 8457; (c, d), ICHUM 8458; (e, f), ICHUM 8462. Scale bars: a–f = 1 mm.

Proboscis apparatus. Descriptions are for the holotype and paratypes (n = 9) (in parentheses). Central stylet 84 μm in length (Fig. 28d, e) (31–93 μm) (Fig. 32a–l), basis 107 μm in length (46–200 μm), anterior-most region 31 μm in width (14–42 μm), posterior region 38 μm in width (12–70 μm); stylet length and basis length ratio 0.79 (0.38–0.87); basis nearly rectangular (Fig. 28d, e) (cylindrical or spindle-like) (Fig. 32a–i), slightly widened at posterior region, anterior half less dense than posterior; two accessory pouches, each with 2–3 accessory stylets (Fig. 28f, g) (up to 4 accessory stylets).

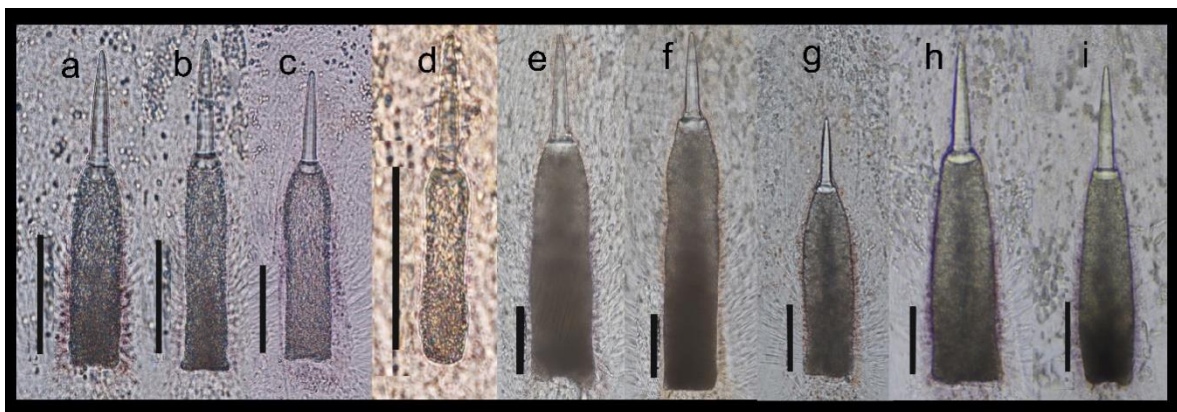


Figure 32. *Tetrastemma luteum* sp. nov., photomicrographs showing the variations of the stylet apparatus in some paratypes. (a), ICHUM 8457; (b), ICHUM 8458; (c), ICHUM 8459; (d), ICHUM 8460; (e), ICHUM 8461; (f), ICHUM 8463; (g), ICHUM 8464; (h), ICHUM 8467; (i), ICHUM 8466. Scale bars: a–l = 50 μm .

Histological features, based on one paratype, ICHUM 8561.

Proboscis. Proboscis nerves 10 in number (Fig. 33f).

Alimentary system. Oesophagus branched off ventrally from rhynchodaeum at level of cerebral organs. Intestinal caecum short, 256 μm in length, anteriorly forked into two branches (Fig. 33g).

Blood vascular system. Mid-dorsal vessel with single vascular plug, latter being extremely flattened (Fig. 33d, e).

Nervous system. Lateral nerve with single neuropil; no accessory lateral nerve (Fig. 33d).

Apical organ and cephalic glands. Single apical organ present, posteriorly leading to basophilic cephalic gland lobules extending backward but not exceeding brain ring (Fig. 33b, c).

Sense organs. A pair of cerebral sense organs present, 40.8–41.5 μm tall and 56.2–81.70 μm wide; open externally ventrolaterally into the anterior pair of cephalic furrows before the brain; reach the brain and extends posteriorly terminating at the ventral commissure; cerebral canals of the organs bifurcate posteriorly (Fig. 33c).

Excretory system. Extends from the rear of the brain to the posterior portion of the foregut, situated ventrolaterally at the rear brain near each side of the stomach region; consists of thick-walled collecting tubules; opening ventrolaterally to the exterior through a pair of slender efferent ducts on each side.

Reproductive system. Sexes are separate; ICHUM 8561 mature female; ovaries do not regularly alternate in the intestinal diverticula; anterior-most ovaries appear near the posterior part of the stomach (Fig. 33h, i).

Remarks. *Tetrastemma candidum* sensu Iwata (1954: 35) from Akkeshi was described as “white, dull yellow, yellowish brown, pale yellowish green or green without any marking” and may have comprised multiple species, including *T. luteum* sp. nov.

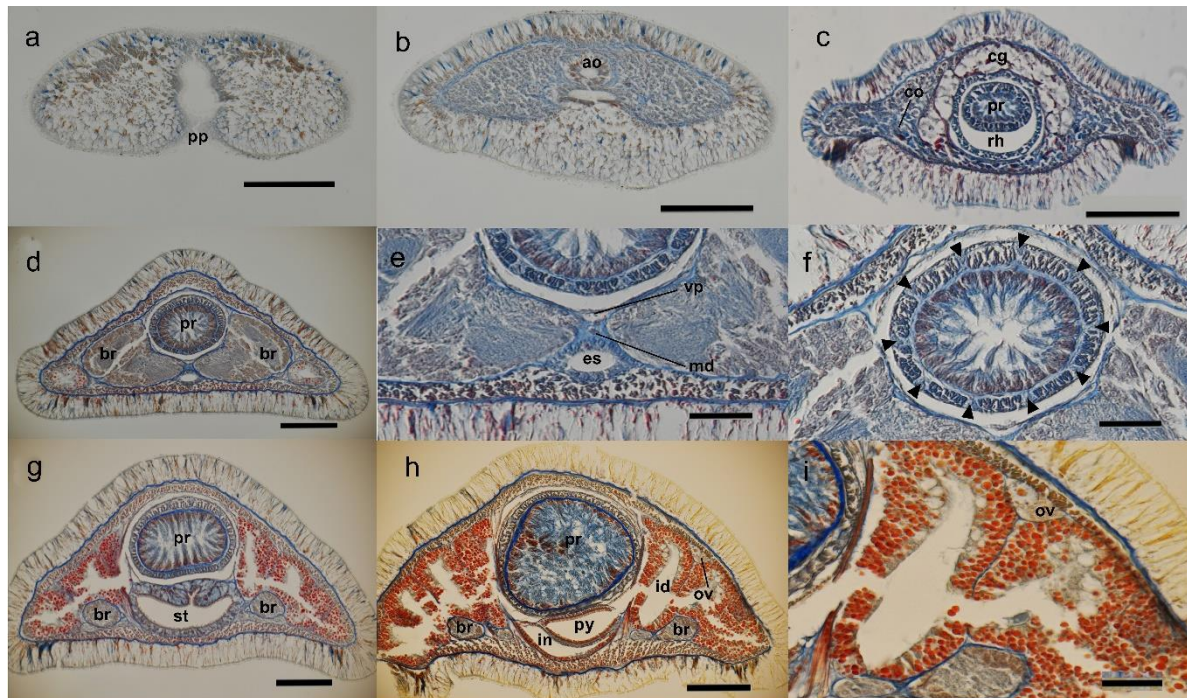


Figure 33. *Tetrastemma luteum* sp. nov., photomicrographs of transverse histological sections, ICHUM 8561, paratype. (a), propodeic pore; (b), apical organ; (c), cerebral

glands; (d), brain; (e), mid dorsal vessel; (f), magnification of the proboscis showing the 10 proboscis nerves (black arrows); (g), stomach region; (h), intestine and pylorus junction; (i), magnification of the ovary. Abbreviations: ao, apical organ; br, brain; cg, cerebral gland; co, cerebral organ; es, esophagus; id, intestinal diverticulum; in, intestine; md, mid-dorsal vessel; ov, ovary; pp, proboscis pore; pr, proboscis; rh, rhynchocoel. Scale bars: a–d, g, h = 100 μ m; e, f, i = 40 μ m.

Tetrastemma phaeobasisae Kulikova, 1987 (Fig. 34a–f).

Tetrastemma phaeobasisae Kulikova 1987: 390–392, table 1, figs. 1, 2, 5a, b.

?*Tetrastemma coronatum*: Iwata 1954: 35–36, in part.

Material examined. Non-type, ICHUM 8508, extracted total DNA, collected by Jamael Abato and Hiroshi Kajihara by washing brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo, found in Oshoro (43.2095°N; 140.8582°E), Hokkaido, Japan on August 30, 2022.

Type locality, distribution, and habitat. The type locality is in Vostok Bay, Russia (Kulikova 1987). Also distributed in Peter the Great Bay, Russia (Chernyshev et al. 2021); and Oshoro, Hokkaido, Japan (present study). Associated with the brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo, collected along with *T. luteum* sp. nov.

Sequences. Sequences from *T. phaeobasisae* were deposited in GenBank with accession numbers shown in Table 4.

Description. External features. Body in anaesthetised state 4.0 mm in length, 0.6 mm in width, nearly flattened, elongated, tapered at posterior end, margin translucent; internal body appears yellowish in a translucent background (Fig. 34a–d); head not demarcated from succeeding body, as wide as the anterior body, elongated, generally translucent; ocelli four in number, nearly squarely-arranged, reddish-orange in color, anterior pair larger, more indented than posterior pair (Fig. 34a–d); anterior cephalic furrows below the anterior ocelli and posterior furrows below the posterior ocelli present but continuity of both at dorsal view not evident in the specimen (Fig. 34c); wide

and deep ventrolateral anterior cephalic furrows extend towards mid passing the anterior ocelli but not connected, thin and connected posterior cephalic furrows run shortly towards mid, immediately above the posterior ocelli (Fig. 21d).

Proboscis apparatus. Central stylet 40 μm in length; basis 40.5 μm in length, anterior most region 15 μm in width; posterior region 22.7 μm in width, cylindrical, gradually widens towards base then narrows, posterior region darker (brownish), anterior lighter; stylet length and basis length ratio 0.99; two accessory pouches present each with 2–3 accessory stylets (Fig. 21e).

Remarks. *Tetrastemma candidum* sensu Iwata (1954: 35) from Akkeshi was described as “white, dull yellow, yellowish brown, pale yellowish green or green without any marking” and may have comprised multiple species, including *T. phaeobasisae*.

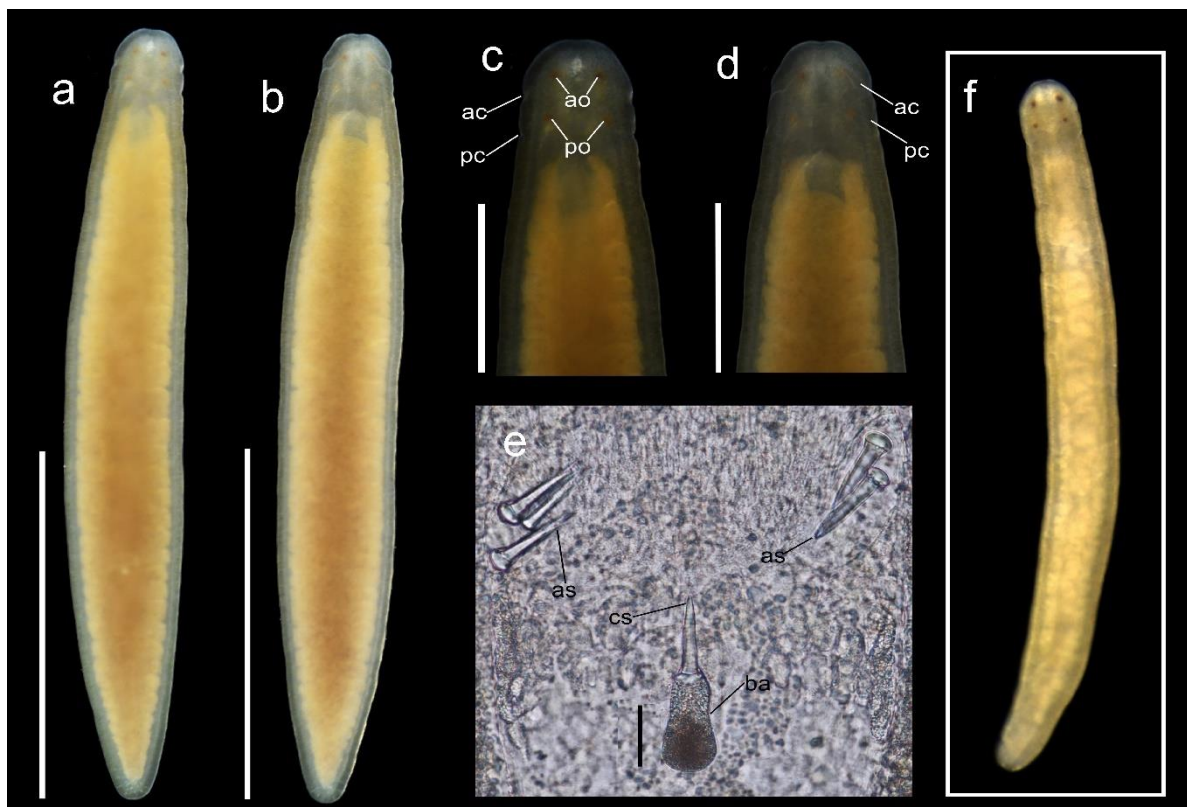


Figure 34. *Tetrastemma phaeobasisae*, non-type (ICHUM 8508), photographs at anaesthetised state, collected in Oshoro Bay, Hokkaido, Japan. Entire worm at (a), dorsal and (b), ventral views; magnification of the head at (c), dorsal and (d), ventral views; (e), photomicrograph of the stylet apparatus. Inserted in f is the same species collected in Peter the Great Bay, Russia (adopted from Chernyshev 2014). Abbreviations: ac, anterior cephalic furrow; ao, anterior ocelli; as, accessory stylets; ba,

basis; cs, central stylet; pc, posterior cephalic furrow; po, posterior ocelli. Scale bars: a, b = 2 mm; c, d = 0.5 mm; e = 25 μ m.

Genetic distance and phylogenetic analyses

A total of 12 *T. luteum* sp. nov. specimens were sequenced in the study. Their conspecificity was determined using their 16S sequences instead of COI since six of the 11 paratypes showed contaminated COI sequences after BLAST search, revealing Amphipoda sequences (either in the genus *Peramphithoe* Conlan and Bousfield, 1982 or genus *Caprella* Lamarck, 1801), a possible indication of the species' preys. Aside from the holotype, uncontaminated COI sequences were generated from ICHUM 8458–8460 and ICHUM 8465–8467 specimens and the uncorrected COI *p*-distance within the 658-bp partial COI region among these individuals ranged from 0–0.91%. The 462-bp 16S based *p*-distance among all the 12 specimens was 0%, suggesting that all specimens are conspecific. On the other hand, the specimen identified as *T. phaeobasisae* from Oshoro is conspecific with a Russian specimen identified as the same with COI and 16S *p*-distances of 0%.

The phylogenetic analyses (Fig. 35) revealed that the new species is a true congener of the genus being included in the clade *Tetrastemma* sensu Chernyshev et al. (2021). *Tetrastemma luteum* sp. nov. is sister to the clade formed by *T. pseudocoronatum* Chernyshev, 1998 and *T. phaeobasisae* with posterior probability of 1.00. The new species and the Oshoro *T. phaeobasisae* are nested within the Asian-Australian Pacific subclade of the genus' Clade C, a clade formed by congeners from the North Atlantic, Asia, and Australia.

Tetrastemma luteum sp. nov.

Taxonomic remarks. Congeners of the genus *Tetrastemma* are identifiable by their body pigmentation and markings. Several species within the genus tend to have different hues of yellow coloration in the body as *T. luteum* sp. nov. *Tetrastemma ambiguum* Riches, 1893; *T. coronatum* (Quatrefages, 1846); *T. dubium* Örsted, 1845; and *T. longistriatum* Wheeler, 1934 are species with yellowish body coloration but can be distinguished from the new species by the presence of notable body markings. Compared to these species, *T. luteum* sp. nov. has a uniformly colored body with no

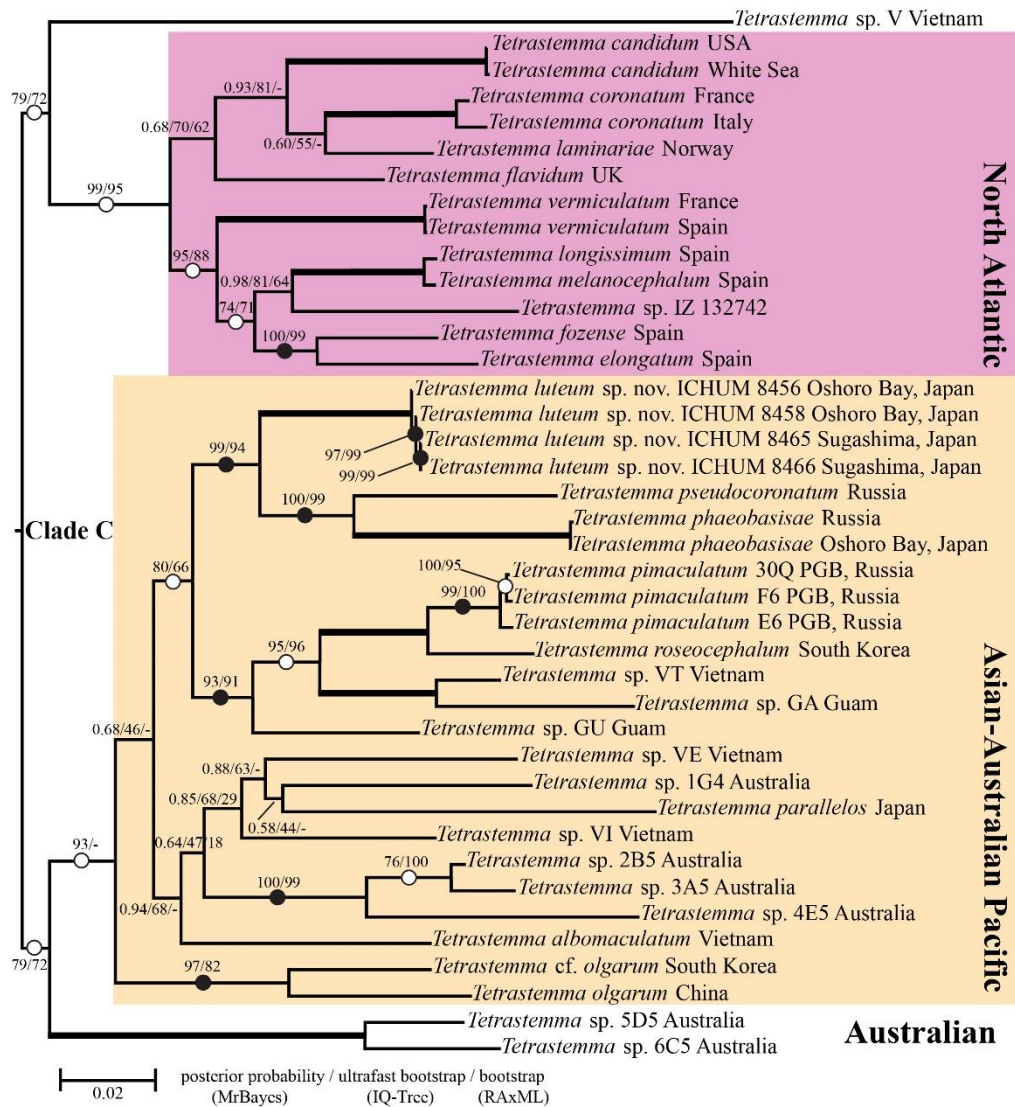


Figure 35. Rooted Bayesian phylogenetic tree showing the position of *Tetrastemma luteum* sp. nov. and *Tetrastemma phaeobasisae* from Oshoro Bay within Clade C of the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent Bayesian posterior probability (PP)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support (1.00 PP/100% UFBoot/100% BS); closed circles indicate 1.00 PP; and open circles indicate 0.99 PP.

other body markings. *Tetrastemma freyae* Chernyshev, Polyakova, Vignesh, Jain, Sanjeevi, Norenburg & Rajesh, 2020; *T. melanocephalum* (Johnston, 1837); *T. pseudocoronatum* Chernyshev, 1998; *T. roseocephalum* (Yamaoka, 1947); *T. stimpsoni* Chernyshev, 1992; and *T. shohoense* Hookabe, Kohtsuka, Fujiwara, Tsuchida & Ueshima, 2023a are species with uniformly yellowish body but can be differentiated from the new species by the presence of cephalic patches. In *T. luteum* sp. nov., the

head has no distinct cephalic patch. *Tetrastemma abberans* Coe, 1901; *T. buxum* Bürger, 1895; *T. cruciatum* Bürger, 1895; *T. flavidum* Ehrenberg, 1828; *T. herthae* Corrêa, 1963; *T. longissimum* Bürger, 1895; *T. phaeobasisae*; *T. quatrefagesi* (Bürger, 1904); and *T. worki* Corrêa, 1961 have bodies that are uniformly yellow (in their own shade) and have no cephalic patches as the new species. However, the new species can be generally distinguished from these species by having a head colored similarly (or lighter) as the body.

Although my phylogenetic analyses show that *T. luteum* sp. nov. is closely related with *T. pseudocoronatum* and *T. phaeobasisae*, my species has a combination of morphological features making it easily distinguishable from the two species. The rounded and wider head similarly colored as (or lighter than) the yellowish body, notched and with bright-orange knobs at the anterior most edges; equally sized, brownish-orange, more indented anterior and bright-orange posterior ocelli are external features that can identify *T. luteum* sp. nov. *Tetrastemma pseudocoronatum* has the typical *Tetrastemma* diamond-shaped head, notched at the anterior edge, translucent to slightly yellowish anteriorly, with notable whitish opaque pigmentation and with transverse brownish cephalic patch bridging the anterior ocelli. The head of *T. phaeobasisae* sometimes appears almost like that of the new species except that it is less round, elongated, as wide as its body, and less pigmented (translucent) and is not notched (Kulikova 1987; Chernyshev 1998). The brownish/darker ocelli of *T. pseudocoronatum* are not equally sized—the anterior pair being always larger than the more indented posterior pair. In *T. phaeobasisae*, the ocelli are slightly larger than the new species, anterior pair larger than posterior pair, all brownish to brownish-orange and nearly squarely arranged. The three species have anterior and posterior cephalic furrows. In *T. coronatum*, the anterior cephalic furrows run shortly towards mid at dorsal view, not connected and situated exactly at the posterior eye region; posterior furrows are connected and V-shaped at dorsal view. Ventrally, the anterior furrows run deeply but are not connected while the posterior furrows connect. In *T. phaeobasisae*, the short, non-connected anterior furrows are found between the anterior and posterior ocelli, dorsally, transverse posterior cephalic furrows are present (Kulikova 1987; Chernyshev 1998). The *T. phaeobasisae* specimen from Oshoro showed ventrolateral anterior cephalic furrows that are not connected. The new species is shorter (8.5 mm in

holotype) compared to the much longer *T. pseudocoronatum* (20 mm in holotype) but slightly longer than *T. phaeobasisae* (7.5 mm in holotype) (Kulikova 1987; Chernyshev 1998).

The central stylet of the new species is always shorter than the basis contrary to a longer central stylet and shorter basis in both *T. pseudocoronatum* and *T. phaeobasisae*. The characteristics of the stylet basis in these two species are almost the same—cylindrical, anterior region is lighter and less wide, posterior region is darker and obviously gradually widens towards the base. The basis of the new species varies from nearly rectangular, cylindrical and spindle shape. The anterior region is nearly the same width or slightly less wide than the posterior region. All the three species have two accessory stylet pouches, however, in *T. pseudoconarum* and *T. phaeobasisae* each pouch may contain 2–3 accessory stylets, but this would vary from 2–3, 2–4, and 4–5 in the new species.

Tetrastemma phaeobasisae

Taxonomic remarks. Molecular sequencing and morphology examination confirmed the occurrence of *T. phaeobasisae* in Oshoro, representing the first record of this species in Japan. The external body features and the stylet apparatus structure of the Japan *T. phaeobasisae* closely resembles that of Kulikova's type species from Vostok Bay (Kulikova 1987) and that of a specimen from Peter the Great Bay (Chernyshev 1998). My specimen is slightly shorter than Kulikova's (1987) and has a slightly widened body. The body margin is remarkably translucent; head is translucent as the body margin except that it has a few tints of yellowish pigmentation at dorsal view. Kulikova (1987) did not mention in her species description the color or appearance of the head, but a specimen from Peter the Great Bay has a head colored as the body (Fig. 34f). Also, the Oshoro specimen slightly tapers from mid body up to the head (Fig. 34a, b) while the widths of the head, anterior body and mid body are nearly the same with respect to the Peter the Great Bay specimen (Fig. 34f). It is also worth mentioning that the central stylet and basis lengths of my specimen are nearly the same while Russian specimens show shorter central stylet and a longer basis. Kulikova (1987) indicated 2 accessory stylets in the Vostok Bay specimens while the Oshoro specimen as 2–3 accessory stylets in each accessory stylet pouch.

The occurrence of *T. phaeobasisae* from Oshoro is likely and can be attributed to several factors. Vostok Bay and Peter the Great Bay are just approximately 660 km and 723 km, respectively, from the new locality. The biodiversity of ribbon worms in the Russian part of the Sea of Japan and adjacent areas is well-studied (Chernyshev 2014, 2020). Water currents in the Sea of Japan such as the Liman and Tsushima Currents (Gamo et al. 2014) could facilitate the material and organismal movements within the sea, and likely to influence the distribution of nemerteans within the region, resulting to a shared nemertean diversity between Russian and Japanese waters (Chernyshev 2014; Kajihara 2017). Hoplonemerteans were reported to produce juvenile-like planktonic larvae with pelagic durations of weeks to months (Maslakova & Hiebert 2014; Maslakova et al. 2022) which can be advected by water currents from Russia to Japan. Ribbon-worm distribution is also be affected by the substrate type of a certain locality. Reports showed that algae, calcareous algae, and other macrophytes provide habitat to most hoplonemerteans such as *Tetrastemma*. Specimens of *T. phaeobasisae* from Peter the Great Bay have been isolated from *Coccophora langsdoeffii* (Turner) Greville; *Corallina* spp.; *Neorhodomela larix* (Turner) Masuda; leaves of *Phyllospadix iwatensis* Makino; and *Zostera* spp. (Chernyshev 2014). In addition to these habitats, using the specimen from Oshoro, I report that *T. phaeobasisae* can also be isolated from sargassums, potentially, *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo—species that are common in the intertidal and subtidal zones of the Oshoro Bay.

Phylogeography

Chernyshev et al. (2021) have identified Clades A, B, and C within the clade they designated as *Tetrastemma*. Within Clades B and C, five geographically cohesive groups or subclades were identified—North Pacific and American Atlantic subclades within Clade B and North Atlantic, Asian-Australian Pacific, and Australian subclades within Clade C. My phylogenetic tree confirms the clade and subclade placements of *T. luteum* sp. nov. Some of the *Tetrastemma* species established from Japanese waters such as *T. parallelus* and *T. roseocephalum*, like the new species, were nested within the Asian-Australian Pacific subclade of the genus (Fig. 35). However, geographic distribution does not always reflect cladogenesis of *Tetrastemma*, since some

congeners established from Japan, especially those that inhabit deep waters, are found to be nested within subclades American Atlantic (e.g., *T. album* and *T. persona*) and North Pacific (e.g., *T. cupido*, *T. shohoense*, and *T. carneum*) of Clade B. This observation should be considered when inferring the cladogenesis of congeners within the genus.

Conclusions

In this study, I described *Tetrastemma luteum* sp. nov., a new polymorphic species of the genus *Tetrastemma* inhabiting on the brown algae from Oshoro, Otsuchi, and Sugashima, Japan using morphology and DNA sequence data. I report that *T. luteum* sp. nov. individuals exhibit variations in the degree of body coloration, body and head pigmentation pattern, head shape, stylet basis shape, and number of accessory stylets. Individuals with bright-yellow, golden-yellow, and pale-yellow pigmentation are currently distributed in Oshoro and Otsuchi (also potentially in Kannonzaki) while those with brownish-yellow coloration are found in Sugashima. As with the other congeners, the shape of the stylet basis and number of accessory stylets vary within the new species, therefore, can't be use for its identification. The new species can be morphologically identified from the rest of the *Tetrastemma* by its yellowish appearance, its round, notched, and knobbed head. DNA sequencing can facilitate the correct, reliable, and rapid description as well as the identification of the *Tetrastemma* species that are uniformly pigmented but show color variations within members. Furthermore, I updated the geographic distribution of *Tetrastemma phaeobasisae*.

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Chapter 3.3. Species description and phylogenetics of *Oerstedtia fuscosparsa* sp. nov. (Nemertea: Monostilifera: Oerstedtiidae) from Japan

Introduction

The phylum Nemertea is composed mostly of marine, unsegmented worms having an eversible proboscis contained in a muscular and fluid-filled chamber called the rhynchocoel (Döhren 2015). The phylum is composed of approximately 1340 ribbon worm species (Chernyshev 2021) classified into >250 genera including *Oerstedtia* Quatrefages, 1846. Norenburg et al. (2022) record 31 species of *Oerstedtia* currently recognised as valid, of which eight are found in Northeastern Asia including Japan (Crandall et al. 2002). Akhmatova et al. (2012) synonymised three nominal species *Oerstedtiella valentinae* Chernyshev, 1993, *Oerstedtiella zebra* Chernyshev, 1993, and *Oerstedtia oculata* Kulikova, 1987 with *Oerstedtia polyorbis* Iwata, 1954 based on 28S rRNA (28S) gene despite this gene being not suitable and not the standard gene marker used to delineate ribbon worm species. Recent phylogenetic analysis of the genus *Oerstedtia* based on 16S rRNA (16S), 18S rRNA (18S), 28S, cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers revealed that *Oerstedtia polyorbis* Iwata, 1954 and *Oerstedtia oculata* Kulikova, 1987 are different species, despite being very similar in external morphology (Chernyshev & Polyakova 2022). From this recent phylogenetics study of the genus, it is more correct to synonymise only *Oerstedtiella valentinae* Chernyshev, 1993 and *Oerstedtiella zebra* Chernyshev, 1993 with *Oerstedtia oculata* Kulikova, 1987, the first two being the junior synonyms of the latter. This synonymisation has not been incorporated in Norenburg et al. (2022). Taking this into account, 29 species of *Oerstedtia* are currently valid.

Identified *Oerstedtia* species reported from Japanese waters include *O. dorsalis* sensu Iwata (1954), *O. polyorbis*, and *O. venusta* Iwata, 1954 (Iwata 1954; Crandall et al. 2002; Kajihara 2007), all from Hokkaido. It should be noted that based on molecular studies, *O. dorsalis* sensu Iwata (1954) is different from *O. dorsalis* (Abildgaard, 1806) s.str. (Akhmatova et al. 2012; Chernyshev & Polyakova 2022). Polyakova & Chernyshev (2022) revealed two, highly supported, reciprocally monophyletic groups in the genus *Oerstedtia*, called the *Oerstedtia* and *Paraoerstedtiella* clades; the former being composed

of eumonostiliferans that are more closely related to *O. dorsalis* s.str. than to *Oerstedia phoresiae* (Kulikova, 1987) and the latter being more closely related to *O. phoresiae* than to *O. dorsalis* s.str. Among the Japanese *Oerstedia*, *O. polyorbis* and *O. dorsalis* sensu Iwata (1954) were positioned in the clade *Paroerstedtiella*. To date, the non-availability of these five gene marker sequences from *O. venusta* makes the clade placement of the species undetermined.

In this taxonomic work I describe the morphology of a new member of the genus *Oerstedia* collected from Chiba, Japan, and infers its phylogenetic position within the genus using partial sequences of 16S, 18S, 28S, COI, and H3 gene markers. I also report pigmentation variation of this new *Oerstedia* species.

Materials and methods

Sample collection and processing

Two specimens of *Oerstedia* reported in this chapter were collected using a biological dredge by the research vessel *Sea Star* of the Marine and Coastal Research Centre, Ochanomizu University (MRCOU), Japan. The specimens were observed alive both before and after anaesthetisation. Microscopy for external and internal morphological examinations and worm anaesthetisation were done as described in Abato et al. (2022). One of the two specimens (eventually designated as a paratype) was then mounted onto a glass slide under a coverslip (squeezed specimen) to observe the internal features of the head and the stylet apparatus. After microscopy, the holotype and the paratype were cut into halves. The anterior halves were fixed in Bouin's fluid for 24 hrs, preserved in 70% ethanol, then embedded in paraffin. Serial sections of 6–7 µm thickness were generated using a Leica 818 microtome blades on a Reichert-Jung 2035 microtome and stained with Mallory's trichome method. The posterior halves were preserved in 99% ethanol for genomic DNA extraction.

DNA extraction, PCR amplification, and sequencing

Procedures for DNA extraction, PCR amplification, sequencing, and maximum-likelihood analyses were based on Abato et al. (2022) with some modifications. This study used a new forward primer Of-16S-F (5'-AAATAGCTGCGGTACTTTGACC-3') designed using Primer3 (Koressaar & Remm 2007; Untergasser et al. 2012) and was

paired with br-H for 16S amplification. Conditions for 16S amplification were 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 60°C [decreasing 1°C each time], 50 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C), followed by 7 min at 72°C. For H3 gene: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 50°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C), followed by 7 min at 72°C. BLAST searches (Altschul et al. 1997), as available on the NCBI website (<http://www.ncbi.nlm.nih.gov/>), were performed to check contamination and if the collected specimens belong to genus *Oerstedtia*. Uncorrected pairwise genetic distance (*p*-distance) with respect to COI was calculated using MEGA ver. 11 (Tamura et al. 2021). Available partial sequences of the selected five gene markers from *Oerstedtia* species and *Emplectonema mitsuui* Yamaoka, 1947 and *Tetranemertes antonina* (Quatrefages, 1846) as outgroup were downloaded from GenBank (Table 5) and were used for the phylogenetic analysis together with the sequences from the holotype specimen.

Maximum-likelihood analyses and Bayesian inference

PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) implementing the greedy algorithm (Lanfear et al. 2012), divided the concatenated sequences into five subsets and models: TRNEF + I for COI (1st codon), 18S, and H3 (2nd codon) (subset 1); HKY + G for COI (2nd codon) (subset 2); TRN + I + G for 28S, COI (3rd codon), and H3 (1st codon) (subset 3); K80 + G for H3 (3rd codon) (subset 4), and K81UF + G for 16S (subset 5). These models were used for ML analysis in IQ-Tree ver. 2.1.3 (Minh et al. 2020) with nodal support values derived from 1000 ultrafast bootstrap (UFBoot) (Minh et al. 2013). For ML analysis in RAxML ver. 8.2.10 (Stamatakis 2015), GTR + G model was applied to all the five subsets; nodal support values were calculated from 1000 bootstrap pseudoreplicates. BI was performed using models GTR + I (subset 1), HKY + G (subset 2), GTR + I + G (subset 3), K2P + G (subset 4), and GTR + G (subset 5) models, launching two parallel runs with four Markov chains in each run (three cold and one hot) for 5×10^7 generations sampling every 100 generations from the chain. Run convergence was evaluated by the standard deviation of split frequencies (0.000536), estimated sample sizes for all parameters (> 200), and potential scale reduction factors for all parameters

(1.000). Results obtained from the ML analyses and BI were used to visualize the best phylogenetic tree using FigTree ver. 1.4.4 (Rambaut 2018).

Voucher material has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan. Nucleotide sequences newly generated in this study have been registered in GenBank.

Table 5. List of *Oerstedtia* species included in the phylogenetic analysis, GenBank accession numbers for 16S, 18S, 28S rRNA, cytochrome c oxidase subunit I (COI), and histone H3 gene markers, and their references.

Species	GenBank accession numbers					Reference
	16S	18S	28S	COI	H3	
<i>Oerstedtia dorsalis</i> sensu Iwata	—	MZ231200	MZ231290	MZ216522	MZ216593	Chernyshev & Polyakova (2022)
<i>Oerstedtia dorsalis</i> 2 sensu Iwata	—	MZ231201	MZ231291	MZ216523	—	Chernyshev & Polyakova (2022)
<i>Oerstedtia dorsalis</i> Spain	OM423017	OM423141	OM423078	OM456721	OM468170	Chernyshev & Polyakova (2022)
<i>Oerstedtia dorsalis</i> Spain EG35n	FJ855379	—	—	FJ855309	—	Sundberg et al. (2009)
<i>Oerstedtia dorsalis</i> Spain EG36n	FJ855380	—	—	FJ855310	—	Sundberg et al. (2009)
<i>Oerstedtia fuscosparsa</i> sp. nov.	OP256567	OP256566	OP271739	OP265741	OP253976	present study
<i>Oerstedtia oculata</i>	MN211495	MN211398	MN211448	MN205512	MN205469	Chernyshev & Polyakova (2022)
<i>Oerstedtia phoresiae</i>	MN211496	MN211399	MN211449	MN205513	MN205470	Chernyshev & Polyakova (2022)
<i>Oerstedtia polyorbis</i>	MZ231137	MZ231202	MZ231292	MZ216524	MZ216594	Chernyshev & Polyakova (2022)
<i>Oerstedtia</i> sp. Bering 1	OM423024	OM423149	OM423086	OM456730	OM468178	Chernyshev & Polyakova (2022)
<i>Oerstedtia</i> sp. Bering 2	OM423025	OM423150	OM423087	OM456731	OM468179	Chernyshev & Polyakova (2022)
<i>Oerstedtia</i> sp. Crimea	OM423028	OM423153	OM423089	OM456734	—	Chernyshev & Polyakova (2022)
<i>Oerstedtia</i> sp. Guam 3	—	—	—	OM456728	OM468177	Chernyshev & Polyakova (2022)

<i>Oerstedia</i> sp. IceAGE	OM423023	OM423148	OM423085	OM456729	—	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Iona 22	OM423020	OM423144	OM423081	OM456724	OM468173	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Iturup	MZ231138	MZ231203	MZ231293	MZ216525	MZ216595	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. IZ-132743	KF935488	KF935320	KF935376	KF935536	KF935432	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Sakhalin 43	OM423022	OM423146	OM423083	OM456726	OM468175	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Shikotan 3	OM423019	OM423143	OM423080	OM456723	OM468172	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Simushir 22	OM423021	OM423145	OM423082	OM456725	OM468174	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. SJ 64	OM423027	OM423152	—	OM456733	OM468181	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Urup 28	OM423018	OM423142	OM423079	OM456722	OM468171	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Vietnam 35	—	OM423147	OM423084	OM456727	OM468176	Chernyshev & Polyakova (2022)
Outgroup						
<i>Tetranemertes antonina</i>	—	KF935318	KF935374	KF935534	KF935430	Kvist et al. (2014)
<i>Emplectonema mitsuii</i>	—	OM423118	OM423056	OM456699	OM468150	Chernyshev & Polyakova (2022)

Results and systematic remarks

Taxonomy

Oerstedtia fuscosparsa sp. nov. Abato, Yoshida & Kajihara (Figs. 36, 37).

urn:lsid:zoobank.org:act:DB8D3AFD-70F2-4688-BAD7-3B2897CC0C94.

Material examined. Holotype, ICHUM 8405, extracted genomic DNA, histological sections; paratype, ICHU 8406. Extracted genomic DNA.

Diagnosis. An *Oerstedtia* with a brownish cephalic color band, a deep brown post-cerebral color band in the posterior head region, and four orange ocelli. Post-cerebrally, the body is sprinkled with brownish aggregated spots or freckles in pale-yellow background forming transverse bands. Internally, the proboscis nerves are not distinct and accessory lateral nerves are present.

Etymology. The new specific name is a participle of the 1st/2nd declension (-us, -a, -um), a combination of the Latin words *fuscus* (meaning ‘dark’, ‘dim’, ‘black’, ‘brown’) and *sparsus* (‘scattered’, ‘sprinkled’, ‘spotted’, ‘freckled’; from the verb *spargo*, meaning ‘[I] scatter’). In combination, *fuscosparsa* pertains to the brownish spots/freckles scattered throughout the body of the species.

Type locality, distribution, and habitat. At present, the species is only known from its type locality along the Pacific coast of Honshu, off Kouyatsu, Tateyema, Chiba, Japan, between 34°59.293' N, 139°48.59' E and 34°59.094' N, 139°48.195' E, 11–18 m deep, mudstone and sandy bottom; the *in-situ* water temperature was 18–20°C when the holotype and the paratype were collected by Ryuta Yoshida. From this place, *Tetrastemma parallelus* Abato, Yoshida & Kajihara, 2022 has also been collected (Abato et al. 2022).

Sequences. From holotype: see Table 5. From paratype: OP256563 (16S), OP256565 (28S), OP265742 (COI), OP253977 (H3).

Description. *External features of holotype in living state (Fig. 36a, b, e, f).* Body 7.9 mm long when stretched, width 0.6 mm, cylindrical, slender, firm, narrowing at anterior and posterior ends, with pale-yellow background postcerebrally (Fig. 36a, b). Head bluntly round anteriorly in dorsal view, not demarcated from body, whitish opaque in background color, ornamented with a few, small, brownish dots on tip; single, wide, brownish, transverse cephalic color band encircling all around head, with posterior edge mid-dorsally directing backward (Fig. 36e, f); another transverse color band encircling posterior portion of head, deep brown in color, mid-dorsally notched at anterior edge, situated post-cerebrally at short distance from cephalic color band except on mid-ventral portion where these two bands are continuous (Fig. 36e, f); cephalic furrow not notable; with four squarely arranged orange ocelli contained within transverse cephalic color band (Fig. 36e). Behind deep-colored post-cerebral transverse color band, additional 18 transverse bands present throughout body from posterior region of head to tail end, tightly arranged one after another, consisting of brown flecks; single, deep brown spot present on mid-dorsal portion of each of 18 bands (Fig. 36a).

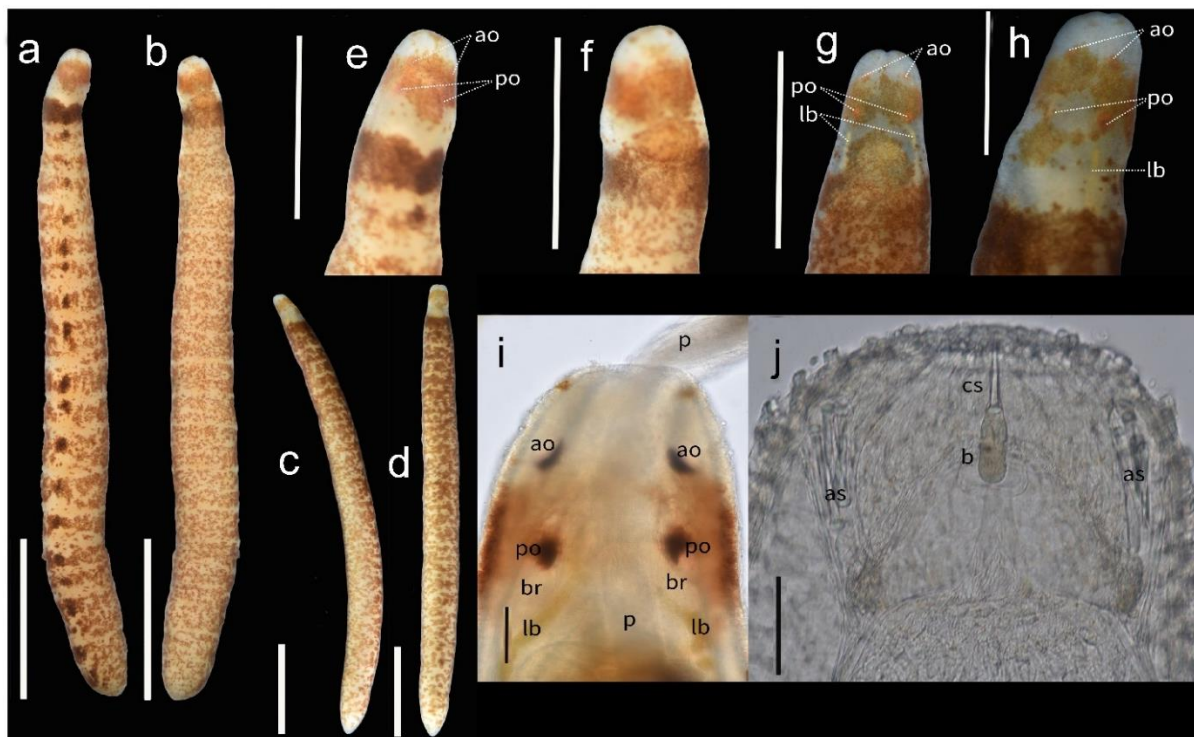


Figure 36. *Oerstedia fuscosparsa* sp. nov., photographs (a–h) and photomicrographs (i, j) taken in anaesthetised state. (a), entire body, dorsal view; (b), entire body, ventral view; (c), entire body, left lateral view; (d), entire body, ventral view; (e, g), magnification of head, dorsal view; (f, h), magnification of head, ventral view; (i), head in squeezed preparation;

(j), stylet apparatus. (a, b, e, f), ICHUM 8405 (holotype); (c, d, g, h–j), ICHUM 8406 (paratype). Abbreviations: ao, anterior ocelli; as, accessory stylet; b, basis; br, brain; cs, central stylet; lb, lateral blood vessel; p, proboscis; po, posterior ocelli. Scale bars: a–d = 2 mm; e–h = 1 mm; i, j = 50 μ m.

External features of paratype in living state (Fig. 36c, d, g, h, i). Body 10 mm in length, 0.6 mm in width; tip of head less pigmented than holotype; posterior end of body narrowed (Fig. 36c, d); cephalic color band light brown; post-cerebral color band lighter than that of holotype, with anterior edge mid-dorsally projected forward but not continuous with cephalic color band on any place (Fig. 36g, h); ocelli more clearly visible with anterior pair smaller than posterior (Fig. 36g, h, i). Body covered with brown patches (Fig. 36c, d), but transverse banding pattern not apparent in posterior body; without mid-dorsal deep brown spots (Fig. 36c).

Internal morphology in paratype. Proboscis nerves not distinct (Fig. 37a). Central stylet 42 μ m in length; basis 36 μ m in length, 11–15 μ m in width, cylindrical in shape, bluntly round posteriorly; two accessory pouches, each containing 5–7 accessory stylets (Fig. 36j). Accessory lateral nerves present (Fig. 37b).

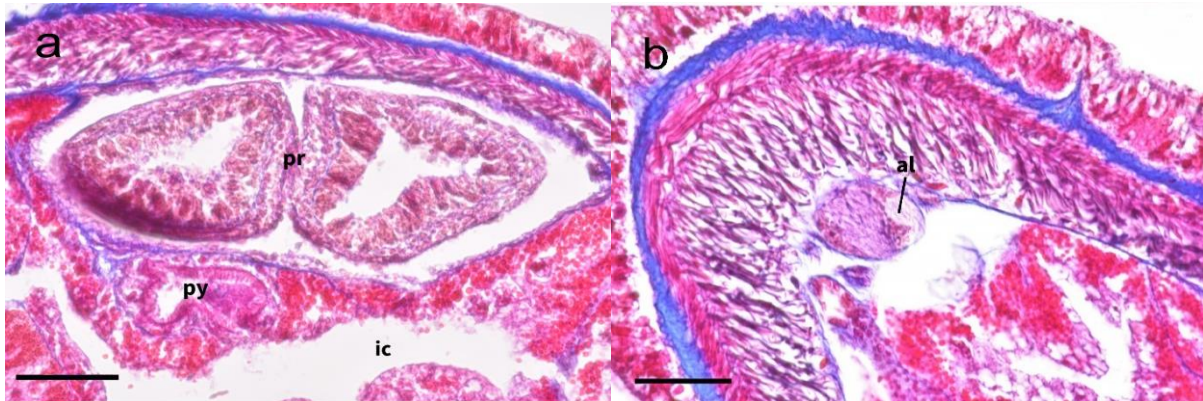


Figure 37. *Oerstedtia fuscosparsa* sp. nov., photomicrographs of transverse histological sections, ICHUM 8405 (holotype). (a), posterior foregut region; (b), intestinal region. Abbreviations: al, accessory lateral nerve; ic, intestinal caecal lumen; pr, proboscis; py, pylorus. Scale bars: a, b, 50 μ m.

Genetic distance and molecular phylogeny

The two specimens of *O. fuscosparsa* sp. nov. reported in this chapter would belong to the same species with uncorrected COI *p*-distance value of 0.004 (0.4%) as to the 658-bp partial COI region, much lower than the 3%-rule COI genetic distance to infer that two ribbon worms are of different species (Sundberg et al. 2016). I therefore regard that the difference in pigmentation pattern observed between the two individuals in this paper is an intraspecific variation commonly observed among other *Oerstedtia* species.

The phylogenetic analysis shows that my species forms a highly supported subclade with *Oerstedtia* species collected from Simushir, *O. dorsalis* sensu Iwata, and *O. phoresiae*. Further, it indicates that *O. fuscosparsa* sp. nov. is sister to *O. phoresiae*, suggesting its placement within the *Paroerstedtiella* clade sensu Chernyshev & Polyakova (2022) of the genus *Oerstedtia* (Fig. 38). This also means that my species is more closely related to *O. phoresiae* than to *O. dorsalis* s.str. and is grouped with other species from the Northwest Pacific. COI *p*-distances between my species and *O. phoresiae* and with *O. dorsalis* s.str. is 8.5% and 11.9%, respectively.

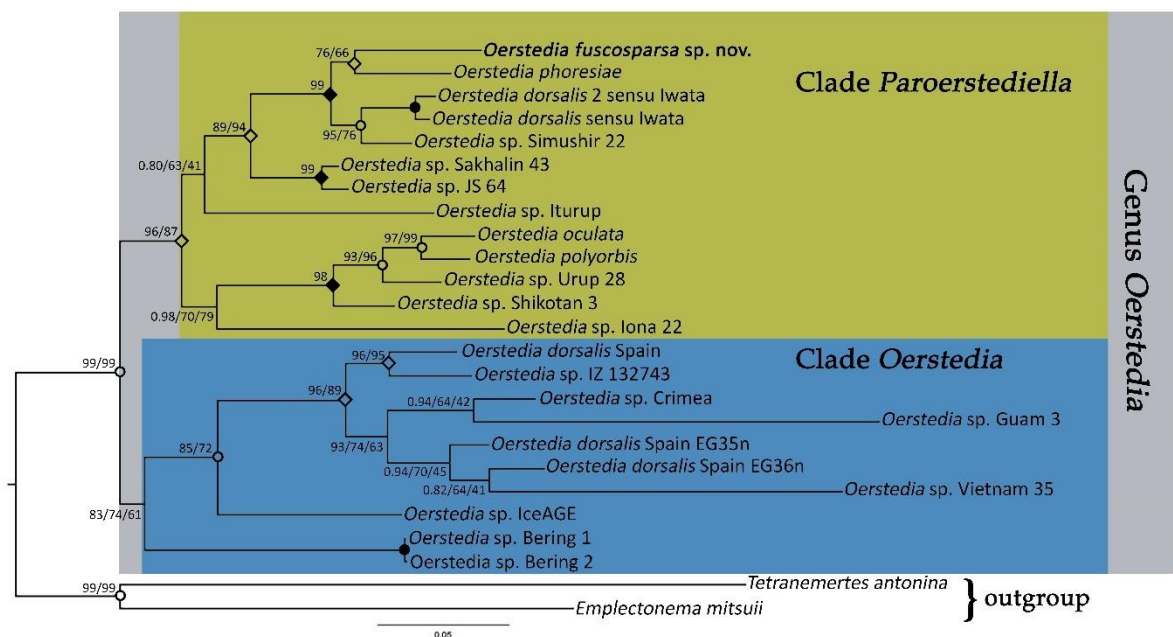


Figure 38. Rooted Bayesian phylogenetic tree showing the position of *Oerstedtia fuscosparsa* sp. nov. within the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Number at each node represents Bayesian posterior probability (PP)/IQTree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Solid circles indicate full support (1.00 PP/100% UFBoot/100% BS); open circles indicate 1.00 PP; solid diamonds mean 1.00 PP/100% UFBoot; open diamonds show 0.99 PP.

Taxonomic remarks. Of the 29 valid species of *Oerstedtia*, two species have previously been known to possess transverse bands in the body; these are *O. oculata* and *Oerstedtia striata* Sundberg, 1988, from both of which *O. fuscosparsa* sp. nov. can be distinguished in external features. *Oerstedtia polyorbis* (Iwata 1954; Chernyshev 1993; Akhmatova et al. 2012) and *O. striata* are banded only dorsally, unlike *O. fuscosparsa* sp. nov. in which the bands are dorsoventrally continuous. In addition, one of the color morphs of *Oerstedtia dorsalis* s.l. (called ‘morph n’) was described to contain reddish-brown stripes/blotches transversally, although details on the continuity of these stripes were not given in Sundberg et al. (2009). However, these two “morph n” specimens are not closely related to the new species as supported by the phylogenetic analysis, with these ‘morph n’ specimens being placed in a different clade (Fig. 38).

DNA-based taxonomy for the members of the genus *Oerstedtia* seems to be indispensable for the reliable establishment of new species since polymorphism and intraspecific variation are predominant among the species in the genus (Bürger 1895; Brunberg 1964; Sundberg 1984, 1988; Envall & Sundberg 1993; Sundberg & Andersson 1995; Zaslavskaya & Chernyshev 2008; Sundberg et al. 2009; Akhmatova et al. 2012). Polymorphism and intraspecific variation in *Oerstedtia* often result in problematic taxonomy; these can hide some cryptic species or mistakenly be used as evidence for species delineation (Sundberg 1998; Sundberg et al. 2009; Akhmatova et al. 2012). The two specimens of *Oerstedtia fuscosparsa* sp. nov. reported in this chapter show distinct pigmentation patterns in the head and body; the holotype has deep brownish cephalic pigmentation, apparent transverse bands in the body, and deep brownish spots mid dorsally while the paratype has light brown cephalic pigmentation, no obvious transverse bands in the posterior body, and no deep brownish spots mid-dorsally.

In traditional *Oerstedtia* taxonomy, these obvious pigmentation differences could have been treated as evidence for different species (Iwata 1954; Kulikova 1987; Chernyshev 1993; Sundberg et al. 2009); however, the COI barcode sequences from the holotype and the paratype strongly suggested that both would belong to the same species despite the differences in external appearance.

Conclusions

Here, I demonstrate, using my species, the necessity and importance of DNA-based taxonomy in the genus *Oerstedia*. The taxonomy of other selected polymorphic congeners of the genus has been verified using barcode sequences of these species (Sundberg & Andersson 1995; Strand & Sundberg 2005; Akhmatova et al. 2012). Utilizing barcode sequence data from these species has resolved inaccurate taxonomic reports established based only on internal and external morphology (Sundberg 1984, 1988; Sundberg & Janson 1988; Sundberg & Andersson 1995; Zaslavskaya & Chernyshev 2008; Akhmatova et al. 2012). With the recent developments in *Oerstedia* taxonomy, it is, therefore, appropriate to investigate polymorphism and intraspecific variation in those already named species using both morphological and barcode sequence data.

Citation: This work has been published in Zootaxa. See and read the published article for use of any information in this chapter.

Abato, J., Yoshida, R., Kajihara, H. 2023. Species description and phylogenetics of *Oerstedia fuscosparsa* sp. nov. (Nemertea: Monostilifera: Oerstedidae) from Japan. *Zootaxa*. 5249(5), 589–597. <https://doi.org/10.11646/zootaxa.5249.5.6>.

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Chapter 3.4. Delimiting the polymorphic congeners of the genus *Oerstedtia* Quatrefages, 1846 (Nemertea, Hoplonemertea), and descriptions of three new species from the Northwest Pacific

Introduction

The monostiliferous hoplonemertean genus *Oerstedtia* Quatrefages, 1846 (Quatrefages 1846), is currently composed of 30 valid ribbon worm species (Norenburg et al. 2023; Abato et al. 2023). As to the external morphology, these worms are characterized by having *i*) a small body, which is usually stout and cylindrical; *ii*) four ocelli, of which the anterior pair are sometimes smaller than the posterior pair; and *iii*) cephalic furrows that are either weakly developed or absent (Envall & Sundberg 1993; Gibson 1994). The internal characteristics include *i*) the presence of accessory lateral nerves and *ii*) the absence of a single mid-dorsal vascular plug, but both are not unique for the genus within Eumonostilifera (Chernyshev 2004; Kajihara 2017). *Oerstedtia* species can be collected from the littoral, sublittoral, and bathyal zones (Iwata 1954; Kulikova 1987; Sundberg & Andersson 1995; Chernyshev & Polyakova 2022; Abato et al. 2023). Some congeners are known to exhibit intraspecific polymorphism in terms of internal and external features (Bürger 1895; Berg 1972; Sundberg 1979; Sundberg 1984; Envall & Sundberg 1993; Sundberg et al. 2009; Akhmatova et al. 2012; Abato et al. 2023). In fact, the two genera *Oerstedtiella* Friedrich, 1935 (Friedrich 1935), and *Paraoerstedtia* Friedrich, 1955 (Friedrich 1955), were synonymized with *Oerstedtia* because the internal characters supposed to define the two were found to vary within a single species (Envall & Sundberg 1993), *Oerstedtia dorsalis* (Abildgaard, 1806) (Abildgaard 1806), the type species of the genus. The polymorphism within the genus (Bürger 1895; Brunberg 1964; Sundberg 1984; Sundberg 1988; Envall & Sundberg 1993; Sundberg & Andersson 1995; Zaslavskaya & Chernyshev 2008; Sundberg et al. 2009; Akhmatova et al. 2012) makes the taxonomy of its members complicated and problematic.

The occurrence of banded and non-banded *Oerstedtia* species in the Northwest Pacific has been reported by several authors. Of these, the banded forms include the nominal species *Oerstedtia fuscosparsa* Abato et al., 2023 (Abato et al. 2023); *Oerstedtia polyorbis* Iwata, 1954 (Iwata 1954); *Oerstedtiella valentinae* Chernyshev, 1993 (Chernyshev 1993); *Oerstedtiella verae* Chernyshev, 1993 (Chernyshev 1993);

Oersteddiella zebra Chernyshev, 1993 (Chernyshev 1993); and the specimen reported as *Oersteddia zebra* by Thollessen & Norenburg (2003). The non-banded forms include the nominal species *Oersteddia venusta* Iwata, 1954 (Iwata 1954); *Oersteddiella oculata* Kulikova, 1987 (Kulikova 1987); *Oersteddiella phoresiae* Kulikova, 1987 (Kulikova 1987); and specimens reported as *Oersteddia dorsalis* by Iwata (1954) and *Oersteddia venusta* by Thollessen & Norenburg (2003). Because of the known polymorphism in the genus, a pioneering species delimitation study on the *Oersteddia* occurring in the Far-Eastern seas of Russia was conducted based on the 28S ribosomal RNA (rRNA) gene, claiming that the nominal species *O. oculata*, *O. zebra*, and the specimens identified by Thollessen & Norenburg (2003) as *O. venusta* and *O. zebra* would be conspecific with *O. polyorbis* (Akhmatova et al. 2012). On the other hand, more recent and comprehensive phylogenetic studies of the genus involving the Pacific and Atlantic congeners—but excluding Thollessen and Norenburg's (- 2003) *O. venusta* and *O. zebra*—showed that *O. oculata* and *O. polyorbis* are different species, despite close similarities in their morphology (Chernyshev & Polyakova 2022; Abato et al. 2023). The non-inclusion of Thollessen and Norenburg's (2003) *Oersteddia* in these recent phylogenetic studies makes the taxonomic identity and the phylogeny of these species within the genus still open for investigation.

Most of the *Oersteddia* species known in the Northwest Pacific were described by Iwata (1954), Kulikova (1987), and Chernyshev (1993), and their distribution is limited in the cold-temperate province (Briggs & Bowen 2012). Only two have so far been reported from the warm-temperate Sino-Japanese province (Briggs & Bowen 2012): *O. fuscosparsa*, established from Honshu, Japan (Abato et al. 2023), and *O. phoresiae*, reported from Jeju Island, South Korea (Akhmatova et al. 2012). Nemertean investigation efforts are therefore needed in the southern areas of the Northwest Pacific. Thus, in this study, members of the genus *Oersteddia* were surveyed in Kanagawa, Honshu, Japan, and Van Phong Bay, Vietnam.

In this chapter, I employ integrative taxonomy to *i*) ascertain the species identity of *O. venusta* and *O. zebra* sensu Thollessen & Norenburg (2003); *ii*) describe new species from Japan and Vietnam; and *iii*) confirm the occurrence of *O. phoresiae* in Japan. Furthermore, I also infer the phylogenetic positions of the new species within the genus using the newly determined 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c

oxidase subunit I (COI), and histone H3 (H3) gene sequences. This taxonomic work highlights the importance of using both traditional and molecular taxonomy in species identification and delimitation of the genus *Oerstedtia*.

Materials and methods

Specimen collection and morphological examination

A total of 13 ribbon-worm individuals were collected *i)* intertidally around Cape Crillon, Aniwa Bay, Sakhalin, Russia (one specimen); *ii)* intertidally among brown algae in Akkeshi Bay, Japan (two specimens), along with the ones used in Thollessen & Norenburg (2003); *iii)* intertidally among brown algae of the family Sargassaceae in Oshoro Bay, Japan (seven specimens); *iv)* by dredging off Misaki, Japan (one specimen); *v)* intertidally among calcareous algae in Manazuru, Japan (one specimen); and *vi)* at a depth of 1 m among calcareous algae off Van Phong Bay, Vietnam (one specimen). The single specimen collected in Aniwa Bay was preserved in 99% ethanol. The two specimens collected in Akkeshi Bay were anesthetized in a MgCl₂ solution and fixed in Bouin's fluid, dehydrated in an ethanol series, cleared in xylene, embedded in paraffin wax, sectioned at 6 µm in thickness, and stained with the Mallory trichrome method. The remaining nine specimens collected in Japan were observed and photographed either alive or in anesthetized condition. For anesthetization, the examined live specimens were placed in a transparent Petri dish, to which a MgCl₂ solution isotonic to seawater was slowly added. Photomicroscopy was mostly done under a Nikon SMZ1500 stereo microscope, with an attached Nikon D5600 digital camera and with external strobe lighting provided by a pair of Morris Hikaru Komachi Di flash units. When successfully extracted, the proboscis of the worms was observed under an Olympus BX51 compound light microscope for the documentation of the stylet apparatus and photographed using the D5600 digital camera. After morphological examination, the worms were preserved in 99% ethanol for genomic DNA extraction. The single specimen collected in Vietnam was cut into two after being photographed; the anterior half was processed in the same manner as for the specimens in Akkeshi Bay.

DNA extraction, PCR amplification, sequencing, species delimitation, and phylogenetic analyses

Nucleotide sequencing was done for 11 of the 13 specimens (the remaining two were fixed in Bouin's fluid for histological sectioning). DNA extraction, PCR amplification, and sequencing followed Abato et al. (2023) except that this study used the primer pairs ar-L/br-H (Palumbi et al. 1991) for 16S amplification and sequencing. BLAST searches (Altschul et al. 1997) on the NCBI website (<http://www.ncbi.nlm.nih.gov/>) were performed to check for possible sequence contamination, to initially verify the genus affiliation of the specimens, and to confirm the identity of the already known *Oerstedtia* species from the collection.

Genetic variation between the 11 specimens was determined by calculating uncorrected pairwise genetic distances (p -distances) based on the 657-bp of COI sequences using MEGA ver. 11 (Tamura et al. 2021). A maximum-likelihood analysis based on the same gene from the specimens that resemble *O. oculata* that were collected from Oshoro Bay (six specimens), Aniwa Bay (one specimen) including the *Oerstedtia* forms (two specimens) in Thollessen & Norenburg (2003), and *O. oculata* (two specimens) from Russia was performed in RAxML ver. 8.2.10 (Stamatakis 2015) using the GTR + G model with branch support values calculated from 1,000 bootstrap pseudoreplicates. The same specimens were subjected to a species delimitation analysis using three methods: Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021), Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), and PTP (Poisson Tree Processes) (Zhang et al. 2013). ASAP was performed in webserver <https://bioinfo.mnhn.fr/abi/public/asap/>, ABGD in <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>, and PTP in the bPTP webserver <https://species.h-its.org/ptp/>—all in default settings. ASAP and ABGD analyses were based on the 657-bp COI sequences, whereas the bPTP analysis used an input tree generated by MrBayes ver. 3.2.6.x64 (parallel version) (Ronquist & Huelsenbeck 2003; Altekar et al. 2004) based on concatenated 16S, 18S, 28S, COI, and H3 genes of selected *Oerstedtia* species.

A TCS haplotype network based on COI was created using PopART (Leigh & Bryant 2015) for visualizing relationships between haplotypes.

Oerstedtia sequences based on the five gene markers used in Abato et al. (2023) and Chernyshev & Polyakova (2022) and those sequences from specimens previously identified as *O. venusta* and *O. zebra* by Tholleson & Norenburg (2003) were downloaded from GenBank (Table 6) and used in the phylogenetic analyses together with the newly generated sequences in the present study. MAFFT ver. 7 online version (Kato et al. 2019) was used for aligning 16S, 18S, and 28S sequences, applying the E-INS-i iterative refinement method in the advanced settings. The COI and H3 sequence datasets were aligned using the MUSCLE algorithm (Edgar 2004) implemented in MEGA ver. 11 (Tamura et al. 2021) in default settings. Uncertainly aligned sites were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al. 2020) resulting in 475-bp 16S, 1,759-bp 18S, 2,880-bp 28S, 657-bp COI, and 330-bp H3 sequences, concatenated in MEGA ver. 11.

The best-fit partitions and substitution models for maximum likelihood (ML) analyses and Bayesian inference (BI) were determined using PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) employing the greedy algorithm (Lanfear et al. 2012) resulting in five subsets and corresponding models: subset 1, 28S, COI (first codon), and H3 (first codon), TRN + I + G; subset 2, COI (second codon), 18S, and H3 (second codon), TRNEF + I; subset 3, COI (second codon), TIM + G; subset 4, H3 (second codon), TVMEF + G; and subset 5, 16S, GTR + G.

ML analyses were executed using IQ-Tree ver. 2.1.3 (Minh et al. 2020) applying the suggested model to each subset with branch support values derived from 1,000 ultrafast bootstrap (UFBoot) (Minh et al. 2013) and RAxML ver. 8.2.10 (Stamatakis 2015) using the GTR + G model to all subsets with branch support values calculated from 1,000 bootstrap pseudoreplicates. BI was performed using MrBayes ver. 3.2.6 x64 (parallel version) (Ronquist & Huelsenbeck, 2003; Altekar et al., 2004) employing models GTR + I + G for subset 1; GTR + I for subset 2; and GTR + G for subsets 3, 4, and 5, launching two parallel runs with four Markov chains in each run (three cold and one hot) for 5×10^7 generations sampling every 100 generations from the chain. Run convergence was assessed by standard deviation of split frequencies (0.000307), estimated sample sizes for all parameters (>5671), and potential scale reduction factors for all parameters (1.000). FigTree ver. 1.4.4 (Rambaut 2014) was used to visualize the trees obtained from the ML and BI analyses.

Voucher materials for the Oshoro and Akkeshi Bay *Oerstedtia* have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan, whereas the specimen collected from Van Phong Bay has been deposited in the Museum of the Institute of Marine Biology (MIMB), National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia. The Aniwa Bay specimen has been deposited in Lomonosov Moscow State University, Moscow, Russia. Nucleotide sequences that were newly generated in this study and used in the phylogenetic analyses have been registered in GenBank.

Table 6. List of *Oerstedtia* species sequenced in the present study and/or included in the phylogenetic analyses, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, catalogue numbers, and their references.

Species	GenBank accession numbers					Voucher	Reference
	16S	18S	28S	COI	H3		
<i>Oerstedtia dorsalis</i> sensu Iwata	–	MZ231200	MZ231290	MZ216522	MZ216593	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia dorsalis</i> 2 sensu Iwata	–	MZ231201	MZ231291	MZ216523	–	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia dorsalis</i> Spain	OM423017	OM423141	OM423078	OM456721	OM468170	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia fuscosparsa</i>	OP256567	OP256566	OP271739	OP265741	OP253976	–	Abato et al. (2023)
<i>Oerstedtia oculata</i> banded PGB	–	–	–	OR794011	–	OzPGB2	present study
<i>Oerstedtia oculata</i> non-banded PGB	MN211495	MN211398	MN211448	MN205512	MN205469	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia phoresiae</i> JI	–	–	–	OR794021	OR805481	OphJj	present study
<i>Oerstedtia phoresiae</i> OB	OR794114	OR794117	OR794118	OR794019	OR805480	ICHUM 8588	present study
<i>Oerstedtia phoresiae</i> PGB	MN211496	MN211399	MN211449	MN205513	MN205470	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia phoresiae</i> PGB2	–	–	–	OR794111	–	OphPGB1	present study
<i>Oerstedtia polyorbis</i>	MZ231137	MZ231202	MZ231292	MZ216524	MZ216594	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia pseudoculata</i> sp. nov. banded AB ^{†,‡}	AJ436802	–	AJ436857	AJ436912	–	–	Tholleson & Norenburg (2003)

<i>Oerstedtia pseudoculata</i> sp. nov. banded OB1 [†]	OR806648	OR806647	OR806656	OR800639	OR805479	ICHUM 8582	present study
<i>Oerstedtia pseudoculata</i> sp. nov. banded OB2	–	–	–	OR800637	–	ICHUM 8583	present study
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded AB ^{†,§}	AJ436801	–	AJ436856	AJ436911	–	–	Thollesson & Norenburg (2003)
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded AnB [†]		–	–	OR800642	–	Kr-Ann077	present study
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded OB1 [†]	OR806645	OR806646	OR806653	OR800640	OR805478	ICHUM 8587	present study
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded OB2	–	–	–	OR800638	–	ICHUM 8586	present study
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded OB3	–	–	–	OR800643	–	ICHUM 8584	present study
<i>Oerstedtia pseudoculata</i> sp. nov. non-banded OB4	–	–	–	OR800644	–	ICHUM 8585	present study
<i>Oerstedtia rugosa</i> sp. nov. Japan	OR806644	OR806649	OR806655	OR800641	OR805482	ICHUM 8590	present study
<i>Oerstedtia rugosa</i> sp. nov. Vietnam [¶]	–	OM423147	OM423084	OM456727	OM468176	MIMB 4718222	Chernyshev & Polyakova (2022)
<i>Oerstedtia viridifusca</i> sp. nov.	OR806643	OR806650	OR806654	OR800645	OR805483	ICHUM 8589	present study
<i>Oerstedtia</i> sp. Bering 1	OM423024	OM423149	OM423086	OM456730	OM468178	–	Chernyshev & Polyakova (2022)
<i>Oerstedtia</i> sp. Bering 2	OM423025	OM423150	OM423087	OM456731	OM468179	–	Chernyshev & Polyakova (2022)

<i>Oerstedia</i> sp. Crimea	OM423028	OM423153	OM423089	OM456734	–	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Guam 3	–	–	–	OM456728	OM468177	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. IceAGE	OM423023	OM423148	OM423085	OM456729	–	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Iona 22	OM423020	OM423144	OM423081	OM456724	OM468173	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Iturup	MZ231138	MZ231203	MZ231293	MZ216525	MZ216595	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. IZ-132743	KF935488	KF935320	KF935376	KF935536	KF935432	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Sakhalin 43	OM423022	OM423146	OM423083	OM456726	OM468175	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Shikotan 3	OM423019	OM423143	OM423080	OM456723	OM468172	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Simushir 22	OM423021	OM423145	OM423082	OM456725	OM468174	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. SJ 64	OM423027	OM423152	–	OM456733	OM468181	–	Chernyshev & Polyakova (2022)
<i>Oerstedia</i> sp. Urup 28	OM423018	OM423142	OM423079	OM456722	OM468171	–	Chernyshev & Polyakova (2022)
Outgroup							
<i>Tetranemertes antonina</i>	–	KF935318	KF935374	KF935534	KF935430	–	Kvist et al. (2014)
<i>Emplectonema mitsuii</i>	–	OM423118	OM423056	OM456699	OM468150	–	Chernyshev & Polyakova (2022)

*AB, Akkeshi Bay; AnB, Aniwa Bay; JI, Jeju Island; OB, Oshoro Bay; PGB, Peter the Great Bay.

†Formerly *O. zebra* (Thollessen & Norenburg 2003).

‡*Oerstedtia pseudoculata* sp. nov. specimens included in the phylogenetic analyses.

§Formerly *O. venusta* (Thollessen & Norenburg 2003).

¶Formerly *Oerstedtia* sp. Vietnam 35 (Chernyshev & Polyakova 2022).

Results and systematic remarks

The 13 *Oerstedtia* specimens reported in this paper are composed of four species, of which three are new to science and the remaining one is already known and herein recorded for the first time in Japan. The new species are described below using an integrative approach, whereas their phylogenetic positions within the genus *Oerstedtia* are inferred. This study updates the number and name of valid *Oerstedtia* species reported from Japanese waters by establishing new species, fixing the identity of the species previously reported with uncertain taxonomic identification, and by reporting a new locality of an already known *Oerstedtia* species.

Taxonomy

Class Hoplonemertea Coe, 1905

Family Oerstedtiidae Chernyshev, 1993

Genus *Oerstedtia* Quatrefages, 1864

***Oerstedtia pseudoculata* sp. nov.** Abato, Chernyshev, Hookabe, Tsuyuki, Kaushik & Kajihara (Fig. 39a–n).

urn:lsid:zoobank.org:act:1C729CE2-82C2-4D16-A915-FEDBBA8EA190.

Oerstedtia polyorbis – Kajihara (2017): fig. 16.5n; Kajihara (2021): fig. 2h, i.

Oerstedtia venusta – Thollessen & Norenburg (2003); Kajihara (2017): fig. 16.5o.

Oerstedtia zebra – Thollessen & Norenburg (2003).

Material examined. Material examined. Holotype, ICHUM 8587, non-banded form (Fig. 26a–h), extracted total DNA, collected by Jamael Abato and Hiroshi Kajihara among the brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo in Oshoro Bay (between 43.2093°N, 140.8585°E and 43.2095°N, 140.8582°E), Hokkaido, Japan, on August 30, 2022. Paratypes, extracted total

DNA from six individuals and serial transverse sections representing two specimens: ICHUM 8586, non-banded form, DNA, collection data same as the holotype; ICHUM 8582 (Fig. 39j–n), 8583, banded forms, DNA, collected by Gauri Kaushik and Hiroshi Kajihara from the topotype on July 7, 2023; ICHUM 8584 (Fig. 39i), 8585, non-banded forms, DNA, collected by Gauri Kaushik and Hiroshi Kajihara from the topotype on 27/04/2023; ICHUM 3287, serial transverse sections, 6 µm, eight slides, non-banded form [originally labelled '*Oerstedtia venusta*'], intertidal, among brown algae, Aininkappu (43.0038°N, 144.8575°E), Akkeshi, Hokkaido, Japan, collected by Svetlana A. Maslakova, Megan Schwartz, Jon L. Norenburg, and Hiroshi Kajihara on July 25, 1999; ICHUM 3288, serial transverse sections, 6 µm, seven slides, banded form [originally labelled '*Oerstedtia zebra*'], collection data same as ICHUM 3287; Kr-Ann077, non-banded form, DNA, collected intertidally by Irina Ekimova and Darya Grishina around Cape Crillon, Aniwa Bay, Sakhalin, Russia, on August 7, 2023.

Diagnosis. An *Oerstedtia* species with a brownish dorsal body pigmentation made up of minute brownish dots or with dorsolateral black transverse bands on a translucent body; anterolateral edges of the head non-pigmented; with bright-orange ocelli visible at the dorsal and ventral sides of the head; and a proboscis pore that opens ventrally near the tip of the head.

Etymology. The new specific name is an adjective (-us, -a, -um), a combination of the Latin words pseudo (meaning 'falsely', 'resembling', or 'deceived') and oculatus (meaning 'adorned with eyes', 'having eyes' or 'eye-shaped'). In combination, pseudoculata pertains to the resemblance of the new species with another already established congener of the genus, *O. oculata*. The specific name directly translates as the new species "not being the true *O. oculata*".

Type locality, distribution, and habitat. The type locality of the species is in Oshoro Bay, Hokkaido, Japan. The species is also distributed in Akkeshi Bay, Hokkaido, and around Cape Crillon, Aniwa Bay, Sakhalin, Russia. The species was collected among the brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo in Oshoro Bay.

Sequences. Sequences from *O. pseudoculata* sp. nov. were deposited in GenBank with accession numbers shown in Table 6.

Description. *External features.* Body cylindrical, widest at middle portion; living worm highly contractile; holotype (ICHUM 8587) significantly shortened when contracted, 1.3 mm in length, 0.45 mm in width (Fig. 39a, b); when anesthetized, holotype 5.6 mm in length, 0.97 mm in width (Fig. 39c, d); paratypes 3.0–4.7 mm in length, 0.5–0.73 mm in width (n = 5, ICHUM 8282, 8283, 8584–8586). Dorsal body either banded or non-banded (Fig. 39a, c, e, j, l). In non-banded form (ICHUM 8584–8587), dorsal surface uniformly dotted with numerous, fine, brownish dots (Fig. 39a, c, e); posterior end opaque white; head similarly dotted with fine, brownish granules except for two, non-pigmented white spots at anterolateral sides, showing an impression of big, whitish, false eyes (Fig. 39a, c, e); ventrally, body less pigmented, lighter than the dorsal, reflects brown-orange hue. In banded form (ICHUM 8582, 8583), background body color translucent; 12–14 dorsolateral, vaguely bordered, dark-brown, transverse bands of varying thickness present; ventral body translucent with a pale-yellow hue (Fig. 39k, m); head dorsally with single, thick, well bordered, dark-brown transverse band, ventrally less pigmented. Ocelli four in number, bright-orange in color, arranged in square. Anterior cephalic furrows short, situated ventrolaterally, slightly notable in the non-banded form (Fig. 39d, f) but not visible in the banded form. Posterior cephalic furrow encircling head (Fig. 39a–f) (furrow positioned on the translucent collar of the banded form) (Fig. 39j–m). Rhynchocoel extending for entire body length (Fig. 39g). Proboscis pore opening at tip of head (Fig. 39b, d, f, k, m).

Stylet apparatus. Central stylet 26 μ m in length in holotype (21 μ m and 27 μ m in paratypes ICHUM 8586 and 8283, respectively; same in the following); basis cylindrical and narrowing at both ends, uniformly light colored, 34 μ m in length (31 μ m and 29 μ m), 13 μ m in width (11 μ m and 11 μ m); stylet to basis length ratio 0.75 (0.68 and 0.91); two accessory pouches each with four to five accessory stylets (5–7 and 4) (Fig. 39h, n). One paratype female specimen (ICHUM 8584) had a strikingly different stylet apparatus morphology: central stylet 35 μ m in length; basis triangular, uniformly light colored, 51 μ m in length, 43 μ m in maximum width at its posterior portion; stylet

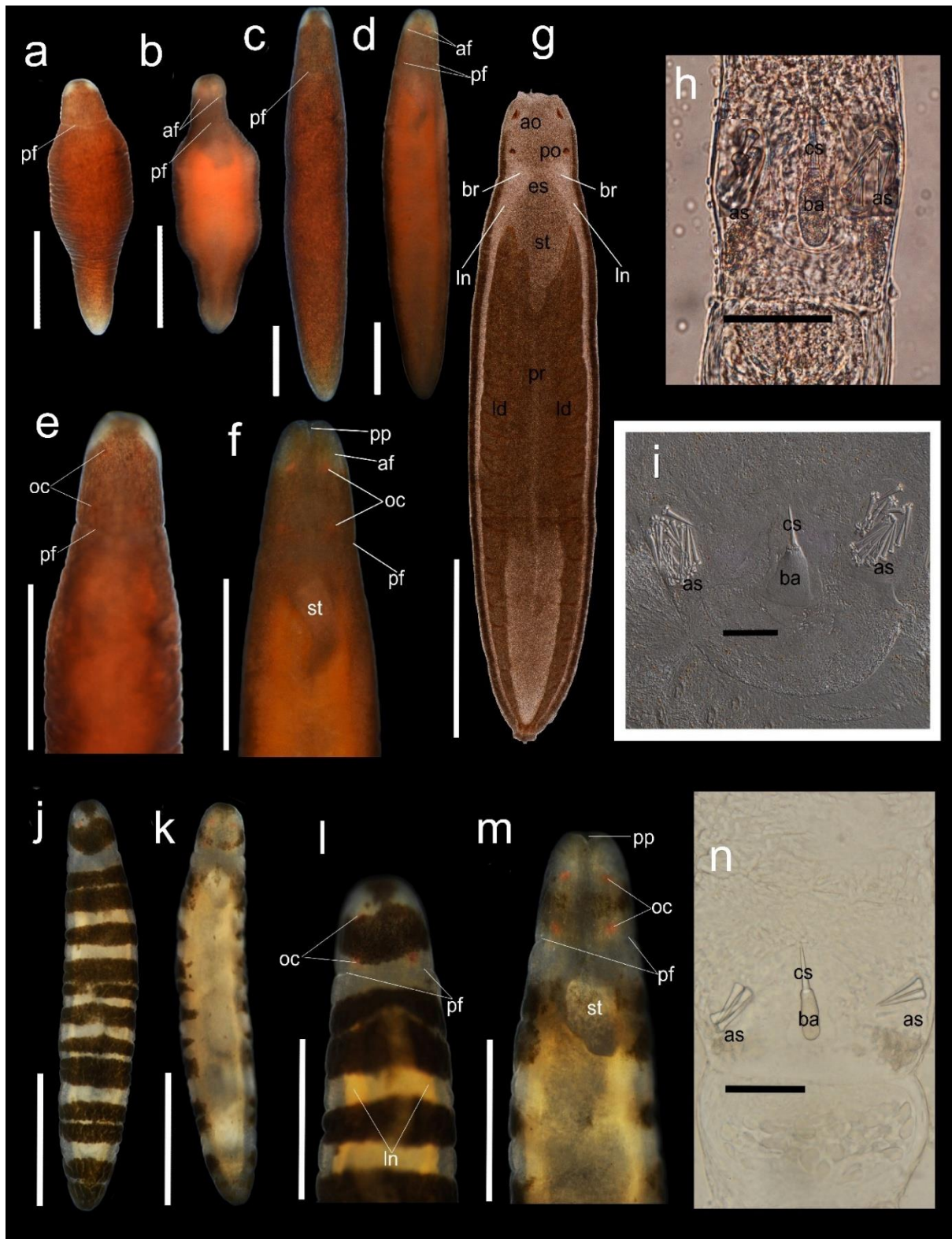


Figure 39. *Oerstedtia pseudoculata* sp. nov., photographs and photomicrographs of the non-banded form, holotype, ICHUM 8587 (a–h); paratype, ICHUM 8584 (i); and the banded form, paratype, ICHUM 8582 (j–n); taken in living and anesthetized states. (a, j), entire body at dorsal view and (b, k), ventral view in non-anesthetized condition; (c), entire body at dorsal view and (d), ventral view in anesthetized condition; (e, l), magnification of the head at dorsal view and (f, m), ventral view in anesthetized state; (g), squeezed specimen at dorsal view; (h, l, n), stylet apparatus. Abbreviations: af, anterior cephalic

furrow; ao, anterior ocelli; as, accessory stylet; ba, stylet basis; br, brain; cs, central stylet; es, esophagus; id, intestinal diverticula; ln, lateral nerves; oc, ocelli; pf, posterior cephalic furrow; po, posterior ocelli; pp, proboscis pore; pr, proboscis; st, stomach. Scale bars: a, b, e, f, l, m = 0.5 mm; c, d, g, j, k = 1 mm; h, i, n = 50 μ m.

to basis length ratio 0.69; two accessory stylet pouches present with 15–18 accessory stylets, base of the stylets remarkably cleaved (Fig. 39i).

Histological features. In paratypes (ICHUM 3287, 3288), proboscis with 10 nerves. Cephalic glands consisting of basophilic lobules, extending posteriorly to reach cerebral ring. Foregut with esophagus, stomach, and pylorus; stomach posteriorly turns dorsally to lead to pylorus; as pylorus slightly extends anteriorly beyond its ventral junction to stomach, there is a short pyloric cecum above stomach (Fig. 40a); intestinal cecum (Fig. 40c) anteriorly extends to reach posterior end of stomach, where it forks into two short dorsolateral pouches (Fig. 40b), not reaching brain. Lateral nerve cords with accessory nerves extending backward to intestinal region (Fig. 40a–c).

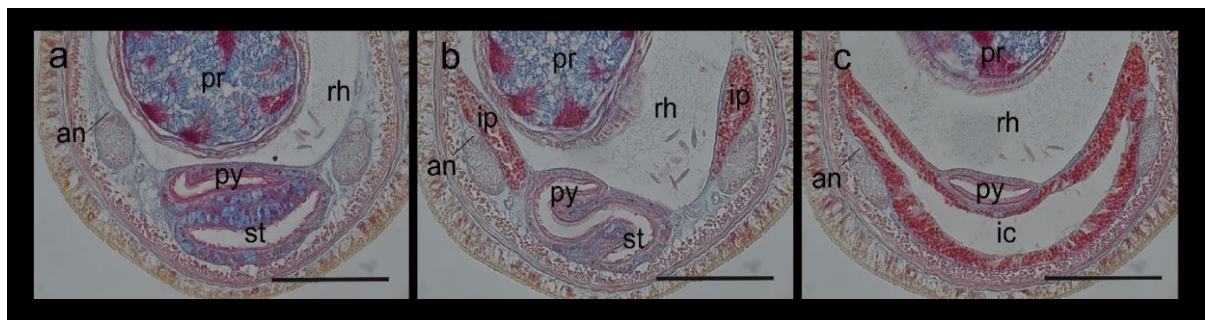


Figure 40. *Oerstedtia pseudoculata* sp. nov., banded form, paratype, ICHUM 3288, photomicrographs of transverse histological sections through the foregut region showing the (a), pyloric cecum above the stomach; (b), the posterior foregut region at the stomach–pylorus junction; and (c), the pyloric region. Abbreviations: an, accessory nerve; ic, intestinal cecum; ip, intestinal-ecum anterior dorsolateral pouch; pr, proboscis; py, pylorus; rh, rhynchocoel, st, stomach. Scale bars: a–c = 100 μ m.

***Oerstedtia rugosa* sp. nov.** Abato, Chernyshev, Hookabe, Tsuyuki, Kaushik & Kajihara (Fig. 41a–h).

urn:lsid:zoobank.org:act:36760C35-3A53-433D-BF09-1C0A3E5520E9.

Oerstedtia sp. Vietnam 35 – Chernyshev & Polyakova (2022).

Material examined. Holotype, ICHUM 8590 (Fig. 41a–d), total DNA extracted from the 99% ethanol-preserved specimen; dredged at a depth of 38.1 m off Sagami Bay (35.1514°N, 139.6050° E), Misaki, Kanagawa, Japan, on June 10, 2022; collected by Natsumi Hookabe. Paratype, MIMB 4718222 (Fig. 41e–h), total DNA extracted from the posterior body preserved in 99% ethanol; anterior body preserved in Bouin’s fluid then in 70% ethanol, sectioned for histology, four slides with transverse sections; collected at a depth of 1 m by Alexei V. Chernyshev, among red calcareous algae, off Van Phong Bay (2.6525°N, 109.3188°E), South China Sea, Vietnam, on May 22, 2010.

Diagnosis. An *Oerstedtia* with carmine or deep-red to brownish-red body, decorated with vaguely bordered, transverse bands composed of white dots, and with prominent longitudinal and intertwined, white lines or wrinkles running from the head to the posterior end of the body; internally, the intestinal cecum has no anterior pouches and with short dorsolateral pouches.

Etymology. The new specific name is an adjective (-us, -a, -um), meaning ‘wrinkled’ in Latin. The specific name *rugosa* pertains to the longitudinal lines or wrinkles on the body of the species giving it a rough surface.

Type locality, distribution, and habitat. The type locality of the species is in Sagami Bay, Misaki, Kanagawa, Japan. It is also distributed in Van Phong Bay, Vietnam. The species in Vietnam was collected among red calcareous algae.

Sequences. Sequences from *O. rugosa* sp. nov. were deposited in GenBank with accession numbers shown in Table 6.

Description. *External features.* Holotype. Living specimen contractile (Fig. 41a–d), 14.4–46.9 mm in length, 2.1 mm in width, tapering at both ends when stretched (tapering sometimes lost when worm contracts) (Fig. 41c, d), firmly cylindrical in cross section, carmine or deep-red in color except white anterior end, with longitudinal, intertwined, white lines appearing as wrinkles, running from posterior head on all sides; entire body sparsely and irregularly decorated with white dots; fuzzily bordered, white, transverse

bands (composed of unevenly sized and dispersed white dots) arranged at irregular interval along body; anterior-most band prominent, thicker than the rest, mid-dorsally and mid-ventrally bisected by a thin deep-red pigmentation (Fig. 41a–d); succeeding bands vague, less thick, more obvious on lateral sides (Fig. 41a–d) and sometimes discontinuous on dorsal surface; number of recognizable transverse bands 17–23, depending on the degree of body contraction (Fig. 41a–d). Head not demarcated from succeeding body; no ocelli observed; cephalic furrow not visible (Fig. 41a–d); stylet apparatus not observed.

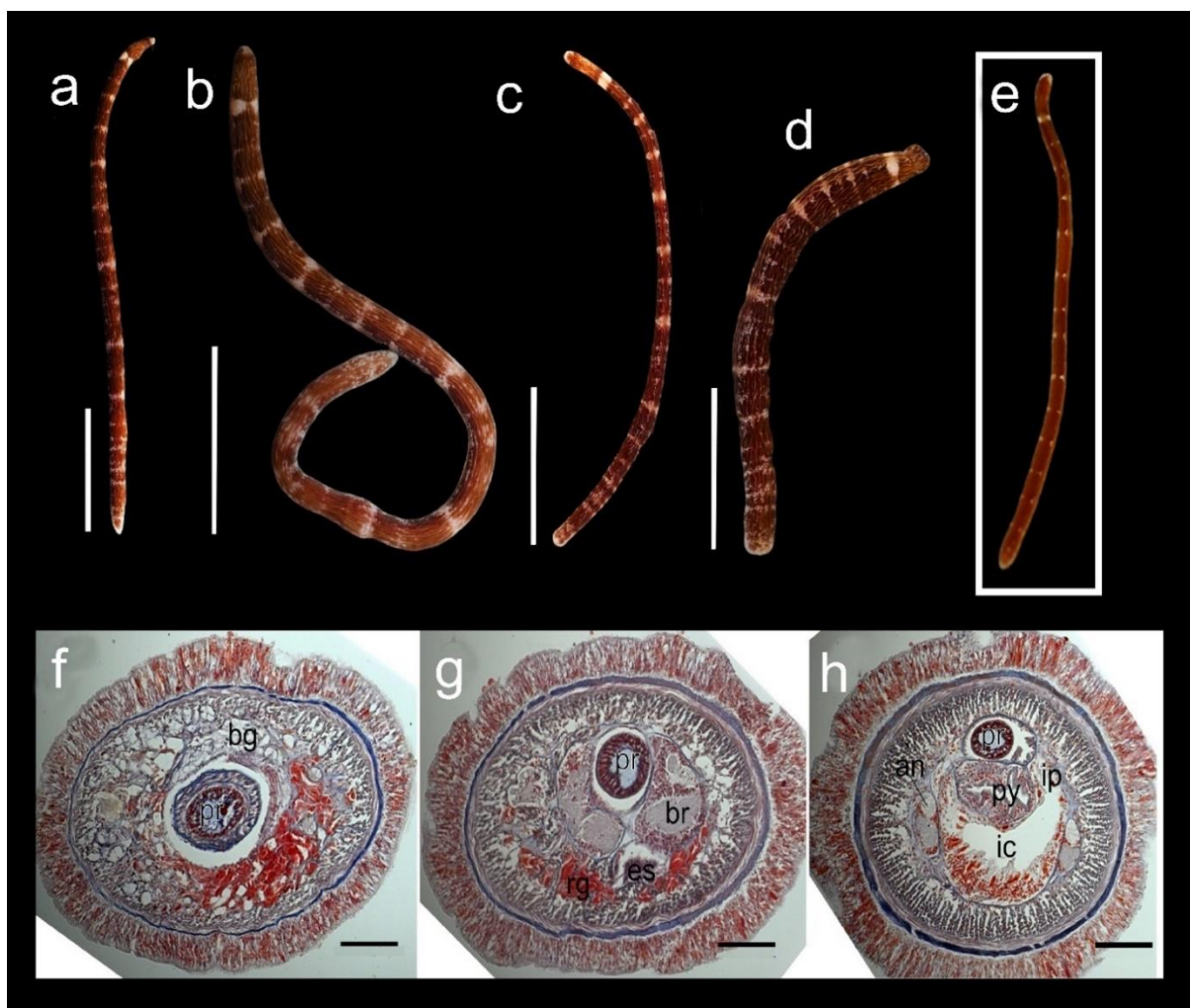


Figure 41. *Oerstedtia rugosa* sp. nov., photographs of the entire worm at living state, (a–d), holotype, ICHUM 8590; (e), paratype, MIMB 4718222; and (f–h), photomicrographs of the transverse histological sections, paratype, MIMB 4718222. (a, b, e), entire body at dorsal (c), ventral, and (d), right lateral view. Transverse histological section at (f), precerebral region; (g), brain region; and (h), anterior pylorus region. Abbreviations: an, accessory nerve; bg, basophilic glands; br, brain; es, esophagus; ic, intestinal cecum; ip, intestinal caecum pouches; py, pylorus; rg, red-stained glands. Scale bars: a = 10 mm; b = 5 mm; c, d = 2.5 mm; f–h = 100 μ m.

Paratype. Body cylindrical, 14 mm in length, with narrow head; body color reddish-brown; with 15 transverse white rings or bands discontinued dorsally and laterally; very small light longitudinal wrinkles and white dots rarely present; anterior tip of head white (Fig. 41e).

Histological features (paratype). Proboscis with 10 nerves and claviform papillae; central stylet around 48 μm in length, basis oval, 50 μm in length and 20 μm in width. Cephalic glands not extending behind brain, including basophilic (colorless) lobules, and redstained glands located ventrally (Fig. 41f, g). Foregut with esophagus, stomach, and narrow pylorus; intestinal cecum without anterior pouches and with short dorsolateral pouches (Fig. 41h); intestine with narrow and unbranched dorsolateral pouches. Lateral nerve cords with large (one and a half to two times less than the main neuropil) (Fig. 41h) and long accessory nerve (at least 1/2 of the body length). The posterior half of the body was not sectioned.

***Oerstedtia viridifusca* sp. nov.** Abato, Chernyshev, Hookabe, Tsuyuki, Kaushik & Kajihara (Fig. 42a–f).

urn:lsid:zoobank.org:act:35279167-5BA1-42DD-A756-5469F4306947.

Material examined. *Holotype.* ICHUM 8589 (Fig. 42a–f), female, total DNA extracted from a single specimen collected intertidally by Aoi Tsuyuki, among calcareous algae in a large tide pool on a rocky shore in Manazuru (35.1418°N, 139.1621°E), Kanagawa, Japan, on April 22, 2023.

Diagnosis. An *Oerstedtia* with greenish-brown freckles on the entire body except for anterolateral edges of the head and a collar-like portion along the posterior cephalic furrow; freckles are denser on the anterior dorsal surface and sparser on the posterior three-fourths of the ventral surface; ocelli brownish-orange.

Etymology. The new specific name is an adjective (-us, -a, -um), a combination of the Latin words *viridis* (meaning ‘green’) and *fuscus* (meaning ‘brown’). In combination, *viridifusca* pertains to the greenish-brown body pigmentation/freckles of the species.

Type locality, distribution, and habitat. The species is currently known only from its type locality—Manazuru, Kanagawa, Japan, among among calcareous algae.

Sequences. Sequences from *O. viridifusca* sp. nov. were deposited in GenBank with accession numbers shown in Table 6.

Description. *External and internal features.* Holotype. Non-anesthetized worm highly contractile, 4.3 mm in length (Fig. 42a); anesthetized body 4.9 mm in length, 0.86 mm in width, cross-sectionally cylindrical in shape, entirely covered with greenish-brown freckles except for two anterolateral edges of head and the collar along posterior cephalic furrow; pigmentation more intense dorsally on anterior body near head and on lateral sides (Fig. 42a, b, d); ventral body less freckled, greenish-brown freckles concentrated only laterally and on anterior one-fourths of ventral body, leaving entire ventral surface lighter than dorsal (Fig. 42c, e); intestinal diverticula and gonads visible except at anterior body before head, (Fig. 42c, e); rhynchocoel running for entire body length (Fig. 42f); head narrower than, but not demarcated from, succeeding body; ocelli prominent at dorsal view, barely visible at ventral view, five in number, brownish-orange in color, and nearly equally sized, with two anterior ones situated more medially than two posterior ones, and additional one (probably representing an abnormal ocellus) situated between anterior pair (Fig. 42a, b, d, f); pair of very short, laterally located, anterior cephalic furrows slightly visible; posterior cephalic furrow encircles the body, visible at the collar; proboscis pore evident at ventral side on tip of head (Fig. 42c, e); stylet apparatus not observed.

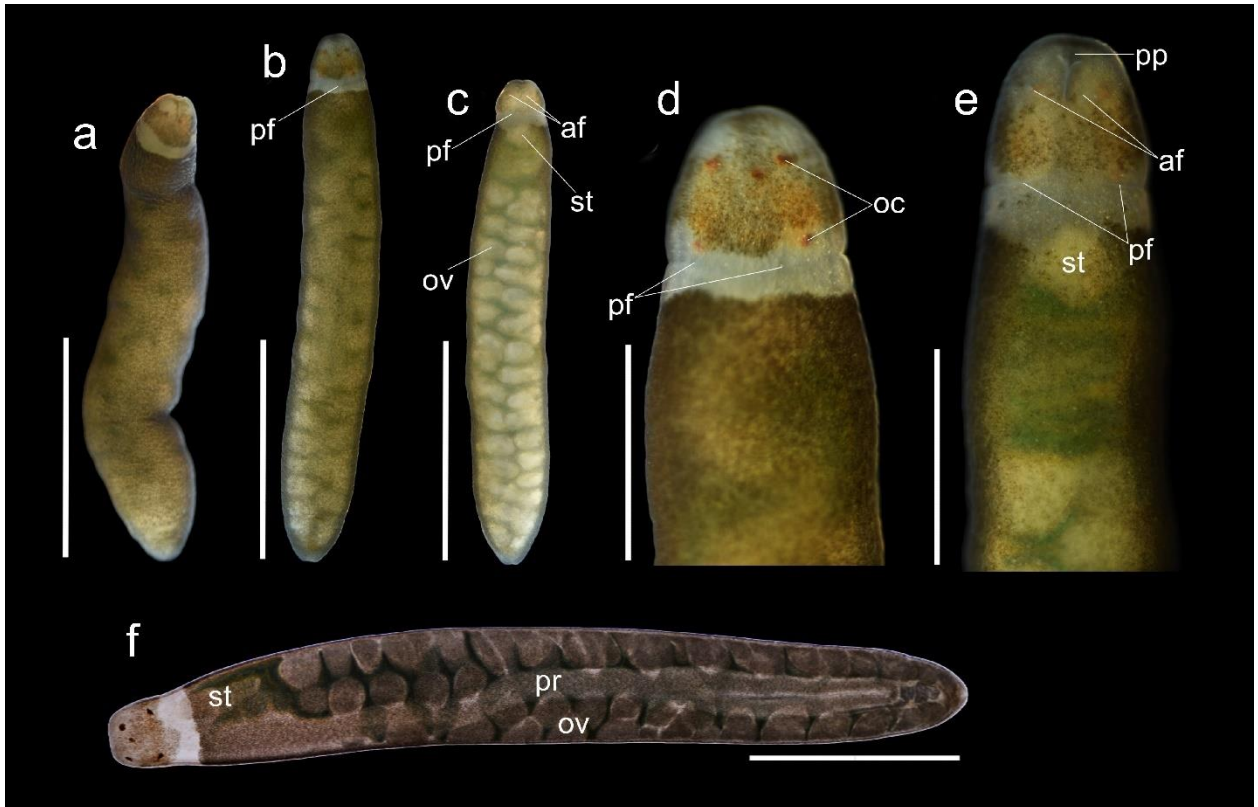


Figure 42. *Oerstedea viridifusca* sp. nov., holotype, ICHUM 8589, photographs of the entire worm in (a), non-anesthetized state at dorsal view; (b–e), anesthetized state, entire animal at (b), dorsal and (c), ventral views, and magnification of the head at (d), dorsal and (e), ventral views; and (f), photomicrograph of the specimen compressed under a coverslip. Abbreviations: af, anterior cephalic furrow; oc, ocelli; ov, ovary; pf, posterior cephalic furrow; pp, proboscis pore; pr, proboscis; st, stomach. Scale bars: a–c = 2 mm; d–f = 1 mm.

Oerstedea phoresiae (Kulikova, 1987) (Fig. 43a–c).

Oerstedella phoresiae Kulikova, 1987 (Kulikova 1987).

Oerstedea phoresiae—Akhmatova et al. (2012).

Material examined. ICHUM 8588 (Fig. 43a–c), total DNA extracted from a single specimen collected by Gauri Kaushik and Hiroshi Kajihara from brown algae in the family Sargassaceae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo in Oshoro Bay (between 43.2093°N, 140.8585°E and 43.2095°N, 140.8582°E), Hokkaido, Japan, on April 27, 2023.

Type locality, distribution, and habitat. The type locality of this species is in Avangard Bight in Vostok Bay, Russia. It is also distributed in Peter the Great Bay, Russia, and Jeju Island, South Korea. A new locality, Oshoro Bay, Hokkaido, Japan, is herein reported. The species in the new locality was collected from brown algae, putatively *Sargassum horneri* (Turner) C. Agardh and/or *Sargassum miyabei* Yendo.

Sequences. Sequences from *O. phoresiae* were deposited in GenBank with accession numbers shown in Table 6.

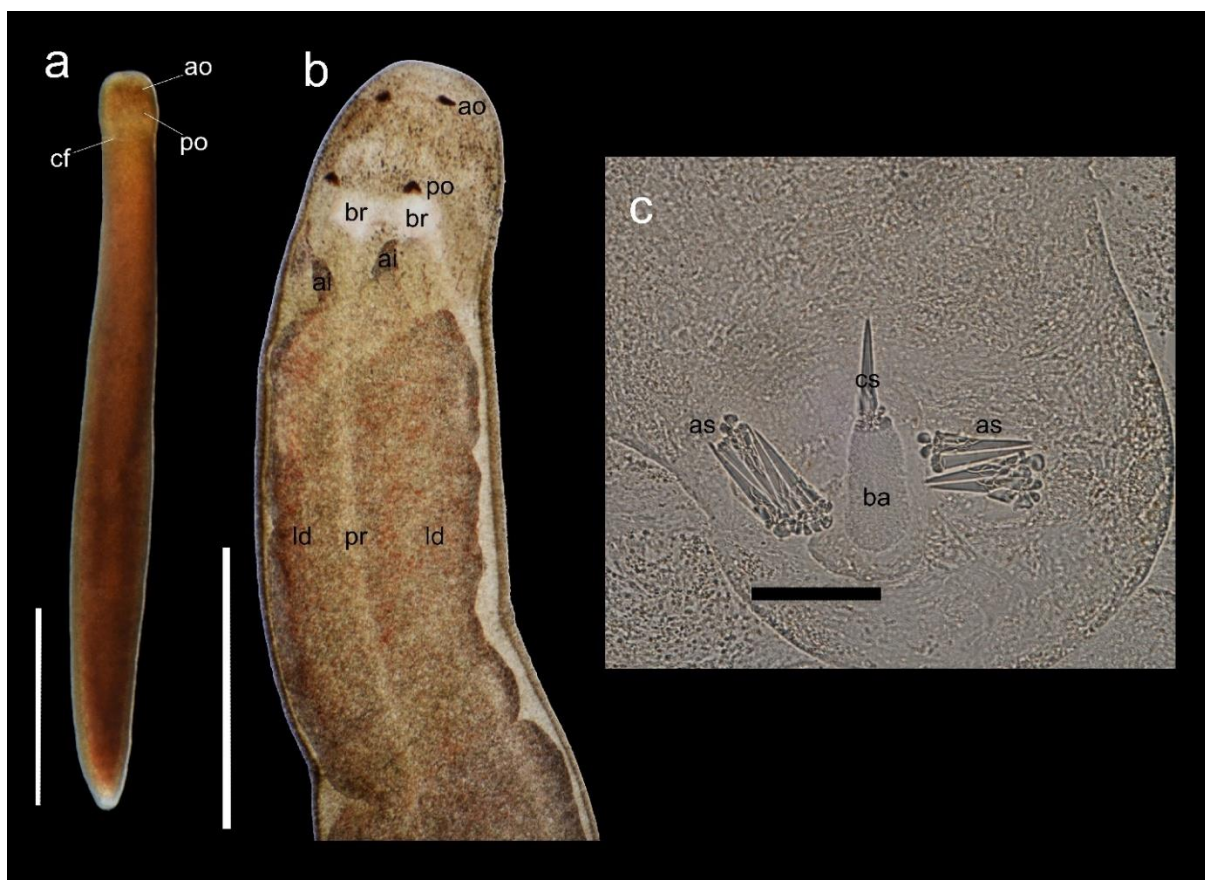


Figure 43. *Oerstedtia phoresiae* (Kulikova, 1987), non-type specimen, ICHUM 8588, (a), photograph of the entire specimen at dorsal view in an anesthetized state; (b), photomicrographs of the compressed specimen showing the anterior half of the dorsal body and (c), its stylet apparatus. Abbreviation: ai, anterior pouch of the intestinal cecum; ao, anterior ocellus; as, accessory stylets; ba, stylet basis; br, brain; cf, cephalic furrow; cs, central stylet; id, intestinal diverticulum; po, posterior ocellus; pr, proboscis. Scale bars: a, b = 1 mm; c = 50 μ m.

Description. *External and internal features.* Body in anesthetized state 4.0 mm in length, widest at middle portion, 0.37 mm in width, cross-sectionally cylindrical in shape, dorsally entirely covered with fine, brownish dots; head nearly square at dorsal view, wider than anterior body, 0.28 mm in width (Fig. 43a); ocelli four in number, brownish in color, squarely arranged, poorly visible at dorsal view but prominent in a squeezed specimen (Fig. 43a, b); posterior cephalic furrow dorsally continuous; ventral surface of head not examined in detail; rhynchocoel not apparent from dorsal body (Fig. 43a) but barely visible in a squeezed specimen (Fig. 43b). Central stylet 39 μm in length; basis cylindrical, narrows at both ends, lightly colored, 42 μm in length, 20 μm in width; stylet to basis length ratio 0.93; two accessory stylet pouches, each with four and six accessory stylets; stylet base cleaved (Fig. 43c).

Genetic distance, species delimitation, haplotype network, and phylogenetics analyses

The result of the uncorrected pairwise COI genetic distance analysis between the species collected in the present study is presented in Table 7. *Oerstedtia oculata* (banded and non-banded forms) from Peter the Great Bay and *O. phoresiae* both from Peter the Great Bay and Jeju Island, *O. venusta* and *O. zebra* sensu Thollessen & Norenburg (2003) from Akkeshi Bay, and *O. polyorbis* from Kuril Island are also included in the genetic distance analysis. The 11 *Oerstedtia* specimens collected and sequenced in the present study are suggested to comprise four species based on the *p*-distance analysis ($\geq 3\%$): i) *Oerstedtia phoresiae*, ii) *Oerstedtia pseudoculata* sp. nov., iii) *Oerstedtia rugosa* sp. nov., and iv) *Oerstedtia viridifusca* sp. nov. The specimen identified as *O. phoresiae* reported from Oshoro Bay and that of specimens from Peter the Great Bay and Jeju Island are suggested to be conspecific (0.49%–0.76% *p*-distance). The remaining six (i.e., two banded and four non-banded) specimens from Oshoro Bay, the two *Oerstedtia* forms from Akkeshi Bay in Thollessen & Norenburg (2003), and a single specimen obtained from Sakhalin Island are all suggested to be conspecific (0%–0.76% *p*-distance) and different from the two *O. oculata* specimens from Peter the Great Bay (*p*-distance of 4.6%–4.8%). The maximum-likelihood analysis based on the partial COI sequences from these specimens (with *O. phoresiae* as outgroup) shows that the former nine specimens forms

Table 7. Uncorrected pairwise genetic distances (*p*-distances) (%) between the *Oerstedtia* species collected in the present study and with some previously described species using the 657-bp cytochrome c oxidase subunit I (COI) barcode sequence. Individuals with *p*-distance $\geq 3\%$ are treated as different species.

<i>Oerstedtia</i> species*	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	0.16											
3	0.05	10.12										
4	10.35	10.44	7.6									
5	10.54	10.44	6.2	1.09								
6	10.88	10.71	4.9	0.97	0.16							
7	6.70	6.85	11.11	11.11	11.16	11.85						
8	4.57	4.67	10.05	10.05	10.39	10.71	6.24					
9	4.87	4.83	10.20	10.20	10.39	10.71	6.39	0.76				
10	4.87	4.83	10.20	10.20	10.39	10.71	6.39	0.76	0			
11	4.72	4.83	9.74	9.74	10.39	10.71	6.39	0.61	0.46	0.46		
12	4.72	4.83	9.74	9.74	10.39	10.71	6.39	0.61	0.46	0.46	0	
13	4.72	4.83	10.05	10.05	10.39	10.71	6.39	0.76	0.61	0.61	0.46	0.46
14	4.72	4.83	10.05	10.05	10.39	10.71	6.39	0.76	0.61	0.61	0.46	0.46
15	4.72	4.83	10.05	10.05	10.39	10.71	6.39	0.76	0.61	0.61	0.46	0.46
16	4.72	4.83	10.05	10.05	10.39	10.71	6.39	0.76	0.61	0.61	0.46	0.46
17	12.33	12.15	13.09	13.39	13.02	13.47	13.24	12.48	12.33	12.33	12.48	12.48
18	11.72	11.56	12.03	12.34	12.34	12.82	13.13	11.88	11.72	11.72	11.72	11.72
19	10.06	10.12	5.79	6.10	5.89	6.33	11.13	10.21	10.37	10.37	10.21	10.21

Table 7. (continuation).

<i>Oerstedtia</i> species*	13	14	15	16	17	18	19
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14	0						
15	0	0					
16	0	0	0				
17	12.63	12.63	12.63	12.63			
18	12.03	12.03	12.03	12.03	2.34		
19	10.21	10.21	10.21	10.21	11.59	10.94	

*1, *Oerstedtia oculata* banded Peter the Great Bay (PGB); 2, *O. oculata* non-banded PGB; 3, *O. phoresiae* Jeju Island; 4, *O. phoresiae* Oshoro Bay (OB); 5, *O. phoresiae* PGB1; 6, *O. phoresiae* PGB2; 7, *O. polyorbis*; 8, *O. pseudoculata* sp. nov. banded Akkeshi Bay (AB) (formerly *O. zebra*); 9, *O. pseudoculata* sp. nov. banded OB1, ICHUM 8582; 10, *O. pseudoculata* sp. nov. banded OB2, ICHUM 8583; 11, *O. pseudoculata* sp. nov. non-banded AB (formerly *O. venusta*); 12, *O. pseudoculata* sp. nov. non-banded Aniwa Bay, Kr-Ann077; 13, *O. pseudoculata* sp. nov. OB1, ICHUM 8587; 14, *O. pseudoculata* sp. nov. non-banded OB2, ICHUM 8586; 15, *O. pseudoculata* non-banded OB3, ICHUM 8584; 16, *O. pseudoculata* sp. nov. non-banded OB1, ICHUM 8585; 17, *O. rugosa* sp. nov. Japan, ICHUM 8590; 18, *O. rugosa* sp. nov. Vietnam (formerly *O. sp. Vietnam* 35); 19, *O. viridifusca* sp. nov. Japan.

a highly supported clade that is sister to another clade composed of *O. oculata*. That *O. pseudoculata* sp. nov. is different from and sister to *O. oculata* is corroborated by the ABGD (prior maximal distance $P = 0.035938$; barcode distance gap = 0.27; distance JC69 Jukes-Cantor MinSlope = 1.500,000); ASAP (ASAP score = 1.00; P-val(rank) = 0.0453(1); W(rank) = 0.378(1); and bPTP (support value = 0.93 for both *O. pseudoculata* sp. nov. and *O. oculata*) species delimitation analyses (Fig. 44b). A TCS haplotype network further shows the relationships among *O. pseudoculata* sp. nov. and *O. oculata* specimens

considered in the present study (Fig. 44a). Based on the three species-delimitation methods, I confirm that *i*) *O. venusta* and *O. zebra* sensu Thollessen & Norenburg (2003), *ii*) the two banded and four non-banded *Oerstedtia* from Oshoro Bay, and *iii*) the non-banded specimen from Aniwa Bay belong to a single species, established herein as *O. pseudoculata* sp. nov. The new species is genetically distinct from *O. oculata* despite a very close resemblance in morphology.

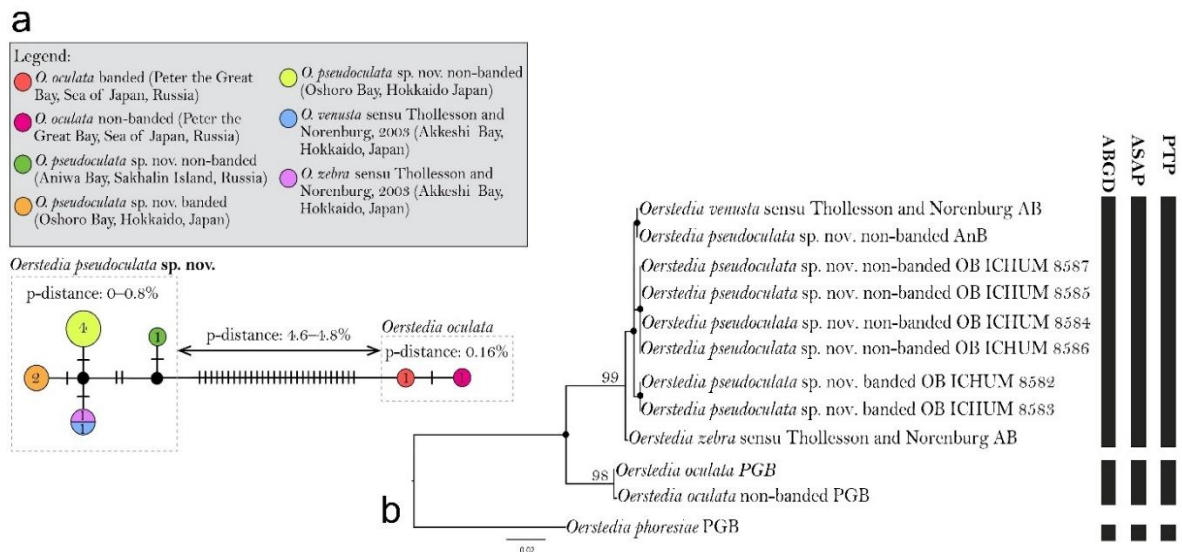


Figure 44. Delimiting *Oerstedtia oculata*, *O. pseudoculata* sp. nov., *O. venusta* and *O. zebra* sensu Thollessen & Norenburg. (a), a TCS haplotype network generated in PopART using partial sequences (657-bp) of the COI. The number of black bars corresponds to the number of nucleotide substitution between haplotypes; numbers inside each circle show the number of specimen(s) representing each haplotype; p-distance value represents the uncorrected pairwise genetic distance calculated within and between species; dashed boxes represent the species. (b), a COI-based ML tree. The number near each node represents the RAxML maximum-likelihood bootstrap value (BS) with closed circles indicated by 100%BS. Vertical bars show the results of the ABGD, ASAP, and PTP species delimitation analyses.

Furthermore, the phylogenetic analyses reveal that the two species exhibit a sister–taxon relationship in the clade *Paroerstedtiella* with high support and are together sister to another dorsally banded species from Kuril Island in the same clade, i.e., *O. polyorbis*, with full support. The holotype Japanese specimen of *Oerstedtia rugosa* sp. nov. is found to be conspecific with what was formerly an unidentified specimen, *Oerstedtia* sp. Vietnam 35 (2.3% p-distance between the Japanese and Vietnamese individuals) (Table

7), nested within the clade *Oerstedtia* of the genus and equally closely related to the undescribed *Oerstedtia* species from Guam and Crimea. *Oerstedtia viridifusca* sp. nov. is within the clade *Paroerstedtiella* and is sister to *O. phoresiae* with full support (Fig. 45).

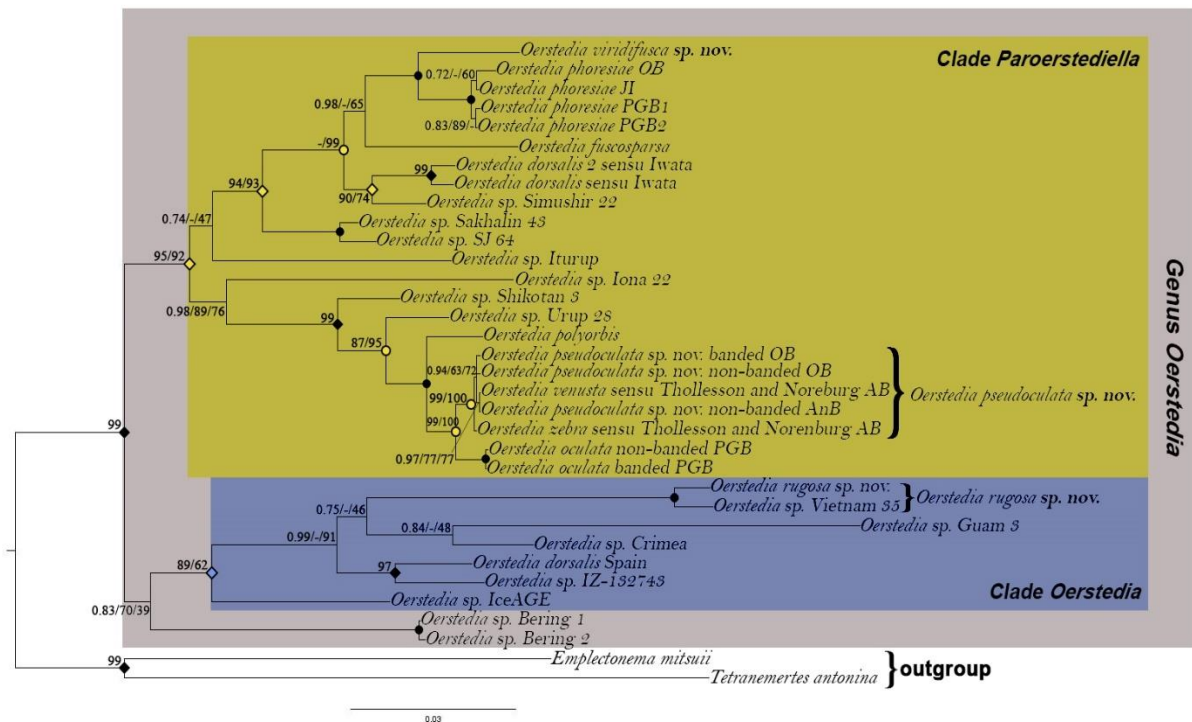


Figure 45. Rooted Bayesian phylogenetic tree showing the position of *Oerstedtia pseudoculata* sp. nov., *Oerstedtia rugosa* sp. nov., and *Oerstedtia viridifusca* sp. nov. within the genus based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Number at each node represents Bayesian posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFB/BS). Solid circles indicate full support (1.00 PP/100% UFB/100% BS); open circles indicate 1.00 PP; solid diamonds indicate 1.00 PP/100% UFB; open diamonds indicate 0.99 PP.

Oerstedtia in the Northwest Pacific region

At present, the species composition of the Japanese *Oerstedtia* include *O. dorsalis* sensu Iwata (1954), *O. fuscosparsa* (Abato et al. 2023), *O. phoresiae* [present study], *O. polyorbis* (Iwata 1954), *O. pseudoculata* sp. nov. [present study], *O. rugosa* sp. nov. [present study], *O. venusta* (Iwata 1954), and *O. viridifusca* sp. nov. [present study]. These species together with *O. oculata* (Kulikova 1987) (with *O. valentinae* (Chernyshev 1993) and *O. zebra* (Chernyshev 1993) being its junior synonyms) and *O. verae* (Chernyshev 1993) constitute the banded and non-banded *Oerstedtia* in the Northwest Pacific region.

I performed species-delimitation analyses using COI (for two non-tree-based methods) and a combined 16S, 18S, 28S, COI, and H3 genes (for a tree-based method) for unknown banded and non-banded forms of *Oerstedtia* from Oshoro Bay and Aniwa Bay that morphologically resemble *O. oculata*. For this purpose, I included in the analysis sequences of *O. oculata* from the Peter the Great Bay, as well as *O. venusta* and *O. zebra* in Thollessen & Norenburg (2003). My analyses revealed that *i*) the *O. venusta* and *O. zebra* sensu Thollessen & Norenburg (2003) and the present specimens from Oshoro Bay and Aniwa Bay are all conspecific (*p*-distance 0–0.76%), and *ii*) these are different from *O. oculata* found in Peter the Great Bay (*p*-distance 4.6%–4.8%), despite very similar morphological features. As I discuss below, *O. venusta* s.str. has anterior cephalic furrows extending dorsally, a character state that is quite unusual among *Oerstedtia* congeners, suggesting that it may not even affiliate with the genus. *Oerstedtia valentinae* and *O. zebra* are now considered synonymous with *O. oculata* (Zaslavskaya & Chernyshev 2008; Akhmatova et al. 2012). *Oerstedtia polyorbis* is distinct from *O. oculata* and *O. pseudoculata* sp. nov. (Chernyshev & Polyakova 2022; Abato et al. 2023; present study). For these reasons, no existing *Oerstedtia* Linnaean binomen can be applied to the species in question, hence, I established *O. pseudoculata* sp. nov. for this species.

Taxonomic history of *Oerstedtia pseudoculata* sp. nov.

The taxonomic history of *O. pseudoculata* sp. nov. has been complicated and its identity has remained uncertain for 21 years (Fig. 46). Iwata (1954) established the earliest *Oerstedtia* in the Northwest Pacific—a dorsally banded *O. polyorbis* (with 30 transverse bands composed of minute black dots) and a non-banded, dorsally brownish *O. venusta*, both from Hokkaido Island. Later, Kulikova (1987) established a non-banded, dorsally brownish to dark-brown *O. oculata* from Vostok Bay. This species has two symmetrical white spots notable on the anterolateral edges of the dorsal head. Chernyshev (1993) described two species of dorsally banded *Oerstedtia* solely based on morphology as *O. valentinae* (with 11–15 dark-brown stripes) and *O. zebra* (with 10–18 bands) from Peter the Great Bay. From their Akkeshi Bay nemertean collections, Thollessen and Norenburg (2003) identified two *Oerstedtia* species. The authors applied the name *O. zebra* to the dorsally banded forms (with 9–16 stripes) and used the name *O.*

venusta to the non-banded forms (Fig. 46). Both forms have the same morphological features, except that one is banded and the other is not (Kajihara 2007,2021).

The first molecular species delimitation including Thollessen and Norenburg's (2003) *Oerstedtia* was conducted by Strand and Sundberg (2005) based on a haplotype network analysis of *Oerstedtia* from the Atlantic Ocean (*O. striata* Sundberg, 1988 (Sundberg 1988) and *O. dorsalis*) and from the Pacific Ocean, including *O. venusta* and *O. zebra* sensu Thollessen and Norenburg (2003), using partial 539-bp COI sequences. Strand and Sundberg (2005) showed that the two *Oerstedtia* forms in Thollessen and Norenburg (2003) differed only by one substitution in their COI sequence (i.e., they belong to the same species); therefore, the former authors regarded these Akkeshi Bay *Oerstedtia* as *O. venusta*; they regarded the name *O. zebra* in Thollessen and Norenburg (2003) as a junior synonym of *O. venusta* (Fig. 46), despite not being consistent with Iwata's external description of *O. venusta*. This synonymization was doubted by Kajihara (2007) since the identity of *O. venusta* sensu Thollessen and Norenburg (2003) was not resolved.

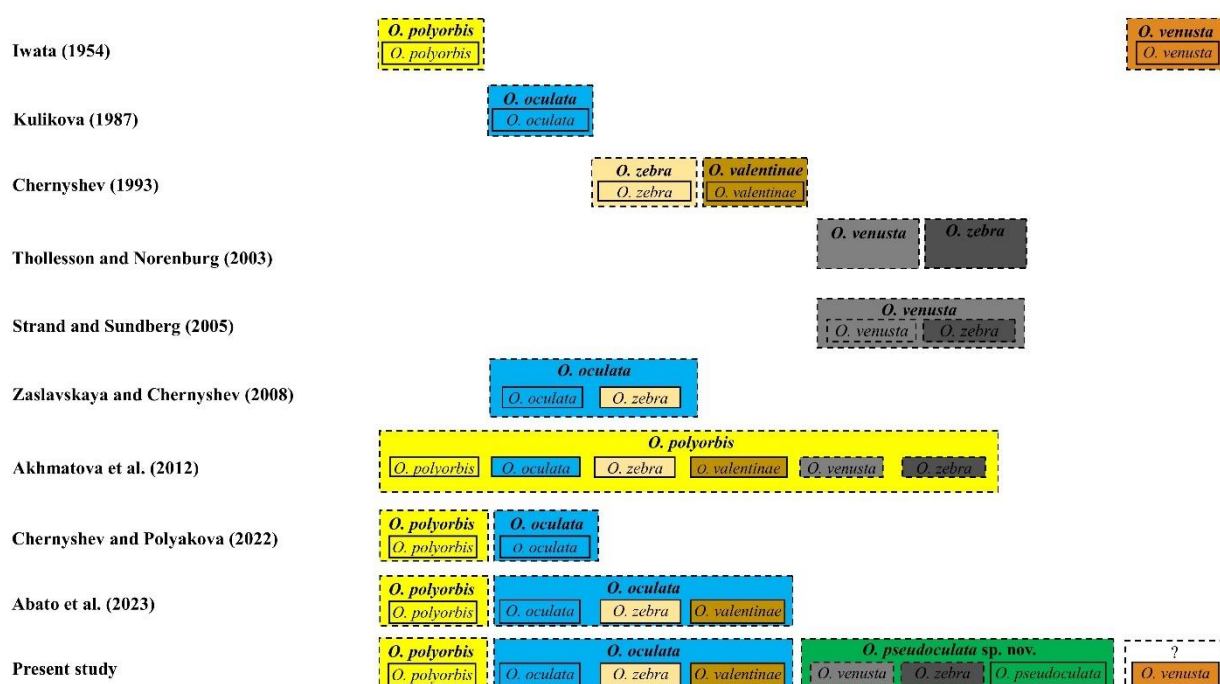


Figure 46. Taxonomic names applied to some banded and non-banded *Oerstedtia* from the Northwest Pacific according to several taxonomic works. Solid box represents nominal species (each denoted by a name bearing type specimen and an available name), and dashed box represents the species concept proposed by the author(s).

In the continued efforts to ascertain the *Oerstedtia* species composition of the waters in the Northwest Pacific, Zaslavskaya and Chernyshev (2008) proposed that *O. zebra* is just a color variation of *O. oculata* based on the similarities of their allozyme markers. Using 28S sequences of the banded and non-banded *Oerstedtia* from Peter the Great Bay and Tholleson and Norenburg's (2003) *Oerstedtia* from Akkeshi Bay, Akhmatova et al. (2012) concluded that the forms identified as *O. venusta* and *O. zebra* sensu Tholleson and Norenburg (2003), the species *O. oculata*, *O. valentinae*, *O. zebra* from Russia and the one identified as *O. polyorbis* from Kuril Island are all conspecific—with the former names considered as junior synonyms of *O. polyorbis*. However, this synonymization was not conclusive since molecular species delimitation in metazoans, including ribbon worms, is accurately and reliably achieved using fast-evolving COI barcode sequences (Hebert et al. 2003a; 2003b; Sundberg et al. 2016). Chernyshev and Polyakova (2022) showed that *O. oculata* and *O. polyorbis* from Kuril Island are two different species based on a phylogenetic analysis using a concatenated 16S, 18S, 28S, COI, and H3 sequences. Having the same phylogenetic evidence, Abato et al. (2023) stressed that it is more correct and valid to only synonymize *O. oculata*, *O. valentinae*, and *O. zebra*, with precedence given to *O. oculata* (Fig. 46). Although the two recent phylogenetic studies of the genus were conclusive (Chernyshev & Polyakova 2022; Abato et al. 2023), both failed to include Tholleson and Norenburg's (2003) *Oerstedtia* in their phylogenetic analyses, making the species' phylogenetic relationship with *O. oculata*, its potential closest relative based on morphological resemblance, not established.

In this study I settled and ascertained the taxonomic identity of a cryptic species hiding within banded and non-banded *Oerstedtia* in the cold-temperate province of the Northwest Pacific. The use of barcode sequence data and its subsequent correct interpretation and application in the taxonomy of the genus *Oerstedtia* (in conjunction with traditional taxonomy) is necessary to prevent taxonomic problems in the genus.

Oerstedtia pseudoculata* sp. nov and *O. oculata

Taxonomic remarks. *Oerstedtia pseudoculata* sp. nov. in Hokkaido/Sakhalin Islands and *O. oculata* in Primorsky Krai are very similar in their external features—both have banded and non-banded forms. However, a few observed morphological differences between the two species are pointed out. In *O. pseudoculata* sp. nov., the ocelli are brilliant-

orange and are notable on the dorsal and ventral sides of the head; the proboscis pore opens at the ventral tip of the head; the dorsally located transverse bands are 9–16 in number; the body is shorter and thinner; the stylet basis is shorter, varies in shaped from cylindrical to triangular; the accessory stylets are usually 4–7 (sometimes as many as 15–18) in number. On the other hand, in *O. oculata*, the black ocelli are not clearly visible on both sides of the head; the proboscis pore is located further below the tip of the head; the dorsally located transverse bands are 10–18 in number; the body is longer and wider; the stylet basis is longer and cylindrical; and the accessory stylets are 1–4 (sometimes up to 9) in number.

Aside from these differences, the geographic distribution and perhaps habitat preference of these two very closely related species are different. *Oerstedtia pseudoculata* sp. nov. seems to be common in Hokkaido Island. The recent collection of a single specimen of the same species from Aniwa Bay, Sakhalin Island, supports my hypothesis that the new species is an island species. This hypothesis on habitat preference is supported by its absence in the Primorsky Krai, despite the intensive ribbon-worm studies in this region. Likewise, *O. oculata* has never been documented in several ribbon-worm surveys conducted in Hokkaido Island, Kuril Islands, and Sakhalin Island, perhaps indicating that this species is not an island species.

***Oerstedtia rugosa* sp. nov.**

Taxonomic remarks. *Oerstedtia rugosa* sp. nov. is defined by its unique body pigmentation, banding patterns, and the presence of wrinkles on its body surface. The COI genetic distance analysis revealed that this new species is conspecific with *Oerstedtia* sp. Vietnam 35. In this regard, this Vietnam specimen is also referred to in this study as *O. rugosa* sp. nov. Although the Japan and Vietnam specimens are conspecific, the two slightly differ in external features—the Japan specimen is longer, with body surface and texture appearing rougher or intensely wrinkled, whereas the Vietnam specimen is less wrinkled, showing a smoother body surface; the most anterior white transverse band in the Japan specimen is bisected at mid by a thinner, deep-red pigmentation whereas it is thicker in the Vietnam specimen; the white bands in the Japan specimen appears completely transverse (i.e., encircling the body) whereas these bands are not completely transverse in the Vietnam specimen (except for the most anterior

band); when viewed dorsally, the bands appear to be situated on the lateral sides only, with middorsal body completely deep red in color in the Vietnam material. The absence of anterior pouches in the intestinal cecum and having short dorsolateral intestinal pouches brings *O. rugosa* sp. nov. closer to the species of the genus *Friedrichia* Kirsteuer, 1965 (Kirsteuer 1965; Chernyshev 1993), which have intestinal cecum with no anterior and lateral pouches. That is, *Friedrichia* is likely a junior synonym of *Oerstedtia*.

***Oerstedtia viridifusca* sp. nov.**

Taxonomic remarks. *Oerstedtia viridifusca* sp. nov. is identifiable by its stout, brownish-green-pigmented body; thick, opaque white collar; and the presence of transverse cephalic band lighter than the body—these make it unique from the currently identified Northwest Pacific *Oerstedtia* and the rest of the congeners. Although closely related to *O. phoresiae*, the two species can be easily discriminated against one another based on body pigmentation, body shape, and head shape (Figs. 42, 43). However, a ‘*viridifusca*-like’ form of *O. phoresiae*, collected from Jeju Island, South Korea (Chernyshev pers. obs.), expresses external features that are quite similar with *O. viridifusca* sp. nov. This specimen is less slender; the body pigmentation pattern in the dorsal and ventral sides appears to be slightly similar to the new species except that the dorsal color is brownish and the ventral body darker; the head shape is similar to the new species but narrower; the tip of the head is opaque white dorsoventrally; and it has an opaque white collar. Other *Oerstedtia* species with white collar include *O. polyorbis*, the banded form of *O. oculata* (Akhmatova et al. 2012), and the banded form of *O. pseudoculata* sp. nov. *Oerstedtia viridifusca* sp. nov. can be distinguished from these collared *Oerstedtia* by its body pigmentation pattern and color. Having three anterior ocelli and two posterior ones could not be a taxonomic character state unique to the new species; instead, this could be a result of a developmental defect that occurred in the collected specimen. *Oerstedtia* species generally only have four ocelli. Whether or not having five ocelli is a trait attributed to *O. viridifusca* sp. nov. can be verified only if multiple specimens of this species are collected in the future. Not until this character state is consistently observed in other *O. viridifusca* sp. nov. specimens, in this work, I consider it an aberration.

Clades *Oerstedtia* and *Paroerstedtiella* in the genus *Oerstedtia*

The two monophyletic clades within the genus *Oerstedtia* are defined based on a close relationship with *O. phoresiae* (i.e., clade *Paroerstedtiella*) or with *O. dorsalis* s.str. (i.e., clade *Oerstedtia*) (Chernyshev & Polyakova 2022). Two members of the clade *Paroerstedtiella* do not have a free-swimming larval stage—*O. phoresiae* and *O. dorsalis* sensu Iwata (Iwata 1960; Chernyshev 2014). *Oerstedtia pseudoculata* sp. nov. and *O. viridifusca* sp. nov. having nested in the clade *Paroerstedtiella*, could have the same larval development as to the two former species. This could lead to their limited dispersal and distribution, and perhaps, faster speciation. Coe (1943) reported that *O. dorsalis* s.str. has a free-swimming larval stage. Having nested in the clade *Oerstedtia*, the developmental pattern of *O. rugosa* sp. nov. could be like that of *O. dorsalis* s.str. With a free-swimming larval stage, the species can be easily dispersed and, thus, widely distributed. This could probably explain its distribution in Japanese and Vietnamese waters.

***Oerstedtia venusta* s.str.**

Taxonomic remarks. Since its establishment in 1954, *O. venusta* s.str. has never been recollected to date. Based on external morphology, it can be identified by the presence of “[t]wo pairs of the cephalic grooves running obliquely from the ventral side toward the dorsal side of the body” (Iwata 1954: 15, 16, fig. 3). Envall and Sunberg (1993) pointed out that it is not possible from Iwata’s (1954) brief description of *O. venusta* to identify it to the genus *Oerstedtia*. The generic diagnosis for *Oerstedtia* includes furrows that are absent or weakly developed (Envall & Sundberg 1993). Having a pair of well-developed anterior cephalic furrows is unusual for a true *Oerstedtia*. The description of Iwata’s (1954) *O. venusta* is vague; hence, its genus affiliation is uncertain. His species description appears ambiguous—color of the ocelli not specified, presence of accessory lateral nerve (a trait common to *Oerstedtia*) not mentioned. Iwata’s (1954) taxonomic concept of *O. venusta* cannot be challenged; however, its genus affiliation can be further studied or verified, only possible when this species is recollected from its type locality and subjected to a more comprehensive internal morphology examination, sequenced, and most importantly, put through a phylogenetic analysis. A phylogenetics study involving Iwata’s (1954) *O. venusta* could result to two possible outcomes that can either resolve

its taxonomy or redefine the diagnoses of the genus *Oerstedia*: *i*) if nested within the genus, it is undoubtedly a genuine member of *Oerstedia*, indicating that the presence of a well-developed anterior cephalic furrows should also define the genus, or *ii*) if not nested within the genus *Oerstedia*, it is definitely not a valid congener and the presence of a well-developed anterior furrows is absolutely not a trait of the genus, and therefore, Iwata's (1954) *O. venusta* needs to be transferred to its proper genus.

Polymorphism in *Oerstedia* and its taxonomic implication

Intraspecific morphological variation or polymorphism is common among *Oerstedia* species. This phenomenon when not carefully considered in the taxonomic study of the genus could result in a taxonomic chaos—polymorphism can either lump two different biological species into a single taxonomic name (as in the case of *O. dorsalis* s.str. and the previously considered form A of the same species in Sundberg (1984), now known as *O. striata*; the taxonomic issue related to Tholleson and Norenburg's *Oerstedia*) or split similar biological species into different taxonomic names, as in the case of *O. oculata*, *O. valentinae*, and *O. zebra* (now under the name *O. oculata* due to synonymy). In extreme cases, as reported in this chapter, polymorphism in the genus could result to two different species with very similar morphology or feature (resemblance between *O. oculata* and *O. pseudoculata* sp. nov.; 'viridifusca-like' *O. phoresiae* and *O. viridifusca* sp. nov.; *O. phoresiae*, non-banded *O. oculata*, and non-banded *O. pseudoculata* sp. nov.; and *O. polyorbis* and the banded forms of *O. oculata* and *O. pseudoculata* sp. nov.). It is noteworthy that the sister species, *O. oculata* and *O. pseudoculata* sp. nov., having exactly very similar pigmentation patterns (both being banded and non-banded in the same manner), likely inherited an ancestral polymorphism. This phenomenon can be explained by Vavilov's law of homologous series (Vavilov 1922; Rozhnov 2006; Trapezov 2007; Bulatova 2020).

Ribbon-worm taxonomists should regard polymorphism when establishing new *Oerstedia* species and even identifying congeners. In *Oerstedia*, both the similarities and differences in external features between closely related members have two implications—they are either conspecific or different species. This confuses taxonomists in species delineation, identification, and the subsequent name application. To avoid such chaos in the genus and to make future species establishment and identification

taxonomically correct and reliable, an integrative approach in their taxonomy is highly recommended, i.e., employing both traditional morphological and modern molecular taxonomy. In the aspect of traditional taxonomy, turbotaxonomy alone coupled with molecular taxonomy is usually sufficient. Molecular species delineation should also rely on the fast-evolving mitochondrial COI gene since the 16S gene of *O. oculata* and *O. pseudoculata* sp. nov. was found to differ only by two substitutions, suggesting that the latter mitochondrial gene is more conservative and does not differ among sibling species. Using my specimens, I have shown the necessity of DNA sequence data in the taxonomy of the genus and in resolving the taxonomic problems arising from the polymorphic species with uncertain identities.

Conclusions

The genus *Oerstedtia* is composed of some congeners that are highly polymorphic. This polymorphism makes the taxonomy and systematics of the genus challenging. Some species in the genus can deceive taxonomists by their variable external morphology, resulting in problematic species demarcation, identification, and name application. Employing the so-called traditional taxonomy for *Oerstedtia* species is undoubtedly inadequate and can possibly result in some taxonomic problems and inconsistencies. The role of DNA sequence data in the taxonomy and systematics of the genus *Oerstedtia* is always indispensable. With these, it is important to revisit those already established *Oerstedtia* and investigate them for potential polymorphism and generate barcode sequence data from a greater representative of the genus for more comprehensive future molecular taxonomy and phylogenetics studies.

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Chapter 3.5. Species diversity of *Tetranemertes* (Nemertea: Monostilifera) in Japan

Introduction

The eumonostiliferous hoplonemertean genus *Tetranemertes* Chernyshev, 1992 currently includes 10 named species (Cherneva et al. 2023). This taxon was first recognized by Friedrich (1955: 171), who applied the name *Nemertes* to it, ascribing its author to Johnston (1837) and indicating *Nemertes antonina* Quatrefages, 1846 as the type species. Because *Nemertes* Johnston, 1837 was a junior homonym of *Nemertes* Cuvier, 1816, while *N. antonina* was not originally included in *Nemertes* when Johnston (1837) established it, Chernyshev (1992) introduced the replacement name *Tetranemertes* to *Nemertes* sensu Friedrich (1955) (Kajihara et al. 2021; Cherneva et al. 2023). The morphological character most highly emphasized to define *Nemertes* sensu Friedrich (1955) (now *Tetranemertes*) was the anteriorly divided body-wall longitudinal muscle layer (Friedrich 1955; Kirsteuer 1974). This feature, however, is also found in many other eumonostiliferous hoplonemertean genera—once grouped as Poseidonemertidae Chernyshev, 2002 (Chernyshev 2005)—while other characters supposedly usable to distinguish such genera (e.g., the way how the body-wall longitudinal muscles contribute to the proboscis insertion) are difficult to trace from serial histological sections and thus prone to be subjective. Besides, no attempt has been made to see the taxonomic usefulness of these characters by examining the degree of variation between and within species/genera; therefore, in my opinion, some genera (such as *Arenogigas* Krämer & von Döhren, 2015; *Correanemertes* Kirsteuer, 1967; *Diopsonemertes* Kajihara, Gibson & Mawatari, 2001; *Fasciculonemertes* Sanchez & Cancino, 1980; and *Poseidonemertes* Kirsteuer, 1967) may even be synonymous with each other. The morphological distinctiveness of *Tetranemertes* from other eumonostiliferous hoplonemertean genera remained unclear even after the type species *Tetranemertes antonina* (Quatrefages, 1846) was included in a molecular phylogenetic analysis by Kvist et al. (2014, 2015). It was only quite recently when the unique characteristics of *Tetranemertes* became obvious by Cherneva et al.'s (2023) high-quality images of living worms in 10 congeners (of which seven were established as new), the monophyly of which was confirmed by a multi-locus molecular phylogenetic analysis. Of the characteristics listed as diagnostic

for *Tetranemertes* in Cherneva et al. (2023)—*i*) filiform body shape, *ii*) diamond-shaped head, *iii*) the ocelli arranged in four rows, *iv*) a single ventral Λ -shaped cephalic furrow, *v*) very short rhynchocoel and proboscis, and *vi*) bilobed to deeply forked central stylet basis—the one that is unique among Monostilifera seems to be the arrangement of the ocelli, which forms four longitudinal rows, with two on each side of the head, the two rows almost on top of one another.

From Japanese waters, only *T. unistriata* Cherneva et al., 2023 has been recorded to date. During my ribbon worm faunal surveys on the other hand, three species of eumonostiliferous hoplonemerteans showing the characteristics of *Tetranemertes*, but not *T. unistriata*, were found. In this study, I describe one of these three species as new, while leaving the remaining two undetermined due to lack of morphological information. In addition, my literature survey indicates that *Emplectonema kandai* Kato, 1939—the only nemertean species known to be bioluminescent—should be transferred to *Tetranemertes*. The purpose of this chapter is to summarize and provide the species diversity of *Tetranemertes* distributed in Japanese waters.

Materials and methods

This study is based on three specimens, collected in 2001, 2007, and 2022, respectively. For anesthetization, each worm was placed in a Petri dish, to which a MgCl_2 solution isotonic to seawater was slowly added. The stylet apparatus was not examined *in vivo* for the first two specimens.

The 2001 specimen was cut into three portions after anesthetization; the anterior and posterior ends were fixed in 10% formalin seawater, whereas the middle portion was preserved in 99% ethanol. The latter had been stored at room temperature for 23 years before DNA extraction.

The 2007 specimen was cut into two portions after anesthetization. The anterior portion was fixed in Bouin's fluid; the posterior portion in 99% ethanol (but the final concentration would be ~60–70%). The latter had been left at room temperature for some years before being stored at -80°C for ~15 years until DNA extraction.

The 2022 specimen was cut in half; the proboscis was extracted and observed under a compound light microscope for the documentation of its stylet apparatus. The posterior half of the worm was preserved in 99% ethanol while the anterior half was fixed

in Bouin's fluid, then preserved in 70% ethanol. The specimen has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Hokkaido, Japan.

DNA extraction from the three specimens was performed followed Abato et al. (2022). PCR amplification of the cytochrome c oxidase subunit I (COI) gene for the 2001 specimen was performed using the primer pairs LCO1490 (Folmer et al. 1994) and TkaCOR (5'-ATCAACCGAACCACCAGCAT-3'); and TkaCOF (5'-TGCCGGTTATGATTGGAGGG-3') and HCO2198 (Folmer et al. 1994). For the 2007 and 2022 specimens, the LCO1490/HCO2198 (Folmer et al. 1994) pair was used.

For the 2022 specimen, the 16S, 18S, and 28S rRNA genes were also sequenced following Abato et al. (2022); sequencing of the histone H3 gene was attempted but failed.

The sequenced genes of the 2022 specimen were used for the maximum likelihood (ML) analyses and Bayesian inference (BI). Moreover, the 16S, 18S, 28S, COI, and H3 sequences of Oerstediiina representatives, including *Tetranemertes* congeners were downloaded from GenBank (Table 8). Sequences from the Amphiporina representatives including *Emplectonema viride* Stimpson, 1857—the type species of the genus *Emplectonema* Stimpson, 1857 (but see Kajihara et al. 2021 for an alternative interpretation)—were included as the outgroup. A concatenated dataset consisting of the 16S (496 bp), 18S (1870 bp), 28S (1989 bp), COI (646 bp), and H3 (330 bp) sequences was partitioned using PartitionFinder ver. 2.1.1 (Lanfear et al. 2016) into seven subsets with corresponding substitution models: *i*) COI (first codon), TRN + I + G; *ii*) COI (second codon) and H3 (second codon), K81UF + I + G; *iii*) COI (third codon), TIM + I + G; *iv*) H3 (first codon) and 18S, TRNEF + I + G; *v*) H3 (third codon), GTR + G; *vi*) 16S, GTR + I + G; and *vii*) 28S, GTR + I + G. ML analyses were executed using IQ-Tree ver. 2.1.3 (Minh et al. 2020) applying the suggested model to each subset with branch support values derived from 1,000 ultrafast bootstrap (UFBoot) (Minh et al. 2013) and RAxML ver. 8.2.10 (Stamatakis 2015) using the GTR + G model to all subsets with branch support values calculated from 1,000 bootstrap pseudoreplicates. BI was performed using MrBayes ver. 3.2.6 x64 (parallel version) (Ronquist & Huelsenbeck 2003; Altekar et al. 2004) employing the models GTR + I + G for subsets 1, 2, 3, 6, and 7; SYM + I for subset 4; and GTR + G for subset 5.

Uncorrected pairwise genetic distances between *Tetranemertes* species were calculated based on COI sequences using MEGA ver. 11 (Tamura et al. 2021). After alignment to the shortest sequence, the dataset had 519 bp.

Sequences determined from the collected *Tetranemertes* have been deposited in GenBank (Table 8).

Table 8. List of Oerstediiina and Amphiporina species included in the phylogenetic analyses, GenBank accession numbers for 16S, 18S, 28S rRNA (16S, 18S, and 28S), cytochrome c oxidase subunit I (COI), and histone H3 (H3) gene markers, and their references.

Species	GenBank accession numbers					Reference
	16S	18S	28S	COI	H3	
<i>Abyssonemertes kajiharai</i> 1V	KY296888	KY296892	KY296897	KY296906	KY296915	Chernyshev & Polyakova (2018)
<i>Acteonemertes orientalis</i> Shimonoseki	OQ532965	OQ532963	OQ532910	OQ466706	–	Hookabe et al. (2023)
<i>Acteonemertes orientalis</i> Tsuruga	OQ532964	OQ532962	OQ532909	OQ466705	–	Hookabe et al. (2023)
<i>Argonemertes australiensis</i> IZ-135314	JF277605	JF293010	HQ856892	HQ848601	JF277750	Andrade et al. (2012)
<i>Argonemertes australiensis</i> IZ-135315	KF935476	KF935309	KF935365	KF935525	KF935420	Kvist et al. (2014)
<i>Alvinonemertes christianeae</i> N253	ON036052	–	–	ON021867	–	Sagorny et al. (2022)
<i>Alvinonemertes claudiae</i> N231	ON036050	–	–	ON021868	–	Sagorny et al. (2022)
<i>Alvinonemertes dagmarae</i> N260	ON036049	ON186254	–	ON021866	ON182034	Sagorny et al. (2022)
<i>Alvinonemertes dariae</i> N262	ON036048	ON186252	–	ON021865	–	Sagorny et al. (2022)
<i>Alvinonemertes tatjanae</i> N119	ON036047	ON186256	–	ON021864	ON182041	Sagorny et al. (2022)
<i>Amphiporus</i> cf. <i>cruentatus</i>	MZ231130	MZ231193	MZ231282	MZ216514	–	Chernyshev et al. (2021)
<i>Amphiporus</i> cf. <i>imparispinosus</i> Kb2hop34	MN211508	MN211414	MN211464	MN205525	MN205486	Chernyshev & Polyakova (2019)
<i>Amphiporus formidabilis</i> SK44	KF935498	KF935331	KF935387	KF935547	KF935443	Kvist et al. (2014)
<i>Amphiporus imparispinosus</i> DNA106137	JF277618	JF293029	HQ856878	HQ848612	JF277696	Andrade et al. (2012)
<i>Amphiporus lactifloreus</i> DNA103901	JF277617	JF293018	HQ856876	HQ848611	–	Andrade et al. (2012)
<i>Amphiporus lactifloreus</i> KB2hop37	MN211511	MN211417	MN211467	MN205528	MN205488	Chernyshev & Polyakova (2019)

<i>Antarctonemertes dilutebasisae</i>	MN211494	MN211397	MN211447	MN205511	–	Chernyshev & Polyakova (2019)
<i>Antarctonemertes phyllospadicola</i>	MN211493	MN211396	MN211446	FJ594419	MN205468	Chernyshev & Polyakova (2019)
<i>Antarctonemertes riesgoae</i> 134229	KF935490	KF935322	KF935378	KF935538	KF935434	Kvist et al. (2014)
<i>Antarctonemertes valida</i> 134228	KF935489	KF935321	KF935377	KF935537	KF935433	Kvist et al. (2014)
cf. <i>Potamonemertes percivali</i> IZ-25172	KF935483	KF935316	KF935372	KF935532	KF935426	Kvist et al. (2014)
<i>Chernyshevia escarpiaphila</i> N235	ON036046	ON186253	–	ON021863	ON182036	Sagorny et al. (2022)
<i>Chernyshevia escarpiaphila</i> N266	ON036043	ON186251	–	ON021860	ON182040	Sagorny et al. (2022)
<i>Diplomma serpentinum</i>	–	MN211400	MN211450	MN205514	MN205471	Chernyshev & Polyakova (2019)
<i>Emplectonema buergeri</i>	JF277616	JF293066	HQ856880	HQ848600	JF277697	Andrade et al. (2012)
<i>Emplectonema gracile</i>	JF277621	JF293022	HQ856883	HQ848620	JF277751	Andrade et al. (2012)
<i>Emplectonema mitsuui</i>	–	OM423118	OM423056	OM456699	OM468150	Chernyshev & Polyakova (2022)
<i>Emplectonema viride</i>	MT647823	–	–	MT649116	–	Mendes et al. (2021)
<i>Eumonostilifera</i> N259	ON036053	ON186269	–	ON021869	ON182042	Sagorny et al. (2022)
<i>Eumonostilifera</i> 2V	–	KY296893	KY296898	KY296907	KY296916	Chernyshev & Polyakova (2018)
<i>Eumonostilifera</i> 5V	–	KY296894	KY296901	KY296910	KY296919	Chernyshev & Polyakova (2018)
<i>Galathenemertes giribeti</i> KB2hop19	–	MN211401	MN211451	MN205515	MN205472	Chernyshev & Polyakova (2018)
<i>Galathenemertes giribeti</i> KB2hop20	MN211497	MN211402	MN211452	MN205516	MN205473	Chernyshev & Polyakova (2019)
<i>Gononemertes</i> 45558	KP270855	KP270805	KP270830	KP270878	–	Kvist et al. (2015)
<i>Gononemertes</i> 6DS	MF512052	MF512079	MF512104	–	MF512146	Chernyshev & Polyakova (2018)

<i>Gononemertes</i> KB2hop21	MN211498	MN211403	MN211453	MN205517	MN205474	Chernyshev & Polyakova (2019)
<i>Gononemertes</i> KB2hop22	MN211499	MN211404	MN211454	MN205518	MN205475	Chernyshev & Polyakova (2019)
<i>Gononemertes parasita</i> 105583	JF277606	JF293014	HQ856889	HQ848607	JF277745	Andrade et al. (2012)
<i>Leptonemertes</i> cf. <i>chalicophora</i>	JF277608	JF293011	HQ856898	HQ848596	–	Andrade et al. (2012)
<i>Monostilifera</i> sp. 2 CNP-2478	–	–	–	OP104783	–	Alfaya et al. (2023)
<i>Monostilifera</i> sp. 2 CNP-2561	–	–	–	OP104831	–	Alfaya et al. (2023)
<i>Nemertellina yamaokai</i>	AJ436797	–	AJ436852	AJ436907	AJ436959	Thollessen & Norenburg (2003)
<i>Oerstedia fuscosparsa</i>	OP256567	OP256566	OP271739	OP265741	OP253976	Abato et al. (2023)
<i>Oerstedia oculata</i> KB2hop16	MN211495	MN211398	MN211448	MN205512	MN205469	Chernyshev & Polyakova (2019)
<i>Oerstedia phoresiae</i> KB2hop17	MN211496	MN211399	MN211449	MN205513	MN205470	Chernyshev & Polyakova (2019)
<i>Oerstedia pseudoculata</i>	OR806645	OR806646	OR806653	OR800640	OR805478	Abato et al. (2024)
<i>Plectonemertidae</i> sp. IZ-25166	KF935477	KF935310	KF935366	KF935526	KF935421	Kvist et al. (2014)
<i>Plectonemertidae</i> sp. IZ-25167	KF935478	KF935311	KF935367	KF935527	–	Kvist et al. (2014)
<i>Plectonemertidae</i> sp. IZ-25169	KF935479	KF935312	KF935368	KF935528	KF935422	Kvist et al. (2014)
<i>Plectonemertidae</i> sp. IZ-25173	KF935480	KF935313	KF935369	KF935529	KF935423	Kvist et al. (2014)
<i>Plectonemertidae</i> sp. IZ-25174	KF935481	KF935314	KF935370	KF935530	KF935424	Kvist et al. (2014)
<i>Plectonemertidae</i> sp. IZ-25175	KF935482	KF935315	KF935371	KF935531	KF935425	Kvist et al. (2014)
<i>Prosorhochmus nelsoni</i>	JF277604	JF293013	HQ856891	HQ848606	JF277744	Andrade et al. (2012)
<i>Pseudotetrastemma</i> KB2hop25	MN211501	MN211407	MN211457	MN205520	–	Chernyshev & Polyakova (2019)
<i>Tetranemertes antonina</i>	–	KF935318	KF935374	KF935534	KF935430	Kvist et al. (2014)
<i>Tetranemertes arabica</i>	–	OQ322651	OQ322596	OQ321721	–	Cherneva et al. (2023)
<i>Tetranemertes aurantia</i> sp. nov.	PP413743	PP413744	PP413745	PP413702	–	present study
<i>Tetranemertes bifrost</i>	KF935484	KF935317	KF935373	KF935533	–	Kvist et al. (2014)

<i>Tetranemertes bifrost</i>	MT581161	MT581203	MT581189	MT578867	–	Cherneva et al. (2023)
<i>Tetranemertes majinbuui</i>	MT581160	MT581201	MT581187	MT578864	–	Cherneva et al. (2023)
<i>Tetranemertes ocelata</i>	MT581159	MT581200	MT581186	MT578863	–	Cherneva et al. (2023)
<i>Tetranemertes pastafariensis</i> isolate CB055 18	–	MT581210	MT581196	MT578892	–	Cherneva et al. (2023)
<i>Tetranemertes pastafariensis</i> isolate SMCP0019	MT581182	–	–	MT578896	–	Cherneva et al. (2023)
<i>Tetranemertes paulayi</i> BOMAN08291	–	OQ322646	OQ322592	–	–	Cherneva et al. (2023)
<i>Tetranemertes paulayi</i> BOMAN07013	–	OQ322649	–	OQ321715	–	Cherneva et al. (2023)
<i>Tetranemertes rubrolineata</i> BOMAN08038	–	OQ322650	OQ322595	OQ321718	–	Cherneva et al. (2023)
<i>Tetranemertes</i> sp. ETP001	MT581183	MT581211	MT581197	MT578897	–	Cherneva et al. (2023)
<i>Tetranemertes</i> sp. AHK-2024-1	–	–	PQ564663		–	present study
<i>Tetranemertes</i> sp. AHK-2024-2	–	–	PQ564664		–	present study
<i>Tetranemertes unistriata</i> 4900709991	MT581158	MT581198	MT581184	MT578861	–	Cherneva et al. (2023)
<i>Tetrastemma elegans</i>	AJ436810	–	AJ436865	AJ436920	AJ436968	Tholleson & Norenburg (2003)
<i>Tetrastemma parallelus</i>	ON112235	ON112234	ON112277	ON098244	ON107495	Abato et al. (2022)
<i>Tetrastemma phaeobasisae</i>	MZ231165	MZ231234	MZ231324	MZ216553	–	Chernyshev et al. (2021)
<i>Tetrastemma vittigerum</i> (132742)	KF935491	KF935323	KF935379	KF935539	KF935435	Kvist et al. (2014)
<i>Tetrastemma vittigerum</i> (25171)	–	KF935324	KF935380	KF935540	KF935436	Kvist et al. (2014)
<i>Tetrastemma vittigerum</i> H3	MZ231192	MZ231272	MZ231362	MZ216585	MZ216663	Chernyshev et al. (2021)
<i>Tetrastemma wilsoni</i>	AJ436811	–	AJ436866	AJ436921	AJ436969	Tholleson & Norenburg (2003)
<i>Vieitezia luzmurubeae</i> (104801)	JF277607	HQ443428	–	HQ443426	JF277746	Andrade et al. (2012)
<i>Vieitezia luzmurubeae</i> (133470)	KF935495	KF935328	KF935384	KF935544	KF935440	Kvist et al. (2014)

Results and systematic remarks

Taxonomy

Tetranemertes Chernyshev, 1992

?*Ommatoplea* Ehrenberg, 1828: pl. IV; type species *Ommatoplea taeniata* Ehrenberg, 1828, fixed by monotypy.

Nemertes: Friedrich 1955: 171 (neither Cuvier, 1816 nor Johnston, 1837); *Nemertes antonina* Quatrefages, 1846 indicated as type species (invalid designation).

Tetranemertes Chernyshev, 1992: 134, type species *Nemertes antonina* Quatrefages, 1846; fixed by original designation.

Taxonomic remarks. The three species names *Ommatoplea taeniata*, *Polystemma adriaticus*, and *Polystemma albicans*, as well as the two genus names *Ommatoplea* and *Polystemma*, appeared for the first time in the caption of plate IV (published in 1828) of Ehrenberg (1828–1831); these names were made available in 1828 under Article 12.2.7 of the International Code of Zoological Nomenclature (hereafter the *Code*) (ICZN 1999). In the text (published in 1831), *P. albicans* was transferred to the newly established genus *Amphiporus* Ehrenberg, 1831. Interpreting the date of publication of *Ommatoplea* Ehrenberg, 1828 and *Polystemma* Ehrenberg, 1828 erroneously as 1831, Verrill (1892: 387) included these two names in the synonymy of *Amphiporus* Ehrenberg, 1831, and, at the same time, recognized the two taxa as subgenera under *Amphiporus* (Verrill 1892: 388). Bürger (1904: 77) regarded *Ommatoplea* and *O. taeniata* as nomina dubia; Gibson (1995: 449, 451) followed this. On the other hand, *Polystemma adriaticum* and *Polystemma albicans* were considered invalid and listed—as valid names—as *Amphiporus adriaticus* and *Amphiporus albicans* (Bürger 1904: 49; Gibson 1995: 486); however, when the priorities of *Polystemma* Ehrenberg, 1828 and *Amphiporus* Ehrenberg, 1831 are considered, these name usages are incorrect.

As *Ommatoplea* and *Polystemma* have unlikely been used as valid after 1898, while *Amphiporus* has been in prevailing usage, the precedence would be reversed under Article 23.9 of the *Code* (which is beyond the scope of this study, though). On the other hand, *Ommatoplea* may actually be a senior synonym of *Tetranemertes*, as some of the characteristics in *O. taeniata*—such as the ocelli arranged in four rows and the short

rhynchocoel (Fig. 47a–d)—suggest. If this is the case, the precedence would not likely be reversed, because the usage of *Tetranemertes* would not satisfy the conditions stipulated in Article 23.9.1.2 of the *Code*. Despite external morphological resemblance with *Tetranemertes*, my observation and opinion on *Ommatoplea* as a senior synonym of the former can be claimed with certainty when fresh *O. taeniata* from the type locality is collected, morphologically thoroughly examined especially its stylet basis morphology, cephalic furrows, and subjected to a phylogenetic study.

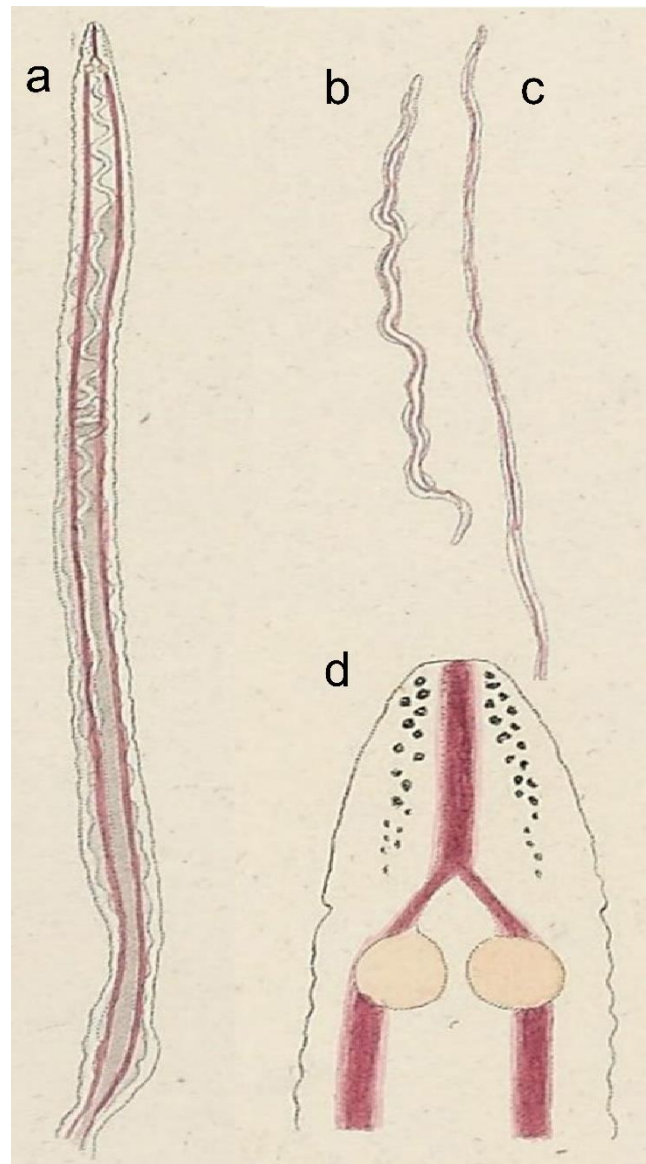


Figure 47. *Ommatoplea taeniata* Ehrenberg, 1828. (a), anterior end of body (Ehrenberg 1828–1831: fig. III f); (b, c), entire animal (Ehrenberg 1828–1831: fig. III a, b); (d), magnification of the head (Ehrenberg 1828–1831: fig. III d). Reproduced from a digitalized copy at Biodiversity Heritage Library (<https://www.biodiversitylibrary.org/item/189273#page/85>).

***Tetranemertes aurantia* sp. nov.** Abato, Hookabe & Kajihara (Figs. 48a–c, 49a–g).

urn:lsid:zoobank.org:act:3C3ADC04-2CDC-4A3B-9E2F-6D5662B1DD3D.

Material examined. Holotype, ICHUM 8719, extracted genomic DNA, posterior half preserved in 99% ethanol, anterior half in Bouin's fluid, then in 70% ethanol, unsectioned; collected by Natsumi Hookabe on June 30, 2022, from a bycaught sample of a fishery gill net deployed around 10 m depth, off Sugashima (Suga Island), Mie, Japan.

Diagnosis. A *Tetranemertes* without mid-dorsal stripe, having ~60 eyes in total, with longitudinally-grooved stylets, basis nearly rod in shape, bifurcated, and with stylet/basis ratio of 0.42.

Etymology. The new specific name, meaning 'orange', is the Latin adjective *aurantius* (m.), *aurantia* (f.), *aurantium* (n.), referring to the body color of the new species.

Type locality and distribution. At present, the species is known only from its type locality, off Sugashima (Suga Island), Mie, Japan.

Sequences. Sequences from *T. aurantia* sp. nov. were deposited in GenBank with accession numbers shown in Table 8.

Description. *External appearance of the holotype at living state.* Body long and thin, thread-like, body uniformly deep orange in color (Fig. 48a–c) on both sides except a semi-translucent head, colorless anterior end, and paler posterior end (Fig. 48b, c), orange appearance due to internal body structures visible through the body, margin colorless, no markings, narrows from anterior region towards head (Fig. 48b, c), when contracted, anterior-most body to head noticeably convoluted (Fig. 49a, b); head not demarcated from the rest of the body, narrow spearhead in shape (i.e., resembles a viper's head), obviously thin when worm stretches at non-anaesthetized state, broadens or bulges immediately after the cerebral ganglia region then narrows anteriorly (Fig. 48b), opaque or colorless at the tip, opaqueness extend mid posteriorly until cerebral ganglia (Fig. 49a); four longitudinal rows of laterally distributed reddish-purple ocelli visible at dorsal view

of the head, two ocelli rows on each side, 26–28 in total number, anterior-most ocelli do not reach head tip, posterior-most immediately in front of the cerebral ganglia (Fig. 49a, b), one row of ocelli on each side of the head visible at ventral view (10–12 in number) (Fig. 49b); cerebral ganglia visible as a pair of round, light orange or pinkish structures at the mid posterior head on both sides (Figs. 48b, 49a, b); cerebral organ not discernable at dorsal view but slightly notable at ventral view (Fig. 49b); cephalic furrow not evident at dorsal view, apparent ventrally as shallow ‘Λ’ with the corner directing anteriorly, located in front of cerebral ganglia, before the posterior-most ocelli (Fig. 49b); posterior tip of the body rounded.

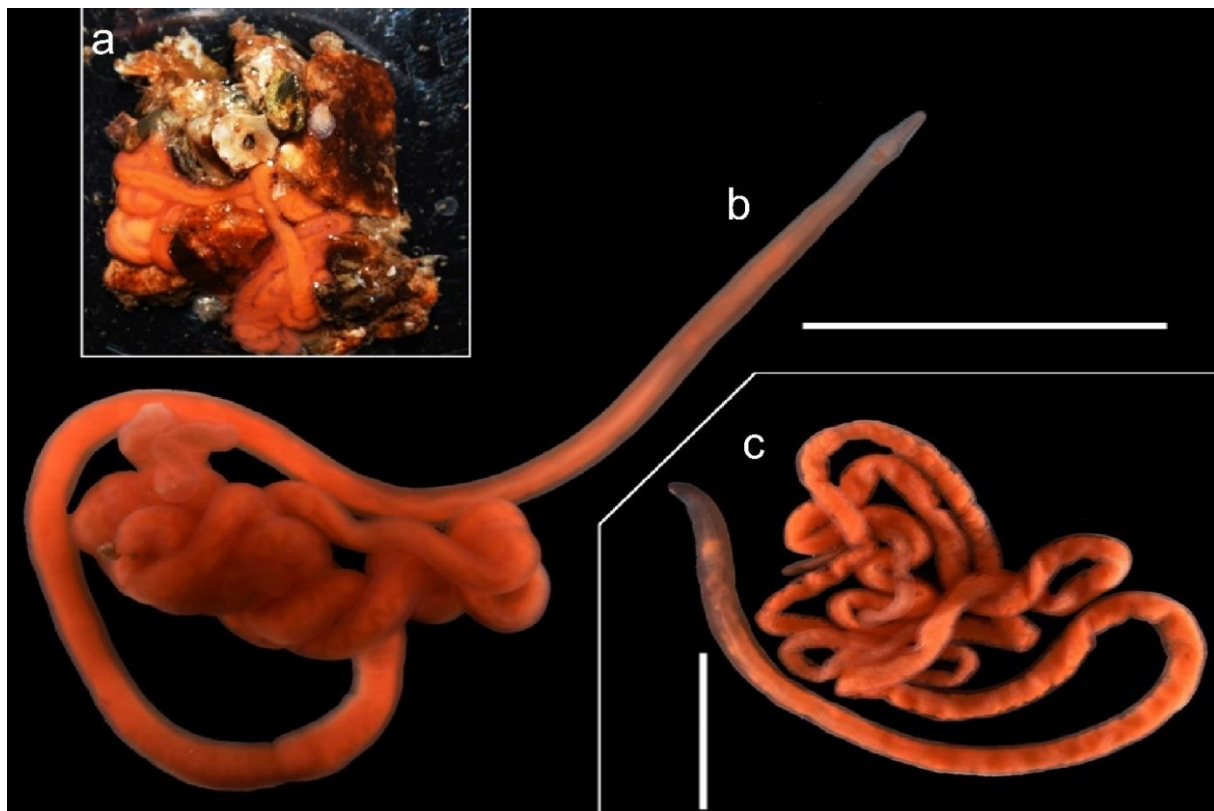


Figure 48. *Tetranemertes aurantia* sp. nov., holotype (ICHUM 8719), photographs in living state. (a, b), before anesthetization; (c), anesthetized state. Scale bars: b, c, 1 = cm (not available for a).

Rhynchocoel and proboscis. Rhynchocoel restricted to anterior-most region of body, approximately within 2 cm from the tip of the head (Fig. 48b); proboscis transparent in color, with two distinct regions separated by a proboscis bulb, diaphragm dark brownish, dotted with reddish-purple pigments at high magnification (Fig. 49g) armed with a single

central stylet (Fig. 49c) and two overlapping pouches, each holding two accessory stylets (Fig. 49e, f); central stylet shaft straight, glassy, fluted, accessory stylets are the same (Fig. 49d–f), 115 μm in length, 19 μm in width; stylet basis rod-shaped, dark brown, narrower anteriorly, posterior appears bifurcated at right lateral view (Fig. 49c, d), 273 μm in length, 53 μm in width; central stylet to basis ratio 0.42.

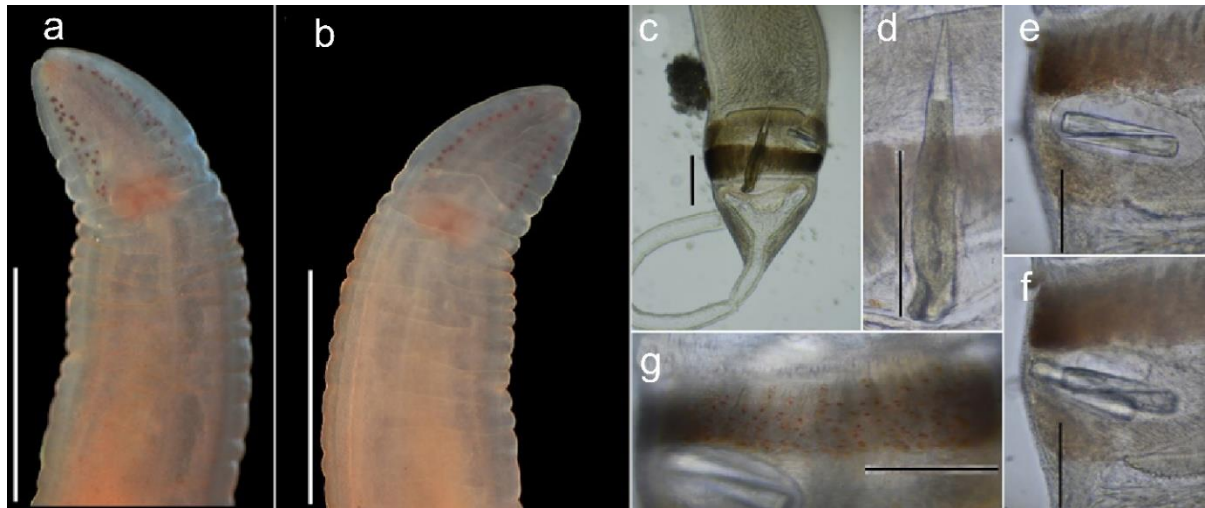


Figure 49. *Tetranemertes aurantia* sp. nov., holotype (ICHUM 8719). (a), magnification of the anterior end of body in anesthetized state, dorsal view; (b), same as A, ventral view, showing 'A'-shaped cephalic furrow; (c), stylet apparatus; (d), magnification of central stylet and basis; (e, f), accessory stylets; (g), pigments around the diaphragm. Scale bars: a, b = 2 mm; c, d = 200 μm ; e–g = 100 μm .

Taxonomic remarks. The new species can be distinguished from the named *Tetranemertes* based on morphology and DNA sequences. Morphological features such as deep orange body color and reddish-purple ocelli are unique to *T. aurantia* sp. nov. These combined morphological characteristics discriminate the new species from the pale to deep pinkish *T. arabica*, the white or yellowish and mid-dorsally striped *T. rubrolineata*, the pale yellowish orange to pale pink and mid-dorsally striped *T. unistriata*, from the dark pink (wine) colored *T. antonina*, from the pale yellow to orangish yellow *T. pastafariensis*, from the uniformly intense pink *T. majinbuui*, from the pale yellow or deep-pinkish orange *T. ocelata*, from the dusky pink *T. hermaphroditica*, and from the multi-colored and stripped and spotted *T. bifrost*. *Tetranemertes aurantia* sp. nov. exhibits color resemblance with the orange-colored *T. paulayi* but both species differ in the number of ocelli and the morphology of the stylet basis.

Tetranemertes kandai (Kato, 1939) comb. nov. (Fig. 50a, b).

Emplectonema kandai Kato, 1939: 251–253, figs. 1, 2, pls. 1–6; Kanda 1939: 166–173, figs. 1–4.

Diagnosis. A luminescent, reddish-orange *Tetranemertes*, anterior end almost colorless while posterior end light yellow, ocelli on each side of the head in linear groups, 15–20 in number per group, with hair-like projections on the anterior and lower lateral edges of the head, central stylet and accessory stylets slender, acutely pointed, longitudinally fluted, basis rounded, and posteriorly bifurcated, stylet to basis ratio 1.45.

Taxonomic remarks. *Emplectonema kandai* was established based on material associated with the solitary ascidian *Chelyosoma siboja* Oka, 1906 living on a sandy or muddy bottom, 30–40 m deep in Aomori Bay near the Asamushi Marine Biological Station. This nemertean can emit a pale green luminescence along the entire body length in response to both chemical and mechanical stimuli (Kanda 1939). In life, *E. kandai* is filiform in shape, dorso-ventrally flat, 10–15 cm to more than 100 cm in length, 0.1 cm in width, uniformly reddish-orange in body color, with the anterior end being almost colorless, posterior end light yellow; the ocelli are arranged in a linear group, 15–20 in number, each dorsally and ventrally on both sides (Fig. 50a); the central stylet is slender, acutely pointed, 0.16 mm in length, glassy, and conspicuously fluted longitudinally; the stylet basis is club-shaped, 0.11 mm in length, with roundly bifurcated terminations; two stylet pouches each has two accessory stylets shorter than the central stylet, fluted as the latter (Fig. 50b). The stylet/basis ratio is 1.45.

While members of the two eumonostiliferous hoplonemertean genera *Emplectonema* and *Tetranemertes* commonly have an elongated body, they differ in the arrangement of the ocelli: four groups or two irregular rows in *Emplectonema* versus four longitudinal rows, with two overlapping dorsoventrally on each side, in *Tetranemertes*. The type species of *Emplectonema* was recently discovered to be *Borlasia camillea* Quatrefages, 1846 (now *Emplectonema neesii* or *Neesia neesii*), instead of *Emplectonema viride* Stimpson, 1857 as had long been accepted among nemertean specialists. In response to this, a case has been submitted to the International Commission on Zoological Nomenclature (ICZN) for a ruling to set aside the original type

fixation and designate *E. viride* as the type species (Kajihara et al. 2021); under Article 82.1 of the *Code* (ICZN 1999), *E. viride* is deemed as the type species until a ruling is published. Irrespective of the overlooked type species, the genus concept for *Emplectonema* has been vague, resulting in almost like a catch-all taxon consisting of any thin-bodied eumonostliferans.

Among 22 species listed as members of *Emplectonema* (Norenburg et al. 2024), the only true congener other than the type species would be *E. gracile* (Johnston, 1837); *Emplectonema friederichi* Sánchez, 1973 may be a junior synonym of the type species *E. viride*. Other species currently in *Emplectonema* will probably be transferred to different genera in future taxonomic studies; *E. mitsuii* (in addition to *E. kandai*) is more closely related to other eumonostliferans than to *E. gracile* and *E. viride* (Kajihara et al. 2011; present study). No matter which of *B. camillea* or *E. viride* becomes the type species of *Emplectonema* upon the ICZN ruling, *E. kandai* should be transferred to *Tetranemertes*. Among the 22 *Emplectonema* species (Norenburg et al. 2024), *E. kandai* is the only one where the ocelli are arranged in four rows, as stated and illustrated in its original description (Kato 1939; fig. 1a, b). I herein propose to transfer the species to *Tetranemertes*, yielding a new combination, *T. kandai* comb. nov.

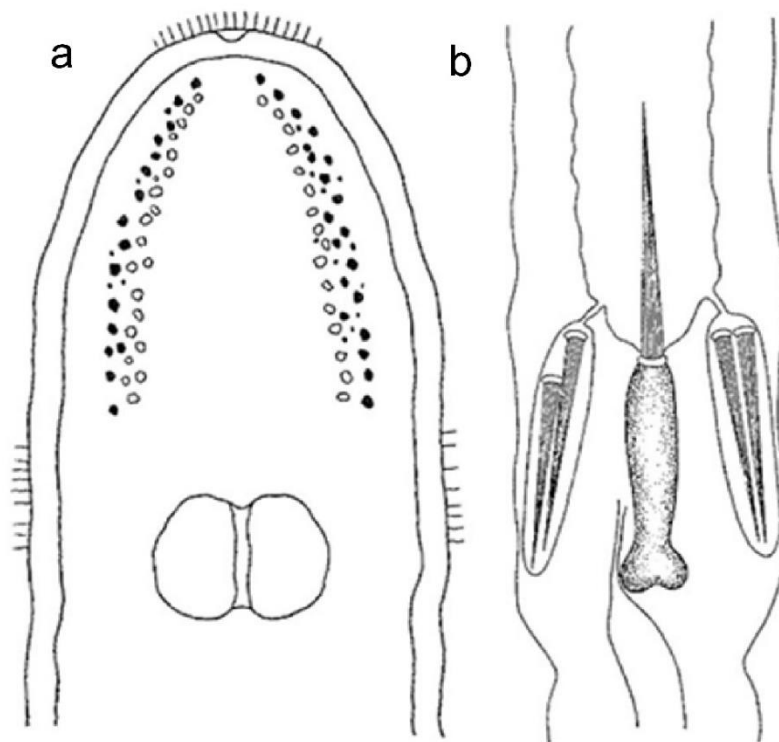


Figure 50. *Tetranemertes kandai* (Kato, 1939) comb. nov. (a), anterior end of body; (b), stylet apparatus. Reproduced from Kato (1939: figs. 1, 2). Copyright © 1938 Japanese Society of Zoology.

Tetranemertes unistriata Cherneva, Ellison, Zattara, Norenburg, Schwartz, Junoy, & Maslakova, 2023 (Fig. 51a–e).

Tetranemertes unistriata Cherneva et al., 2023: 188, fig. 7a–h.

Taxonomic remarks. *Tetranemertes unistriata* was established based on specimens from Oman and Japan (COI *p*-distance is 3.1%), the latter was found among calcareous algae taken on Engetsu Island, Shirahama, Japan (Cherneva et al. 2023). It is one of two congeners that have a mid-dorsal reddish stripe (Fig. 51a, d, e), the other being *T. rubrolineata* (Kirsteuer, 1965). The body color in life varies from pale yellowish orange to pale pink. A pair of ventral anterior cephalic furrows nearly fused mid-ventrally to form a shallow ‘Λ’, with the corner directing anteriorly (Fig. 50b). The central stylet basis is cylindrical and deeply forked posteriorly (Fig. 51c).

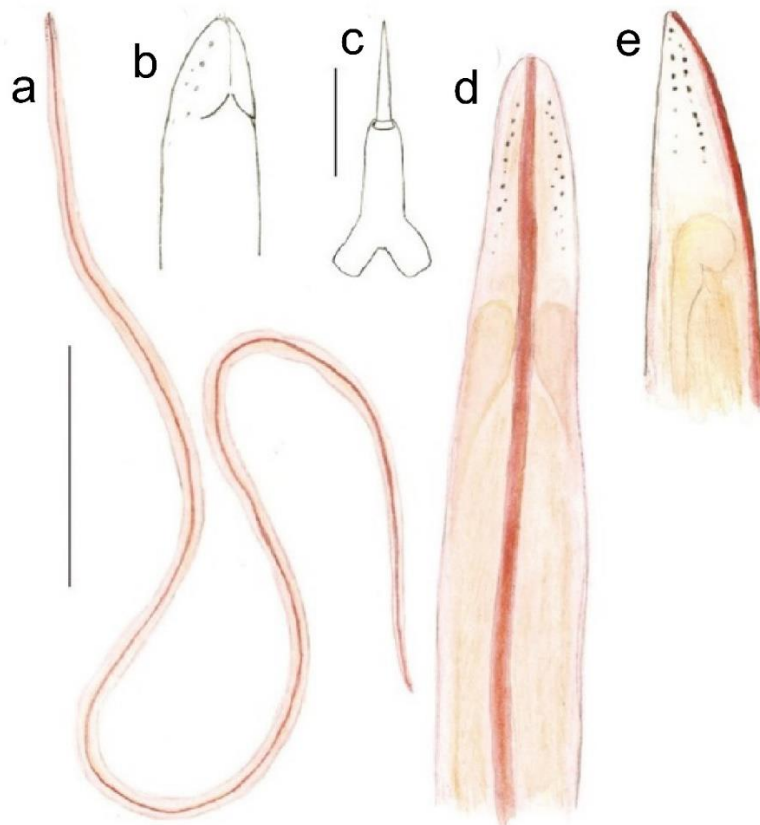


Figure 51. *Tetranemertes unistriata* Cherneva et al., 2023, drawings made by Svetlana A. Maslakova based on three specimens collected from among calcareous algae taken at Engetsu Island, Shirahama, Wakayama, Japan in July 1999. Reproduced from Cherneva et al. (2023: fig. 7a–e). Copyright © 2023 Irina Cherneva et al. (CC BY 4.0).

***Tetranemertes* sp. AHK-2024-1 (Fig. 52).**

Material examined. One specimen, collected on May 27, 2001 from between the base of a sponge and its attaching rock, obtained by beam-trawling from a depth of 288 m at 28°08.95'N, 129°6.85'E, approximately 6–7 km west of Kakeroma Island in the Amami Islands, which is part of the Ryukyu Islands, in the East China Sea, during the 3rd research cruise in F.Y. 2001 of the TR/V *Toyoshio-maru*.

Sequence. PQ564663, COI (658 bp).

Description. Body 40 cm in length and 1.3 mm in width, transparent and color-less; rhynchocoel extending for 1 cm from anterior end; intestine reddish orange; eyes orange in color, ~67 in total number, arranged in four lines (Fig. 52); brain whitish; pair of ventral cephalic furrows meeting medially, forming 'Λ'-shape with apex pointing anteriorly.

Remarks. Due to the lack of certain morphological data, especially those of the stylet apparatus, the material is left undescribed despite being morphologically and molecularly distinct from the named *Tetranemertes*.

The specimen of *T. sp.* AHK-2024-1, preserved in 99% ethanol, had been left at room temperature for 23 years before DNA extraction. The DNA has likely been fragmented, as PCR amplification using the LCO1490/HCO2198 primer pair, expected to yield a 685-bp fragment, failed. To obtain the COI sequence from this specimen, I designed the specific primers TkaCOF/TkaCOR based on aligned sequences from the congeners. In combination with the LCO1490/HCO2198 primers, I obtained 339-bp and 434-bp fragments of the Folmer region. This suggests that the application of so-called next-generation sequencing (such as Illumina MiSeq) would retrieve a greater amount of

sequence information even from ethanol-preserved specimens kept at room temperature for ~20 years.



Figure 52. *Tetranemertes* sp. AHK-2024-1, anterior end of body, dorsal view.

Tetranemertes sp. AHK-2024-2 (Fig. 53).

Emplectonema kandai: Kajihara 2017: 435, fig. 6.4n–p.

Material examined. One specimen, collected intertidally from a tidal flat near the mouth of Aburatsubo Cove, Misaki, Kanagawa, Japan, by Dr Yuji Ise on May 18, 2007.

Sequence. PQ564664, COI (538 bp).

Description. Body 50 cm in length, 1 mm in width; anterior end yellowish white, intestinal region red to reddish brown (Fig. 53a). Ocelli arranged in four rows, with two rows above the other two, each consisting of ~10–13 eyes (Fig. 53b). Central stylet—albeit deformed after fixation—approximately 60 μ m in length; basis 150 μ m in length, posteriorly bilobed (Fig. 53c); stylet/basis ratio 0.4.

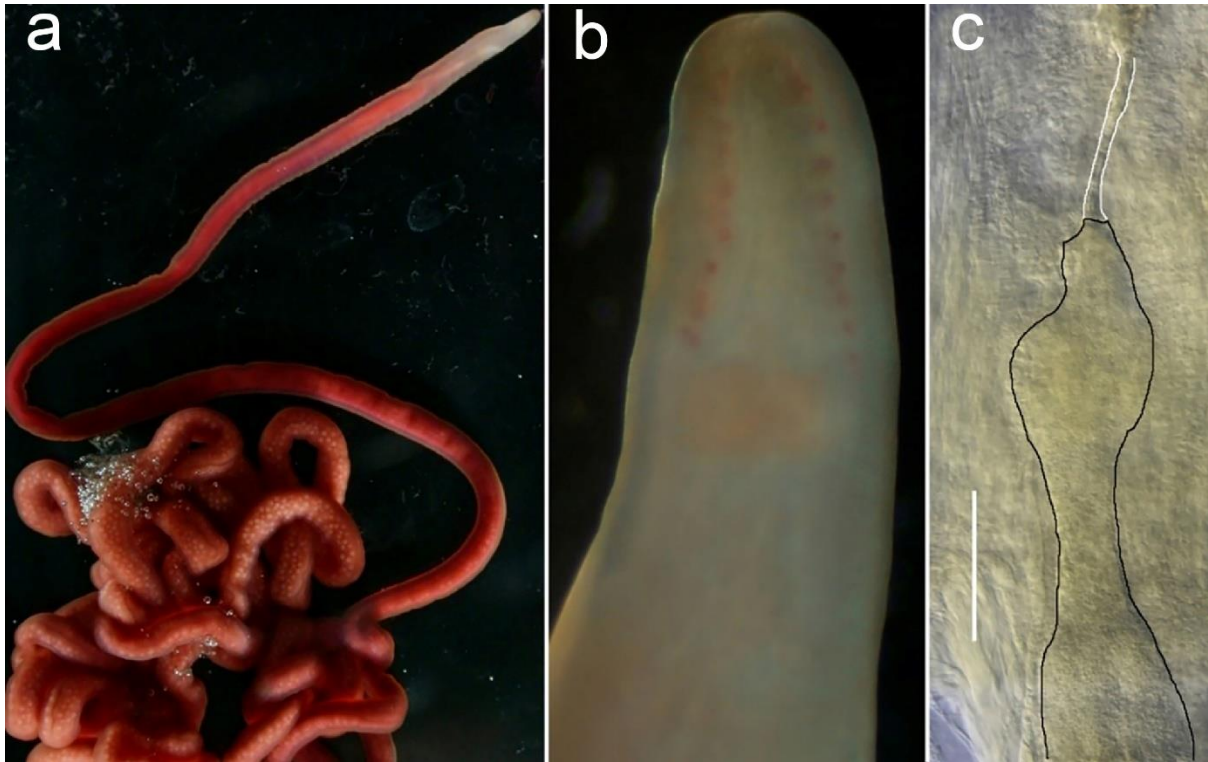


Figure 53. *Tetranemertes* sp. AHK-2024-2. (a), entire specimen; (b), magnification of head, dorsal view; (c), central stylet (outlined in white) and basis (outlined in black) (deformed after fixation), cleared in xylene, taken from Bouin-fixed specimen. Scale bar: c = 50 μ m.

Taxonomic remarks. Kajihara (2017) misidentified the specimen from Misaki as *E. kandai* (now *T. kandai*) without examining the central stylet and basis. In this study, the stylet/basis ratio in this Misaki specimen (0.4) is shown to be quite different from that of *T. kandai* (1.45). This species should be fully described when more complete information is obtained.

Results and systematic remarks

Phylogeny and genetic distance analysis

Tetranemertes aurantia sp. nov. forms a highly supported clade along with other members of *Tetranemertes* and is sister to a clade consisting of *T. arabica* Cherneva et al., 2023, *T. rubrolineata* (Kirsteuer, 1965), and *T. unistriata* Cherneva et al., 2023 (Fig. 54) with uncorrected pairwise genetic distances of 11%, 12%, and 11.4–12.8%, respectively (Table 9). *Tetranemertes* sp. AHK-2024-1 forms a clade with *T. antonina* (11%) and *T. paulayi* (14.1%), and is sister to the former while *T.* sp. AHK-2024-2 forms a clade with *T.*

pastafariensis (10.8%) and with another undescribed congener *T. sp.* CNP-2478 (12.2%) from Argentina and is closest to *T. aurantia* sp. nov., *T. bifrost*, *T. majinbuui*, *T. rubrolineata*, and *T. unistriata* from Oman (11.4%) (Table 9).

Species diversity. My study expanded the number of known *Tetranemertes* species in Japanese waters from one to five, although two of them were left undescribed. Certain congeners are quite common in some parts of the world and habitats—associated with coral rubble in the Caribbean and Oman (Cherneva et al. 2023), Madagascar (Kirsteuer 1965), Red Sea (Maslakova, unpublished). Moreover, several potentially new species resembling *T. rubrolineata* in the Red Sea, another new species in Guam (Maslakova, unpublished), another in Australia (Audrey Falconer, pers. comm.), and another new species in Argentina (Alfaya et al. 2023). Despite being common in some parts of the world, my impression is that ribbon worms of *Tetranemertes* occur quite rarely in Japan and it will take a long time to fully understand the actual species diversity of this genus in Japanese waters.

Systematics. The monophyly of the genus *Tetranemertes* was shown by Cherneva et al (2023) by subjecting nine named and one undescribed species to a multi-locus phylogenetic analysis. However, the phylogenetic position of *Tetranemertes* within Oerstediiina was not settled. Chernyshev et al. (2021) initially showed that *T. antonina* is closely related to *Amphiporus* cf. *cruentatus*. In Cherneva et al. (2023), *Tetranemertes* was sister to the clade formed by *Antarctonemertes riesgoae* Taboada, Junoy, Andrade, Giribet, Cristobo & Avila, 2013, *Vieitezia luzmurubeae* Junoy, Andrade & Giribet, 2010, and *Oerstedtia* sp. My phylogenetic analysis confirmed the placement of the genus *Tetranemertes* within Oerstediiina, being closely related to *A. cf. cruentatus*, both form a clade with *Nemertillina yamaokai* Kajihara, Gibson & Mawatari, 2000. This relationship was not shown in Cherneva et al. (2023) since the authors did not include *A. cf. cruentatus* and *N. yamaokai* in their phylogenetic analysis. The ratio of analyzed species of *Tetranemertes*/Oerstediiidae was 1/32 in Cheryshev et al. (2021), 21/24 in Cherneva et al. (2023), and 18/56 in my analysis. Additionally, I included in my analysis COI sequences of a new and undescribed species of the genus from Argentina, identified as *Monostilifera* sp. 2 CNP-2478 and *Monostilifera* sp. 2 CNP-2561 (Alfaya et al. 2023). It is

no doubt that this species is another undescribed *Tetranemertes*. Morphologically, the oerstediid affinity of *Tetranemertes* was assumed by Kajihara (2021) based on the descriptions of *T. rubrolineata* by Kirsteuer (1965) and *T. hermaphroditica* by Gibson (1982), where these keen observers did not find a single mid-dorsal vascular plug.

Based on the morphological analyses in Cherneva et al. (2023) and in the present study, it can be assumed that *i*) the arrangement of the eyes in two lines on the ventral and dorsal sides of the head, and *ii*) the basis posteriorly slightly bilobed or deeply forked (i.e., in fully grown individuals) are indeed possible synapomorphies of the genus *Tetranemertes*. Of the named congeners, only *T. paulayi* has the non-bifurcated, rounded stylet basis. Although this is also true with the type species of the genus, *T. antonina*, future specimens of this species should be re-examined for their stylet basis morphology (see Cherneva et al. 2023). Nonetheless, the bilobed stylet basis appears to be common in most *Tetranemertes* having it observed also in *T. aurantia* sp. nov. and *T. kandai* comb. nov. Additionally, current congeners are the same when it comes to body shape, cephalic furrow morphology, and length of the rhynchocoel and proboscis (Cherneva et al. 2023). These characters can be used to morphologically identify future members of *Tetranemertes*.

Ommatoplea ophiocephala was originally described from South Africa by Schmarda (1859), as reaching 1 m in body length, dark yellow in color, with a laceolate head, on each side of which eyes are arranged in two longitudinal lines. Wheeler (1934) redescribed this species as *Emplectonema ophiocephala* using specimens collected from Saldanha Bay, South Africa, and provided a more detailed morphological description—head distinct from the body, flattened, lanceolate, posteriorly broader than succeeding body parts, anteriorly tapers to an acute snout; a pair of faintly marked sloping lateral grooves apparent behind the head; with elongated groups of small eyespots on each side of the head, about 20 in each group, spreading out posteriorly, when seen partly from the side or from above, each group can be divided into two rows (Wheeler 1934, fig. 11a, b); brain pinkish, brownish or greyish, bilobed, visible through the skin, behind the groups of eyespots (Wheeler 1934, fig. 11a); the rhynchocoel does not extend beyond the anterior third of the body, greatly restricted by the closely opposed ganglia; proboscis short, and thin (Wheeler 1934, fig. 11c). The morphological characteristics of *E. ophiocephala* presented by Wheeler (1934) and herein emphasized

are combined morphological features common to ribbon worms in the genus *Tetranemertes*. Although its stylet basis is rounded and non-bifurcated (Wheeler 1934, fig. 11c), as in *T. antonina* and *T. paulayi*, the arrangement of the ocelli (in combination to the morphological features herein emphasized) in *E. ophiocephala* (as with *O. taeniata*) makes it more morphologically related to *Tetranemertes* rather than *Emplectonema*. This morphology-based claim that *E. ophiocephala* is likely a species in the genus *Tetranemertes* can be further backed up by a phylogenetic analysis using new material from which DNA is extracted.

Cherneva et al. (2023) established the species *T. unistriata* based on material collected from Japan and Oman, designating one of the Japan specimens as the holotype. Despite the substantial habitat separation and a COI *p*-distance of 3.1% between the Japan and Oman specimens they opted to recognize the specimens conspecific. The Japan specimen has more ocelli, non-fluted or grooved central stylet, and deeply forked stylet basis while the Oman specimen has fewer ocelli, fluted central stylet (and accessory stylets), and posteriorly non-bifurcated and rounded stylet basis. Only one species of Eumonostilifera has been reported from both the Pacific and Indian Oceans, *Tetrastemma freyae* Chernyshev, Polyakova, Vignesh, Jain, Sanjeevi, Norenburg & Rajesh, 2020 with a COI *p*-distance of 1.3%, confirming conspecificity, despite the significant geographical range separating the species. The findings of a eumonostiliferan considered conspecific, despite significant COI genetic distance (see Sundberg et al. 2016), in the Pacific and Indian Oceans is very unusual. Bottom-dwelling hoplonemerteans are not characterized by a very wide distribution, unlike most of heteronemerteans (Chernyshev, pers. comm.).

Table 9. Uncorrected pairwise *p*-distances (%) in terms of 519-bp COI sequences between the current *Tetranemertes* congeners.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. <i>T. antonina</i> (KF935534)															
2. <i>T. arabica</i> (OQ321709)	14.1														
3. <i>T. aurantia</i> sp. nov. (PP413702)	14.1	11.0													
4. <i>T. bifrost</i> (MT578867)	13.5	12.8	12.0												
5. <i>T. majinbuui</i> (MT578865)	11.4	10.8	12.4	11.2											
6. <i>T. ocelata</i> (MW021887)	11.8	11.0	11.6	12.0	11.4										
7. <i>T. pastafariensis</i> (MT578887)	13.0	11.6	11.2	11.2	12.2	11.0									
8. <i>T. paulayi</i> (OQ321715)	10.4	12.0	12.8	14.1	11.6	12.2	14.7								
9. <i>T. rubrolineata</i> (OQ321723)	13.0	10.1	12.0	11.6	12.8	11.0	11.6	13.5							
10. <i>T. unistriata</i> Japan (MT578861)	13.9	9.3	12.8	10.8	12.0	10.8	11.8	15.1	9.5						
11. <i>T. unistriata</i> Oman (OQ321719)	12.8	9.3	11.4	10.6	11.2	11.0	11.8	14.7	8.9	3.1					
12. <i>T. sp.</i> AHK-2024-1 (PQ564663)	11.0	14.7	13.7	13.5	12.4	12.6	11.8	14.1	13.9	13.5	12.4				
13. <i>T. sp.</i> AHK-2024-2 (PQ564664)	14.5	11.8	11.4	11.4	11.4	12.2	10.8	12.8	11.4	13.3	11.4	13.5			
14. <i>T. sp.</i> CNP-2478 (OP104783)*	12.8	11.8	12.6	11.4	13.5	11.4	9.5	13.3	12.0	12.8	11.2	14.1	12.2		
15. <i>T. sp.</i> ETP001 (MT578897)	12.6	9.9	11.8	7.7	9.7	11.8	11.8	11.8	12.2	11.8	11.4	13.5	11.8	12.0	

*Originally identified as *Monostilifera* sp. 2 CNP-2478 in Alfaya et al. (2023), treated in this manuscript as *Tetranemertes* based on the phylogenetic analysis.

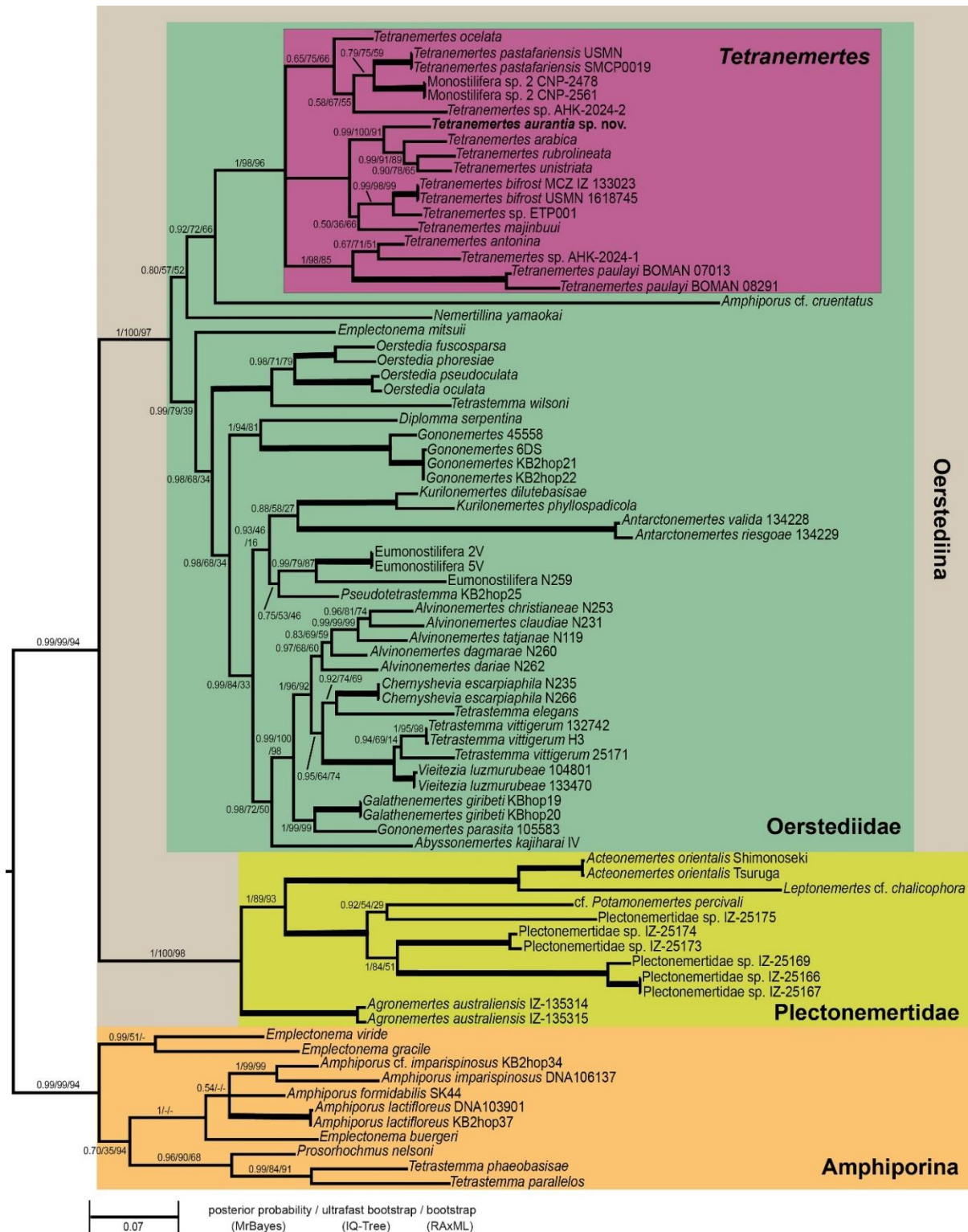


Figure 54. Rooted Bayesian phylogenetic tree showing the position of *Tetranemertes aurantia* sp. nov. within the genus *Tetranemertes* based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers represent branch support—Bayesian posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branch indicates full support (1.00 PP/100% UFBoot/100% BS).

Bioluminescence. None of the three specimens in this study (*T. aurantia*, *T. sp.* AHK-2024-1, *T. sp.* AHK-2024-2) was examined as to bioluminescence simply because the collector of each specimen had no idea that the material would be potentially bioluminescent. This study urges future researchers to test bioluminescence of any fresh *Tetranemertes* samples upon collection by putting them in a dark place and applying “mechanical, chemical, thermal or electrical” stimuli (Kanda 1939: 167). Not only *T. kandai* but also other congeners may be bioluminescent.

Conclusions

I expanded the diversity of the genus *Tetranemertes* in Japanese waters by reporting a new species, *T. aurantia* sp. nov. and two other undescribed ones such as *T. sp.* AHK-2024-1 and *T. sp.* AHK-2024-2 and transferring *T. kandai* comb. nov., in addition to the previously described *T. unistriata*. The possible synapomorphy within the genus is the arrangement of the ocelli, which forms four longitudinal rows, with two on each side of the head, the two rows almost on top of one another. I proposed that *Ommatoplea* could be a senior synonym of *Tetranemertes* and that *O. taeniata* could possibly be a *Tetranemertes*. Similarly, *Emplectonema ophiocephala* could be more related to *Tetranemertes* and should be transferred to the small genus.

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Chapter 4: Taxonomy and systematics of deep-sea nemerteans from Japan

Exploration of deep-sea nemertean diversity (i.e., species diversity at depths ≥ 1000) is hampered by the challenging and resource-demanding procedures to access deep-sea habitats. In addition, the inherent delicate and fragile bodies of nemerteans often result in damaged and incomplete or fragmented specimens upon being retrieved from the deep, rendering them often unidentified and unnamed. There are only limited studies on deep-sea nemerteans. Such studies included specimens from the Vema Fracture Zone in the Atlantic Ocean; Costa Rica margin and Juan de Fuca Ridge, off the Pacific coast of the United States; Nishi-Shichito Ridge; Kuril-Kamchatka Trench (KKT); Japan Trench (JT), all in the western Pacific region. Most of the information I currently know with respect to deep-sea nemertean diversity was obtained from faunal surveys conducted in the last decade (Kajihara & Lindsay 2010; Chernyshev 2013; Chernyshev et al. 2015; Chernyshev & Polyakova 2018a, 2018b, 2019, 2022, 2023; Hookabe et al. 2022a, 2022b; Sagorny et al. 2022; Chernyshev & Kuznetsov 2024).

In Japanese waters, only two deep-sea species have been established since 2010: *i) Dinonemertes shinkaii* Kajihara & Lindsay, 2010 from JT and *ii) Genrokunemertes obesa* Hookabe et al., 2022a from Nishi-Shichito Ridge. Although the portion of the KKT in the Russian waters was extensively surveyed for deep-sea nemerteans, the part of the trench within Japanese territory has never been explored for this taxon. Similarly, deep-sea nemertean diversity in JT has never been explored following the discovery of *D. shinkaii*, making the knowledge of nemertean diversity in this region unknown to the present.

This chapter I present the deep-sea nemerteans collected in the JT and KKT onboard the R/V *Hakuho-maru* in 2022 and 2023. Here, a comprehensive deep-sea nemertean species in the JT is presented and the species composition in the KKT is updated. A total of 80 nemertean specimens composed of 30 putative species were collected from the surveys. From the 30 species, two deep-sea monostiliferous hoplonemerteans are herein established as new: *i) Nemertovema kojimai* sp. nov. collected from JT and *ii) Profundonemertes hakuhoae* gen. et sp. nov., from the JT and KKT. This study contributed to the knowledge on the diversity of nemerteans from the deep.

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Chapter 4.1. Ribbons from the deep: deep-sea nemerteans collected along the Japan Trench and the adjacent Kuril-Kamchatka Trench onboard the R/V *Hakuho-maru* with descriptions of *Nemertovema kojimai* sp. nov. and *Profundonemertes hakuhoae* gen. et sp. nov. in Hoplonemertea

Introduction

The phylum Nemertea, commonly known as nemerteans or ribbon worms, comprises approximately 1350 species (Norenburg et al. 2024). Most described species inhabit the littoral and sublittoral zones of the world's oceans, with 33 known species of deep-sea representatives, despite evidence of high species diversity below depths of 3000 m (Chernyshev 2020). The challenges of sampling deep-sea nemerteans, combined with their delicate, soft, and fragile bodies, often result in damaged, incomplete, or fragmented specimens that are difficult to identify and unsuitable for traditional species descriptions. As a result, early studies frequently referred to such specimens as 'Nemertea sp.' (Belyaev 1966, 1989; Mokievsky 1954), a practice that obscured the true diversity of deep-sea nemerteans. For example, the abyssal and hadal specimens collected during the Soviet deep-sea expeditions from the 1950s to the 1970s remain unstudied (Belyaev 1989).

In the last decade, deep-sea explorations have significantly advanced the understanding of nemertean diversity in deep habitats. These research efforts have revealed a more accurate picture of the group's diversity and suggested that many undiscovered species remain hidden from the bathyal to hadal zones (Chernyshev 2013; Chernyshev et al. 2015;; Chernyshev & Polyakova 2018a, 2018b, 2019, 2022, 2023; Hookabe et al. 2022a, 2022b; Sagorny et al. 2022; Chernyshev & Kuznetsov 2024). This progress has been facilitated by the advent of molecular taxonomy, enabling researchers to identify both intact and fragmented specimens down to the species level through the use of cytochrome c oxidase subunit I (COI) barcode sequence data from public databases.

The so-far-known nemerteans in deep-sea environments are predominantly pelagic, with 101 species identified to date, all belonging to Hoplonemertea Coe, 1905. This includes two named and two undescribed species in Monostilifera Brinkmann, 1917 (Wheeler 1934; Korotkevich 1961; Crandall & Gibson 1998; Chernyshev 2005; Crandall

2006), while the remaining 99 species are classified in *Pelagica* Brinkmann, 1917, a subgroup of *Polystilifera* Brinkmann, 1917 (Maslakova & Norenburg 2001; Kajihara & Lindsay 2010). Members of *Pelagica* are abundant in depths of 600 to 3000 m (Roe & Norenburg 1999; Maslakova & Norenburg 2001; Kajihara & Lindsay 2010), although they can be found as deep as 9000 m (Chernyshev 2013).

Before 2013, only 13 species of deep-sea benthic nemerteans were known from depths of ≥ 1000 m (Chernyshev 2013). However, intensive deep-sea research expeditions over the past decade have led to the description of 18 new species, alongside the discovery of two known species not initially considered deep-sea inhabitants. These include two species in *Palaeonemertea* Coe, 1905, four in *Pilidiophora* Thollessen & Norenburg, 2003, and 14 in *Hoplonemertea* (Table 10). This has brought the current number of known deep-sea benthic nemertean species to 33 (Chernyshev 2013; Chernyshev et al. 2015; Chernyshev & Polyakova 2018a, 2018b, 2019, 2023; Hookabe et al. 2022a; Kajihara et al. 2022a; Sagorny et al. 2022).

The deepest recorded pelagic nemertean is *Plenanemertes rubella* Coe, 1954, collected from depths of 4000–6000 m, while the deepest known benthic nemertean is *Nemertovema hadalis* Chernyshev & Polyakova, 2018a, found at 8336–8339 m. Even so, benthic nemerteans have been recorded at even greater depths, with specimens retrieved from as deep as 9577 m, the current maximum depth for benthic ribbon worms (Chernyshev & Polyakova 2019).

Prior to this study, the only documented nemertean from the Japan Trench (JT) was *Dinonemertes shinkaii* Kajihara & Lindsay, 2010, collected in 2000 at a depth of 2343 m. While deep-sea nemertean expeditions have been extensively conducted in the adjacent Kuril-Kamchatka Trench (KKT)—notably during the KuramBiol expedition in 2012, SokhoBio in 2015, and KuramBiolII in 2016 (Chernyshev 2020)—providing valuable insights into the diversity of ribbon worms in that region, the species composition in the JT remains largely unknown. To address this gap and enhance the data on ribbon worm diversity for both the JT and KKT (i.e., part of the KKT within the Japan territory) specimens were collected from the two trenches during research expeditions aboard the R/V *Hakuho-maru* in October 2022 and September 2023. I identified the collected nemerteans to the species level when possible, based on DNA barcode sequences and morphological characteristics. In this study I describe two new species and establish one

new genus of Eumonostilifera based on molecular data alongside brief external morphological descriptions. In addition, histological sections were examined to provide further morphological details for the new species placed in the new genus. I also report four previously known ribbon-worm species, recorded for the first time in Japanese waters, updating their geographical and depth-range distributions in the deep ocean. An integrative taxonomic approach was employed to describe the new species and identify the nemerteans collected from the JT and adjacent KKT.

Table 10. Known benthic nemertean species collected from depths ≥ 1000 meters since 2013 to present. In bold are new species established in this chapter.

Species	Depth (m)	Locality	Reference
Palaeonemertea			
<i>Cephalothrix iwatai</i>	1494–3334	NW Pacific (Sea of Japan)	Chernyshev (2013)
<i>Tubulanus cf. lutescens</i>	966–1045	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
Pilidiophora			
<i>Baseodiscus profundus</i>	3300–3306	NW Pacific (Sea of Okhotsk)	Kajihara et al. (2022a)
<i>Micrura bathyalis</i>	2670–3426	NW Pacific (Sea of Japan)	Chernyshev (2013)
<i>Sonnenemertes cantelli</i>	3306–4869	NW Pacific (Sea of Okhotsk and Kuril-Kamchatka Trench)	Chernyshev et al. (2015)
<i>Heteronemertes longifissa</i>	370–1459	Antarctic waters	Chernyshev & Polyakova (2023)
Hoplonemertea			
<i>Abyssonemertes kajiharai</i>	5510–5514	NW Atlantic (Vema Fracture Zone)	Chernyshev & Polyakova (2018a)
<i>Alvinonemertes christianeae</i>	1887–2184	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
<i>Alvinonemertes claudiae</i>	2250	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
<i>Alvinonemertes dagmarae</i>	1410	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
<i>Alvinonemertes dariae</i>	1407	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
<i>Chernyshevia escarpiaphila</i>	1801–1909	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)

<i>Galathenemertes giribeti</i>	7256	NW Pacific (Kuril-Kamchatka Trench)	Chernyshev & Polyakova (2019)
<i>Genrokunemertes obesa</i>	2084	NW Pacific (Nishi-Shichito Ridge, Japan)	Hookabe et al. (2022a)
<i>Nemertovema hadalis</i>	8336–8339	NW Atlantic (Puerto Rico Trench)	Chernyshev & Polyakova (2018a)
<i>Nemertovema kojimai</i> sp. nov.	6211	NW Pacific (Japan Trench)	present study
<i>Nemertovema norenburgi</i>	8221–8282	NW Pacific (Kuril-Kamchatka Trench)	Chernyshev & Polyakova (2019)
<i>Oerstedtia sashae</i>	1407	NW Pacific (Emperor Seamounts)	Chernyshev & Kuznetsov (2024)
<i>Proamphiporus crandalli</i>	5495	NW Pacific (Kuril-Kamchatka Trench)	Chernyshev & Polyakova (2019)
<i>Profundonemertes hakuhoae</i> gen. et sp. nov.	6807–7607	NW Pacific (Japan Trench)	present study
<i>Tetrastemma strandae</i>	1908	NE Pacific (Costa Rica margin)	Sagorny et al. (2022)
<i>Uniporus alisae</i>	3301	NW Pacific (Sea of Okhotsk)	Chernyshev & Polakova (2018b)

Materials and methods

Specimen collection

Samples were collected along the JT and KKT aboard the R/V *Hakuho-maru* during the KH-22-8 and KH-23-5 cruises in October 2022 and September 2023, respectively. Bottom sediments were obtained from 58 sampling stations using one of the three gear types: *i*) a beam trawl (BT) with a 4-m-wide aperture; *ii*) an Agassiz trawl (AGT) with a 3-m-wide aperture, and *iii*) an epibenthic sledge (EBS). Each trawl was equipped with inner nets of 330–500 µm mesh size (Akiyama et al. 2008; also see Brandt et al. 2024). The 58 stations were distributed into six transects designated as: *i*) ‘A line’; *ii*) ‘C line’; *iii*) ‘D line’; *iv*) ‘F line’; *v*) St. G-BT; and *vi*) ‘H line’ (Table 11). Sampling stations along the ‘A line’ were 20–30 km northeast of the Erimo Seamount, while those along the ‘C line’ were 99–138

km northeast, thus placing the stations along the KKT. The remaining stations were distributed along the JT.

On the deck, trawled muddy sediments were washed with seawater in plastic containers, and the supernatant was filtered through a sieve basket with a mesh size of 420 μm . The residues on the sieves were examined either with the naked eye or under a stereomicroscope to sort the collected invertebrates into higher taxa. Nemertean specimens were photographed using a Nikon D5700 camera equipped with either an AF-S Micro NIKKOR 40 mm lens or attached to a stereomicroscope. After microscopy, fragmented specimens were preserved directly in 99% ethanol for DNA extraction, while intact specimens were halved, with the anterior preserved in Bouin's fluid and the posterior in 99% ethanol. Voucher materials have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the ethanol-preserved specimens using a modified silica gel method (Boom et al. 1990) or the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. When possible, five gene markers were partially amplified from the extracted genomic DNA: *i*) 16S; *ii*) 18S; *iii*) 28S ribosomal RNA (rRNA); *iv*) cytochrome c oxidase subunit I (COI); and *v*) histone H3. Polymerase chain reaction (PCR) amplification was performed using primer pairs described in Abato et al. (2022). The COI gene was amplified in an Applied Biosystems 2720 thermal cycler under the following standard PCR conditions: 1 min at 98°C, 35 cycles of (10 sec at 98°C, 30 sec at 50°C, 40 sec at 72°C) and 7 min of 72°C. When this did not work, a touch-down PCR using a TaKaRa Thermal Cycler Dice Gradient was performed under the following conditions: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 45°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 35°C, 40 sec at 72°C), followed by 7 min at 72°C. Touch-down PCRs were also performed for the amplification of 16S, 18S, 28S, and H3. The conditions for 16S amplification were as follows: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 50°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C), followed by 7 min at 72°C. Amplification of 18S and 28S followed these conditions: 2 min at 94°C, 10 cycles of (1 min at 94°C, 30 sec at 60°C [decreasing 1°C each time] 90 sec at 72°C), 25 cycles of (1

min at 94°C, 30 sec at 50°C, 90 sec at 72°C), followed by 7 min at 72°C. For H3 amplification: 1 min at 98°C, 10 cycles of (10 sec at 98°C, 30 sec at 50°C [decreasing 1°C each time], 40 sec at 72°C), 25 cycles of (10 sec at 98°C, 30 sec at 40°C, 40 sec at 72°C), followed by 7 min at 72°C. Downstream procedures, including gel electrophoresis, PCR product purification, sequencing, and sequence processing, were performed as described by Abato et al. (2022). The processed sequences were analyzed using MegaBLAST (Morgulis et al. 2008) on the NCBI website (<http://www.ncbi.nlm.nih.gov/>) to initially determine the species or higher taxonomic classification of the collected specimens. Based on the MegaBLAST search results, the sequenced nemerteans were grouped into the classes Palaeonemertea, Pilidiophora, and Hoplonemertea.

Genetic distance calculation and species delimitation analysis

The uncorrected COI *p*-distances within each class were calculated using MEGA ver. 11 (Tamura et al. 2021) to determine genetic variation. Molecular species delimitation analyses were performed to estimate the number of putative species present in each nemertean class, using Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021) and Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012). The species delimitation analyses used the aligned COI sequences of specimens within each class. ASAP was performed on the webserver (<https://bioinfo.mnhn.fr/abi/public/asap/>) with default settings, while ABGD was run on the web interface (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>). For the pilidiophoran COI dataset, ABGD was executed with default parameters, whereas for the palaeonemertean and hoplonemertean COI datasets, the X parameter (relative gap width) was set to 1.0.

Phylogenetic analysis

Partial sequences for the 16S, 18S, 28S, COI, and H3 gene markers from the specimens used in the phylogenetic analysis were generated. Three nucleotide supermatrices, corresponding to Palaeonemertea, Pilidiophora, and Hoplonemertea, were constructed in MEGA ver. 11 (Tamura et al. 2021). Gene sequences from Operational Taxonomic Units (OTUs) for each class were retrieved from GenBank (Table 12) and combined with the newly generated sequences in the present study (Table 13). Per supermatrix, each of the 16S, 18S, and 28S sequence datasets was aligned separately in

MAFFT ver. 7 online version (Kato et al. 2019), applying the E-INS-i iterative refinement method in the advanced settings. COI and H3 sequences were aligned in MEGA ver. 11 using the MUSCLE algorithm (Edgar 2004). Uncertainly aligned sites in each dataset were trimmed using ClipKIT ver. 1.3.0 (Steenwyk et al. 2020). The sizes of the trimmed gene marker dataset for each supermatrix were as follows: *i*) Palaeonemertea, 592 bp for 16S, 2370 bp for 18S, 4633 bp for 28S, 683 bp for COI, and 326 bp for H3; *ii*) Pilidiophora, 644 bp for 16S, 1824 bp for 18S, 2768 bp for 28S, 657 bp for COI, and 337 bp for H3; and *iii*) Hoplonemertea, 561 bp for 16S, 1986 bp for 18S, 1533 bp for 28S, 673 bp for COI, and 324 bp for H3. The alignments for each gene marker dataset per supermatrix were concatenated in MEGA ver. 11.

The best-fit partitions and substitution models for maximum likelihood (ML) and Bayesian inference (BI) analyses were determined using PartitionFinder ver. 2.1.1 (Lanfear et al. 2016), applying the greedy algorithm (Lanfear et al. 2012) (Table 14). ML analyses were conducted in IQ-Tree ver. 2.1.3 (Minh et al. 2020), with branch support values derived from 1000 ultrafast bootstrap replicates (UFBoot) (Minh et al. 2013) and in RAxML ver. 8.2.10 (Stamatakis 2015), where branch support was calculated from 1000 bootstrap pseudoreplicates (BS).

BI was performed using MrBayes ver. 3.2.6 x64 (parallel version) (Ronquist & Huelsenbeck 2003; Altekari et al. 2004), employing two parallel runs, each with four Markov chains (three cold and one hot), for 5×10^7 generations, sampling every 100 generations. Convergence diagnostics indicated that sufficient trees and parameters were sampled (Table 14). Based on the convergence of likelihood scores, 25% of the sampled trees were discarded as burn-in, and the remaining trees were used to generate a consensus tree.

Table 11. Locations (coordinates and depths), type of gears, and sampling dates of the 58 stations surveyed during the cruises KH-22-8 and KH-23-8 of the R/V *Hakuho-maru*.

Station ^a	On-bottom location			Off-bottom location			Gear ^b	Date
	Latitude	Longitude	Depth (m)	Latitude	Longitude	Depth (m)		
A0-BT	41°31.358'N	144°08.595'E	1987	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
A1-BT	41°28.076'N	144°33.195'E	4026	41°28.533'N	144°33.273'E	4028	BT	2 October 2022
A1-EBS	41°27.984'N	144°33.182'E	4028	41°28.617'N	144°33.287'E	4007	EBS	2 October 2022
A2-BT	41°18.499'N	144°43.189'E	5030	41°18.514'N	144°43.974'E	5013	BT	2 October 2022
A2-EBS	41°18.502'N	144°43.038'E	5034	41°18.504'N	144°44.275'E	5015	EBS	3 October 2022
A3-BT	41°20.786'N	144°56.455'E	6116	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
A3-EBS	41°20.808'N	144°56.404'E	6116	41°20.133'N	144°58.291'E	6136	EBS	3 October 2022
A4b-BT	41°11.786'N	144°43.042'E	6799	41°12.354'N	144°43.439'E	6807	AGT	6 October 2022
A5-BT	41°14.024'N	145°01.931'E	7184	41°14.126'N	145°01.108'E	7185	AGT	6 October 2022
A5-EBS	41°14.033'N	145°01.813'E	7185	41°14.225'N	145°00.497'E	7186	EBS	6 October 2022
A7-BT	41°06.287'N	145°00.898'E	6667	41°06.474'N	145°01.808'E	6697	BT	16 September 2023
A7-EBS	41°06.079'N	144°59.117'E	6660	41°05.941'N	144°58.499'E	6660	EBS	17 September 2023
A8b-BT	41°08.465'N	145°39.391'E	6102	41°08.340'N	145°39.034'E	6093	BT	18 September 2023
A8-BT	41°07.030'N	145°35.557'E	6124	41°07.275'N	145°36.368'E	6113	BT	17 September 2023
A9-BT	41°02.076'N	145°47.142'E	5576	41°01.921'N	145°46.385'E	5584	BT	18 September 2023
A9-EBS	41°02.481'N	145°47.613'E	5583	41°02.357'N	145°47.059'E	5585	EBS	18 September 2023
C1-BT	41°39.892'N	145°41.025'E	6374	41°39.661'N	145°40.837'E	6370	BT	20 September 2023
C2-BT	41°38.132'N	145°43.062'E	6835	41°38.096'N	145°42.631'E	6806	BT	20 September 2023
C4-BT	41°38.115'N	145°54.407'E	7288	41°38.254'N	145°54.876'E	7285	AGT	20 September 2023
C5-BT	41°28.411'N	146°06.803'E	6186	41°28.519'N	146°07.632'E	6183	BT	21 September 2023
C7-BT	42°12.080'N	145°40.725'E	3943	42°11.456'N	145°40.909'E	3934	BT	19 September 2023
C7-EBS	42°11.226'N	145°41.188'E	3937	42°10.356'N	145°41.473'E	3938	EBS	19 September 2023
C8-BT	42°05.280'N	145°51.386'E	5083	42°05.250'N	145°50.912'E	5074	BT	19 September 2023
D2-BT	40°55.796'N	144°05.318'E	5110	40°55.800'N	144°06.103'E	5059	AGT	9 October 2022

D3-BT	40°50.207'N	144°21.171'E	6169	40°49.560'N	144°21.543'E	6211	AGT	15 October 2022
D4-BT	40°48.568'N	144°31.361'E	6793	40°49.211'N	144°31.565'E	6756	AGT	14 October 2022
D6-BT	40°48.211'N	144°39.576'E	7455	40°48.899'N	144°39.972'E	7426	AGT	12 October 2022
D7-BT	40°42.724'N	144°41.596'E	7119	40°41.735'N	144°41.975'E	7040	AGT	13 October 2022
D8-BT	40°44.217'N	144°54.575'E	6246	40°44.208'N	144°55.613'E	6242	AGT	13 October 2022
D9-BT	40°23.208'N	144°59.350'E	5991	40°22.690'N	144°58.790'E	5976	BT	14 September 2023
D9-EBS	40°23.258'N	144°59.186'E	5993	40°23.720'N	144°59.701'E	5993	EBS	15 September 2023
D10-BT	40°42.423'N	144°47.597'E	6533	40°41.807'N	144°47.595'E	6551	BT	15 September 2023
D10-EBS	40°42.607'N	144°47.369'E	6538	40°41.812'N	144°47.376'E	6562	EBS	15 September 2023
F0-BT	39°27.733'N	143°42.462'E	3443	39°27.240'N	143°42.189'E	3428	BT	27 September 2023
F0-EBS	39°27.584'N	143°42.647'E	3446	39°27.036'N	143°42.422'E	3430	EBS	28 September 2023
F1-BT	39°27.885'N	143°45.623'E	3693	39°27.285'N	143°45.397'E	3685	BT	27 September 2023
F2-BT	39°28.555'N	143°47.347'E	3893	39°27.934'N	143°47.240'E	3890	BT	29 September 2023
F3-BT	39°26.718'N	143°48.128'E	4116	39°26.687'N	143°48.111'E	4117	BT	28 September 2023
F4-BT	39°26.667'N	143°50.128'E	4358	39°26.962'N	143°49.470'E	4348	BT	29 September 2023
F5-BT	39°32.516'N	143°51.305'E	4556	39°31.887'N	143°51.285'E	4565	BT	27 September 2023
F5-EBS	39°33.583'N	143°51.137'E	4561	39°34.374'N	143°52.026'E	4609	EBS	29 September 2023
F6-BT	39°32.375'N	143°59.769'E	4840	39°31.821'N	143°59.920'E	4845	BT	27 September 2023
F6-EBS	39°32.400'N	144°00.076'E	4826	39°31.767'N	144°00.214'E	4843	EBS	27 September 2023
F7b-BT	39°40.441'N	144°04.508'E	5314	39°40.955'N	144°04.122'E	5313	AGT	30 September 2023
F7-BT	39°39.126'N	144°03.521'E	5170	39°38.847'N	144°03.148'E	5174	BT	26 September 2023
F8-BT	39°41.404'N	144°05.279'E	5738	39°41.023'N	144°05.056'E	5702	BT	26 September 2023
F8-EBS	39°41.385'N	144°05.050'E	5713	39°41.022'N	144°04.833'E	5700	EBS	26 September 2023
F9-BT	39°32.540'N	144°09.524'E	6539	39°31.961'N	144°09.705'E	6523	BT	26 September 2023
F10-BT	39°34.961'N	144°11.014'E	7038	39°34.320'N	144°10.353'E	6871	AGT	25 September 2023
F11-BT	39°26.581'N	144°13.336'E	7504	39°25.883'N	144°12.478'E	7505	AGT	24 September 2023
F12-BT	39°25.798'N	144°18.888'E	7083	39°25.139'N	144°18.559'E	7108	AGT	24 September 2023
F13-BT	39°27.708'N	144°24.086'E	6669	39°27.149'N	144°24.086'E	6676	BT	24 September 2023

F13-EBS	39°28.217'N	144°23.883'E	6675	39°27.674'N	144°23.876'E	6680	EBS	24 September 2023
F14-BT	39°27.983'N	144°48.982'E	5795	39°27.482'N	144°48.726'E	5798	BT	23 September 2023
F14-EBS	39°27.847'N	144°48.418'E	5804	39°27.414'N	144°48.199'E	5802	EBS	23 September 2023
G-BT	38°33.673'N	144°05.371'E	7651	38°32.980'N	144°05.381'E	7654	AGT	15 October 2022
H-BT	36°04.148'N	142°44.075'E	8007	36°03.412'N	142°43.704'E	8014	AGT	13 September 2023
H-EBS	36°04.303'N	142°43.649'E	8013	36°03.631'N	142°43.317'E	8010	EBS	13 September 2023

^a Alpha-numeric station names beginning with 'A' were along a transect designated as 'A line' in the project, running perpendicular to the trench axis and 20–30 km northeast to the Erimo Seamount; 'C' were along a transect designated as 'C line', 99–138 km northeast to the seamount, thus, both were in the Kuril-Kamchatka Trench; 'D' were along a transect line designated as 'D line', 59–101 km southwest to the Erimo Seamount; 'F' were in a transect designated as 'F line', 180–215 km southwest to the seamount, both were in the Japan Trench; station G-BT was off Onagawa, about 270 km south-southwest to the Erimo Seamount, on the axis of the Japan Trench.

^b AGT, Agassiz trawl with a 3-m-width aperture; BT, beam trawl with a 4-m-width aperture.

Table 12. List of species included in the phylogenetic analyses with 16S, 18S, 28S, COI, and H3 GenBank accession numbers and their references.

Species	GenBank accession numbers					Reference
	16S	18S	28S	COI	H3	
Palaeonemertea						
<i>Balionemertes australiensis</i>	–	MK309617	MK309624	MK307892	MK309328	Chernyshev et al. (2019)
<i>Baseodiscus mexicanus</i>	KF935449	KF935281	KF935337	KF935503	KF935393	Kvist et al. (2014)
<i>Callinera grandis</i>	JF277570	JF293067	HQ856881	HQ848626	JF277709	Andrade et al. (2012)
<i>Callinera kasyanovi</i>	KP270840	KP270790	KP270816	KP270865	–	Kvist et al. (2015)
<i>Callinera</i> sp. 17 Alaska	MZ744983	MZ744958	MZ744978	–	MZ772888	Chernyshev et al. (2022)
<i>Callinera</i> sp. 7 Vostok	–	MZ744957	MZ744977	MZ772874	MZ772887	Chernyshev et al. (2022)
<i>Callinera</i> sp. IZ 45635	KP270839	KP270789	KP270815	KP270864	–	Kvist et al. (2015)
<i>Carinina ochracea</i>	JF277631	–	HQ856896	HQ848627	JF277753	Andrade et al. (2012)
<i>Carinina plecta</i>	–	EU495307	–	EU489493	–	Sundberg et al. (2009)
<i>Carinina yushini</i>	KP270838	KP270788	KP270814	KP270863	MN205439	Kvist et al. (2015); Chernyshev & Polyakova (2019)
Carininidae 13DS	MF512037	MF512062	MF512088	–	MF512131	Chernyshev & Polyakova (2018b)
Carininidae 26DS	MF512039	MF512064	MF512090	MF512114	–	Chernyshev & Polyakova (2018b)
Carininidae KuramBio1 3-9	–	MN211365	–	MN205490	MN205441	Chernyshev & Polyakova (2019)
Carininidae KuramBio2 10	MN211468	MN211366	MN211419	MN205491	–	Chernyshev & Polyakova (2019)
Carininidae KuramBio2 103	–	MN211364	MN211418	MN205489	MN205440	Chernyshev & Polyakova (2019)
Carininidae KuramBio2 97/2	–	MN211368	MN211421	MN205493	–	Chernyshev & Polyakova (2019)

Carininidae KuramBio2 97/3	–	MN211367	MN211420	MN205492	–	Chernyshev & Polyakova (2019)
Carininidae sp. 39DS	MF512041	MF512066	MF512092	MF512116	MF512134	Chernyshev & Polyakova (2018b)
<i>Carinoma hamanako</i>	KF935446	KF935278	KF935334	KF935500	KF935390	Kvist et al. (2014)
<i>Carinoma mutabilis</i>	AJ436832	–	AJ436887	AJ436942	AJ436985	Tholleson & Norenburg (2003)
<i>Carinoma</i> sp. DNA105597	–	JF293047	HQ856863	HQ848628	JF277715	Andrade et al. (2012)
<i>Carinoma tremahoros</i>	JF277602	–	HQ856865	HQ848630	JF277713	Andrade et al. (2012)
<i>Cephalothrichella echinicola</i>	MK309618	MK309614	MK309621	MK307889	MK309325	Chernyshev et al. (2019)
<i>Cephalothrix bipunctata</i>	KF935447	KF935279	KF935335	KF935501	KF935391	Kvist et al. (2014)
<i>Cephalothrix filiformis</i>	MW136194	MW136199	MW136152	MW118019	MW118028	Chernyshev & Polyakova (2021)
<i>Cephalothrix hongkongiensis</i>	JF277591	JF293057	HQ856839	HQ848614	JF277739	Andrade et al. (2012)
<i>Cephalothrix iwatai</i>	KP270850	KP270800	KP270825	KP270873	–	Kvist et al. (2015)
<i>Cephalothrix simula</i>	AJ436836	–	AJ436891	AJ436945	AJ436988	Tholleson & Norenburg (2003)
<i>Cephalothrix rufifrons</i>	JF277592	JF293056	HQ856841	HQ848604	JF277741	Andrade et al. (2012)
<i>Parahubrechtia kvisti</i>	KP270848	KP270798	–	KP270871	–	Kvist et al. (2015)
<i>Parahubrechtia</i> sp.	KP270844	KP270794	KP270820	KP270869	MN205445	Kvist et al. (2015); Chernyshev & Polyakova (2019)
<i>Parahubrechtia peri</i>	MZ744984	MZ744959	–	MZ772875	MZ772889	Chernyshev et al. (2022)
<i>Parahubrechtia rayi</i>	MZ744987	MZ744961	–	MZ772878	MZ772892	Chernyshev et al. (2022)
<i>Parahubrechtia rayi</i> 29 Vostok	–	MZ744962	–	MZ772879	MZ772893	Chernyshev et al. (2022)
<i>Parahubrechtia rayi</i> MS 2008	–	EU495309	–	EU489499	–	Sundberg et al. (2009)
Tubulanidae KuramBio2 17	MN211470	MN211371	MN211424	MN205495	MN205444	Chernyshev & Polyakova (2019)

Tubulanidae KuramBio2 77/1	MN211469	MN211369	MN211422	MN205494	MN205442	Chernyshev & Polyakova (2019)
Tubulanidae KuramBio2 77/4	–	MN211370	MN211423	–	MN205443	Chernyshev & Polyakova (2019)
Tubulanidae KuramBio2 90	MN211471	MN211372	MN211425	–	MN205446	Chernyshev & Polyakova (2019)
Tubulanidae sp. 14DS	MF512038	MF512063	MF512089	MF512113	MF512132	Chernyshev & Polyakova (2018b)
Tubulanidae sp. 33DS	MF512040	MF512065	MF512091	MF512115	MF512133	Chernyshev & Polyakova (2018b)
Tubulanidae sp. 33Q California	MZ744988	MZ744963	MZ744979	–	MZ772894	Chernyshev et al. (2022)
Tubulanidae sp. IZ 45557	KP270846	KP270796	KP270822	–	–	Kvist et al. (2015)
Tubulanidae sp. IZ 45651	KP270845	KP270795	KP270821	–	–	Kvist et al. (2015)
Tubulanidae Vema3	–	–	KY296899	KY296908	KY296917	Chernyshev & Polyakova (2018a)
Tubulanidae Vema4	KY296889	MF512060	KY296900	KY296909	KY296918	Chernyshev & Polyakova (2018a, 2018b)
Tubulanidae Vema6	KY296890	MF512061	KY296902	KY296911	KY296920	Chernyshev & Polyakova (2018a, 2018b)
<i>Tubulanus annulatus</i>	JF277599	JF293060	HQ856901	HQ848622	JF277717	Andrade et al. (2012)
<i>Tubulanus</i> cf. <i>ezoensis</i>	MZ744989	MZ744964	–	MZ772880	MZ772895	Chernyshev et al. (2022)
<i>Tubulanus</i> cf. <i>lutescens</i>	ON036062	ON186261	–	ON021852	ON182043	Sagorny et al. (2022)
<i>Tubulanus ezoensis</i>	MZ744990	MZ744965	MZ744980	MZ772881	MZ772896	Chernyshev et al. (2022)
<i>Tubulanus izuensis</i>	MT809229	MT809204	–	MT811763	LC581475	Hookabe et al. (2020b)
<i>Tubulanus pellucidus</i>	JF277595	JF293062	HQ856900	HQ848625	JF277708	Andrade et al. (2012)
<i>Tubulanus polymorphus</i>	JF277598	JF293061	HQ856899	HQ848621	JF277716	Andrade et al. (2012)
<i>Tubulanus punctatus</i>	JF277597	JF293063	AY210473	HQ848624	JF277748	Andrade et al. (2012)
<i>Tubulanus punctatus</i> 3 Vostok	MZ744991	MZ744966	–	MZ772882	MZ772897	Chernyshev et al. (2022)
<i>Tubulanus rhabdotus</i>	AJ436839	–	AJ436894	AJ436948	AJ436990	Tholleson & Norenburg (2003)

<i>Tubulanus riceae</i>	MZ744992	MZ744967	–	MZ772883	MZ772898	Chernyshev et al. (2022)
<i>Tubulanus sexlineatus</i>	JF277596	JF293064	HQ856895	HQ848623	JF277747	Andrade et al. (2012)
<i>Tubulanus</i> sp. B Sakhalin	MZ744993	MZ744968	–	MZ772884	MZ772899	Chernyshev et al. (2022)
<i>Tubulanus</i> sp. D Arctica	MZ744994	MZ744969	MZ744981	MZ772885	MZ772900	Kvist et al. (2015); Chernyshev et al. (2022)
<i>Tubulanus</i> sp. E Arctica	–	MZ744970	MZ744982	MZ772886	MZ772901	Chernyshev et al. (2022)
<i>Tubulanus</i> sp. IZ 45552	KP270843	KP270793	KP270819	KP270868	MZ772902	Kvist et al. (2015)
<i>Tubulanus</i> sp. IZ 45559	KP270847	KP270797	KP270823	KP270870	–	Kvist et al. (2015)
<i>Tubulanus tamias</i>	LC042091	–	AB854621	LC042093	LC042094	Kajihara et al. (2015)
Pilidiophora						
<i>Apatronemertes albimaculosa</i>	JF277587	JF293030	HQ856860	HQ848584	JF277733	Andrade et al. (2012)
<i>Baseodiscus mexicanus</i>	KF935449	KF935281	KF935337	KF935503	KF935393	Kvist et al. (2014)
<i>Baseodiscus profundus</i>	MF512045	MF512070	MF512096	–	MF512138	Chernyshev & Polyakova (2018b)
<i>Baseodiscus punnetti</i>	LC178679	LC178601	LC178631	LC190953	–	Kajihara et al. (2022a)
<i>Baseodiscus quiquelineatus</i>	LC178683	LC178602	–	LC190955	LC178650	Kajihara et al. (2022a)
<i>Baseodiscus takakurai</i>	LC178653	–	LC178611	LC190937	LC178644	Kajihara et al. (2022a)
<i>Baseodiscus unicolor</i>	KF935451	KF935284	KF935340	KF935505	KF935396	Kvist et al. (2014)
<i>Cephalomastax brevis</i>	LC178689	–	–	LC190961	LC178651	Kajihara et al. (2022a)
<i>Cerebratulus lacteus</i>	JF277575	JF293044	HQ856857	HQ848576	JF277728	Andrade et al. (2012)
<i>Cerebratulus marginatus</i>	JF277576	JF293042	HQ856858	HQ848575	JF277729	Andrade et al. (2012)
<i>Cerebratulus mordukhovichi</i>	OM422971	OM423090	OM423029	OM456681	OM468125	Chernyshev & Polyakova (2022)
<i>Cerebratulus orochoi</i>	LC538101	LC538103	LC538104	LC538102	LC538105	Kajihara (2020)
<i>Cerebratulus</i> sp. Antarctica 28	OQ449306	OQ449292	OQ449324	OQ450482	OQ446609	Chernyshev & Polyakova (2023)
<i>Cerebratulus</i> sp. BOLD ACM5920	–	–	–	KP697728	–	Strand (unpubl.)
<i>Cerebratulus</i> sp. DH 2009 A4pilidia04	GU227013	–	–	GU227125	–	Heimeier et al. (2010)

Cf. <i>Heteronemertea</i> sp. DH-2009 A4larva04	GU227014	–	–	GU227126	–	Heimeier et al. (2010)
Cf. <i>Heteronemertea</i> sp. DH-2009 D4pilidia04	GU227013	–	–	GU227120	–	Heimeier et al. (2010)
<i>Corsoua takakurai</i>	LC520112	–	LC520126	LC520106	LC520128	Hookabe & Kajihara (2020a)
<i>Dushia</i> cf. <i>nigra</i>	LC389832	LC389840	LC389844	LC389867	LC389851	Hookabe et al. (2019)
<i>Dushia wijnhoffae</i>	EF124878	–	EF178494	EF124967	–	Hookabe et al. (2019)
<i>Eopilidion misakiense</i>	LC178690	LC178608	LC178641	LC190962	–	Kajihara et al. (2022a)
<i>Euborlasia maycoli</i>	LC520114	LC520121	LC520125	LC520108	–	Hookabe & Kajihara (2020b)
<i>Gorgonorhynchus albocinctus</i>	–	LC010650	LC010651	LC010649	–	Kajihara (2015)
<i>Gorgonorhynchus</i> cf. <i>bermudensis</i>	KF935467	KF935300	KF935356	KF935517	KF935412	Kvist et al. (2014)
<i>Gorgonorhynchus</i> cf. <i>repens</i>	LC520115	LC520122	LC520123	LC520105	LC520131	Hookabe et al. (2021)
<i>Heteronemertea</i> gen sp. 4 TCH-2015	KU197548	–	KU365690	KU197835	–	Heibert (2016)
<i>Heteronemertes longifissa</i> 11	–	OQ449293	OQ449325	OQ450483	–	Chernyshev & Polyakova (2023)
<i>Heteronemertes longifissa</i> 20	OQ449307	OQ449294	–	OQ450484	OQ446610	Chernyshev & Polyakova (2023)
<i>Heteronemertes longifissa</i> 25	OQ449308	OQ449295	OQ449326	OQ450485	OQ446611	Chernyshev & Polyakova (2023)
<i>Heteronemertes longifissa</i> 78	OQ449309	OQ449296	OQ449327	OQ450487	OQ446612	Chernyshev & Polyakova (2023)
<i>Heteronemertea</i> sp. 17 HA-2021	LC625672	LC625688	LC625699	LC625640	LC625729	Kajihara et al. (2022b)
<i>Heteronemertea</i> sp. IZ 45556	KP270851	KP270801	KP270826	KP270874	–	Kvist et al. (2015)
<i>Heteronemertea</i> sp. 2DS	MF512042	MF512067	MF512093	–	MF512135	Chernyshev & Polyakova (2018b)
<i>Heteronemertea</i> sp. 5DS	MF512044	MF512069	MF512095	–	MF512137	Chernyshev & Polyakova (2018b)

<i>Hinumanemertes kikuchii</i>	OQ449310	OQ449297	OQ449328	OQ450491	OQ446613	Chernyshev & Polyakova (2023)
<i>Hubrechtella dubia</i>	JF277630	JF293051	HQ856897	HQ848631	JF277699	Andrade et al. (2012)
<i>Hubrechtella juliae</i>	KP270852	KP270802	KP270827	KP270875	–	Kvist et al. (2015)
<i>Kulikovia alborostrata</i>	KU821503	–	KU856679	KU821529	KU821552	Chernyshev et al. (2018)
<i>Kulikovia cf. montgomeryi</i>	OM422978	OM423098	OM423037	OM456685	OM456685	Chernyshev & Polyakova (2022)
<i>Kulikovia manchenkoi</i>	KU821497	KY468934	KU856671	KU821523	KU821546	Chernyshev et al. (2018)
<i>Kulikovia torquata</i>	KU821486	KY468935	KU856673	KU821511	KU821534	Chernyshev et al. (2018)
Lineidae KuramBio1 125	MN211473	MN211375	MN211427	MN205497	MN205448	Chernyshev & Polyakova (2019)
Lineidae KuramBio2 85	MN211475	MN211377	MN211429	MN205498	–	Chernyshev & Polyakova (2019)
Lineidae KuramBio2_77.2	MN211481	MN211383	MN211434	MN205504	MN205454	Chernyshev & Polyakova (2019)
Lineidae KuramBio2_77.3	MN211480	MN211382	MN211433	MN205503	MN205453	Chernyshev & Polyakova (2019)
Lineidae KuramBio277	MN211481	MN211383	MN211434	MN205504	MN205454	Chernyshev & Polyakova (2019)
Lineidae SokhoBio 10-7	MN211476	MN211378	–	MN205499	MN205450	Chernyshev & Polyakova (2019)
Lineidae sp. 41DS	MF512050	MF512076	MF512102	–	MF512144	Chernyshev & Polyakova (2018b)
Lineidae sp. Antarctica 1	OM422984	OM423104	OM423043	OM456687	OM468138	Chernyshev & Polyakova (2022)
Lineidae sp. antarctica 14	OM422986	OM423106	OM423045	OM456689	OM468140	Chernyshev & Polyakova (2022)
Lineidae sp. Antarctica 16	OM422987	OM423107	OM423046	OM456690	OM468141	Chernyshev & Polyakova (2022)

Lineidae sp. Antarctica 18	OM422985	OM423105	OM423044	OM456688	OM468139	Chernyshev & Polyakova (2022)
Lineidae sp. Antarctica 5	OM422988	OM423108	–	OM456691	OM468142	Chernyshev & Polyakova (2022)
Lineidae sp. G06 Bering	OM422979	OM423099	OM423038	–	OM468133	Chernyshev & Polyakova (2022)
Lineidae sp. H02 Ice AGE	OM422982	OM423102	OM423041	–	OM468136	Chernyshev & Polyakova (2022)
Lineidae sp. KGK 2	LC625651	–	–	LC625624	LC625709	Kajihara et al. (2022b)
Lineidae sp. KGK 4	LC625653	LC625683	–	LC625626	LC625711	Kajihara et al. (2022b)
Lineidae sp. KGK 6	LC625656	–	LC625690	LC625627	LC625714	Kajihara et al. (2022b)
Lineidae sp. KGK 7	LC625657	–	LC625691	–	–	Kajihara et al. (2022b)
Lineidae sp. KGK 8	LC625658	–	–	LC625628	LC625715	Kajihara et al. (2022b)
Lineidae sp. Kuril 011	OM422989	OM423109	OM423047	OM456692	OM468143	Chernyshev & Polyakova (2022)
<i>Lineus acutifrons</i>	JF277573	JF304778	HQ856855	GU590937	JF277727	Andrade et al. (2012) Cherneva et al. (2019);
<i>Lineus clandestinus</i>	MK064103	OQ449298	OQ449329	MK078739	OQ446614	Chernyshev & Polyakova (2023)
<i>Lineus flavescens</i>	KP682165	–	EF178497	KP682050	–	Hiebert & Maslakova (2015); Schwartz (2009)
<i>Lineus lacteus</i>	JF277584	JF293065	HQ856850	HQ848583	JF277725	Andrade et al. (2012)
<i>Lineus longissimus</i>	MW073006	KY468932	MW077245	KY561813	KY606234	Chernyshev et al. (2018) Chernyshev et al. (2021)
<i>Lineus ruber</i>	MK064093	KY468933	KY468929	MK078684	KY606235	Chernyshev et al. (2018, 2019)
<i>Lineus sanguineus</i>	KF935468	KF935301	KF935301	KF935518	KF935413	Kvist et al. (2014) Cherneva et al. (2019);
<i>Lineus viridis</i>	MK064101	OQ449299	OQ449330	MK078733	OQ446615	Chernyshev & Polyakova (2023)

<i>Macalaura aquilonia</i>	OQ449311	OQ449300	OQ449331	OQ450492	OQ446616	Chernyshev & Polyakova (2023)
<i>Macalaura</i> sp.	–	OQ449301	OQ449332	OQ450493	OQ446617	Chernyshev & Polyakova (2023)
<i>Micrura bathyalis</i>	MN211479	MN211381	MN211432	MN205502	–	Chernyshev & Polyakova (2019)
<i>Micrura bella</i>	OQ449312	OQ449302	OQ449333	OQ450494	OQ446618	Chernyshev & Polyakova (2023)
<i>Micrura callima</i>	MN211472	MN211374	MN211426	MN205496	MN205447	Chernyshev & Polyakova (2019)
<i>Micrura chlorapardalis</i>	KF935459	KF935292	KF935348	KF935512	KF935404	Kvist et al. (2014)
<i>Micrura dellechiaiei</i>	KF935461	KF935294	KF935350	KF935514	KF935406	Kvist et al. (2014)
<i>Micrura fasciolata</i>	JF277585	JF293038	HQ856846	HQ848578	JF277721	Andrade et al. (2012)
<i>Micrura ignea</i>	OQ449313	OQ449303	OQ449334	OQ450495	OQ446619	Chernyshev & Polyakova (2023)
<i>Micrura purpurea</i>	JF277577	JF293036	HQ856845	HQ848586	JF277726	Andrade et al. (2012)
<i>Micrura rubramaculosa</i>	KF935460	KF935293	KF935349	KF935513	KF935405	Kvist et al. (2014)
<i>Micrura</i> sp. 3 TCH-2015 E1H8	KU197563	–	KU365710	KU197841	–	Hiebert (2016)
<i>Micrura</i> sp. 4 TCH-2015 E3B2	KU197581	–	KU365711	KU197857	–	Hiebert (2016)
<i>Micrura</i> sp. <i>albocephala</i>	KU197574	–	KU365712	KU197849	–	Hiebert (2016)
<i>Micrura</i> sp. dark	KU197586	–	KU365713	KU197858	–	Hiebert (2016)
<i>Micrura</i> sp. IceAGE	OM422994	OM423114	OM423052	OM456696	OM468146	Chernyshev & Polyakova (2022)
<i>Micrura</i> sp. IZ 132529	KF935458	KF935291	KF935347	KF935511	KF935403	Kvist et al. (2014)
<i>Micrura</i> sp. IZ 132532	KF935457	KF935290	KF935346	KF935510	KF935402	Hiebert (2016)
<i>Micrura verrilli</i>	KF935455	KF935288	KF935344	KF935508	KF935400	Kvist et al. (2014)
<i>Micrura wilsoni</i>	KU197535	–	KU365716	KU197827	–	Hiebert (2016)
<i>Nippnomicrura uchidai</i>	KU821509	–	KY468930	KY561815	KY561819	Chernyshev et al. (2018)

<i>Nipponomicrura</i> sp.	OQ449314	OQ449304	OQ449335	OQ450496	OQ446620	Chernyshev & Polyakova (2023)
<i>Notospermus geniculatus</i>	KF935462	KF935295	KF935351	–	KF935407	Kvist et al. (2014)
<i>Notospermus mitellatus</i>	LC625660	LC625685	LC625693	LC625629	LC625717	Kajihara et al. (2022b)
<i>Oxypolella alba</i>	AF103767	–	–	–	–	Sundberg & Saur (1998)
<i>Oxypolella hiebertae</i>	KU748595	–	–	–	–	Hiebert (2016)
<i>Parbolasia corrugata</i>	JF277578	JF293037	HQ856851	EU194826	JF277732	Andrade et al. (2012); Thornhill et al. (2008)
<i>Parbolasia corrugata</i> 111	OQ449315	OQ449305	OQ449336	OQ450497	OQ446621	Chernyshev & Polyakova (2023)
<i>Parvicirrus dubius</i>	AJ436830	–	AJ436885	AJ436940	–	Tholleson & Norenburg (2003)
<i>Pseudomicrura afzelii</i>	GU445914	GU445924	GU445919	GU392013	–	Strand & Sundberg (2011)
<i>Riseriellus occultus</i>	JF277581	JF293031	HQ856848	HQ848581	JF277724	Andrade et al. (2012)
<i>Siphonenteron nakanoi</i>	LC625678	–	LC625706	LC625646	LC625737	Kajihara et al. (2022b)
<i>Sonnenemertes cantelli</i>	MF512048	MF512073	MF512099	MF512118	MF512141	Chernyshev & Polyakova (2019)
						Schwartz (2009);
<i>Tenuilineus bicolor</i>	AJ436823	–	EF124960	AJ436933	AJ436980	Tholleson & Norenburg (2003)
<i>Valencinura bahusiensis</i>	–	–	–	GU392026	–	Strand & Sundberg (2011)
<i>Valencinura jambio</i>	LC178692	–	LC178643	LC190964	–	Kajihara et al. (2022a)
<i>Yininemertes pratensis</i>	KY274025	KY274047	KY274069	KY274003	KY274091	Kvist et al. (2014)
<i>Zygeupolia rubens</i>	JF277574	JF293045	HQ856861	HQ848585	JF277735	Andrade et al. (2012)
Hoplonemertea						
<i>Abyssonemertes kajiharai</i>	KY296888	KY296892	KY296897	KY296906	KY296915	Chernyshev & Polyakova (2018b)
<i>Agronemertes australiensis</i>	JF277605	JF293010	HQ856892	HQ848601	JF277750	Andrade et al. (2012)
<i>Alvinonemertes christianeae</i>	ON036051	–	–	–	–	Sagorny et al. (2022)

<i>Alvinonemertes christianeae</i> N253	ON036052	–	–	ON021867	–	Sagorny et al. (2022)
<i>Alvinonemertes claudiae</i> N231	ON036050	–	–	ON021868	–	Sagorny et al. (2022)
<i>Alvinonemertes dagmarae</i> N260	ON036049	ON186254	–	ON021866	ON182034	Sagorny et al. (2022)
<i>Alvinonemertes tatjanae</i>	ON036047	ON186256	–	ON021864	ON182041	Sagorny et al. (2022)
<i>Alvinonemertes dariae</i>	ON036048	ON186252	–	ON021865	–	Sagorny et al. (2022)
<i>Amphiporus</i> cf. <i>imparispinosus</i>	MN211508	MN211414	MN211464	MN205525	MN205486	Chernyshev & Polyakova (2019)
<i>Amphiporus formidabilis</i>	KF935498	KF935331	KF935387	KF935547	KF935443	Kvist et al. (2014)
<i>Amphiporus lactifloreus</i>	MN211511	MN211417	MN211467	MN205528	MN205488	Chernyshev & Polyakova (2019)
<i>Amphiporus lactifloreus</i>	JF277617	JF293018	HQ856876	HQ848611	–	Andrade et al. (2012)
<i>Antarctonemertes valida</i>	KF935489	KF935321	KF935377	KF935537	KF935433	Kvist et al. (2014)
<i>Antarctonemertes delutibasisae</i>	MN211494	MN211397	MN211447	MN205511	–	Chernyshev & Polyakova (2019)
<i>Antarctonemertes phyllospadicola</i>	MN211493	MN211396	MN211446	FJ594419	MN205468	Chernyshev & Polyakova (2019); Maslakova & Döhren (2009)
<i>Antarctonemertes riesgoae</i>	KF935490	KF935322	KF935378	KF935538	KF935434	Kvist et al. (2014)
<i>Armaueriidae</i> sp. 13	MN211489	MN211392	MN211442	–	MN205464	Chernyshev & Polyakova (2019)
<i>Armaueriidae</i> sp. 81	MN211488	MN211391	MN211441	–	MN205463	Chernyshev & Polyakova (2019)
<i>Asteronemertes</i> sp.	MN211500	MN211406	MN211456	MN205519	MN205477	Chernyshev & Polyakova (2019)
<i>Chernyshevia escarpiaphila</i> N235	ON036046	ON186253	–	ON021863	ON182036	Sagorny et al. (2022)
<i>Chernyshevia escarpiaphila</i> N266	ON036043	ON186251	–	ON021860	ON182040	Sagorny et al. (2022)
<i>Collarenemertes</i> cf. <i>bimaculata</i>	MN211492	MN211395	MN211445	MN205510	MN205467	Chernyshev & Polyakova (2019)
<i>Cratenemertea</i> 25DS	MF512058	MF512086	MF512111	MF512127	–	Chernyshev & Polyakova (2018b)

Cratenemertidae Guam	MN211490	MN211393	MN211443	MN205508	MN205465	Chernyshev & Polyakova (2019)
Cratenemertidae sp. IZ 45644	KP270854	KP270804	KP270829	KP270877	–	Kvist et al. (2015)
<i>Dinonemertes arctica</i>	–	–	–	FJ602542	–	Bucklin et al. (2010)
<i>Diplomma serpentina</i>	–	MN211400	MN211450	MN205514	MN205471	Chernyshev & Polyakova (2019)
Drepanophoridae sp. 1 Vietnam	MN211484	MN211386	MN211437	–	MN205457	Chernyshev & Polyakova (2019)
Drepanophoridae sp. 4 Vietnam	MN211483	MN211385	MN211436	–	MN205456	Chernyshev & Polyakova (2019)
<i>Drepanophorus spectabilis</i>	JF277627	JF293009	HQ856868	HQ848610	JF277710	Andrade et al. (2012)
<i>Emplectonema gracile</i>	JF277621	JF293022	HQ856883	HQ848620	JF277751	Andrade et al. (2012)
Eumonostilifera N259	ON036053	ON186269	–	ON021869	ON182042	Sagorny et al. (2022)
Eumonostilifera sp. Vema2B	–	KY296894	KY296901	KY296910	KY296919	Chernyshev & Polyakova (2018a)
Eumonostilifera sp. Vema7	–	KY296896	KY296904	KY296913	–	Chernyshev & Polyakova (2018a)
Eureptantia sp. 2 Vietnam	MN211482	MN211384	MN211435	–	MN205455	Chernyshev & Polyakova (2019)
<i>Galathenemertes giribeti</i> 11	MN211497	MN211402	MN211452	MN205516	MN205473	Chernyshev & Polyakova (2019)
<i>Galathenemertes giribeti</i> 12	–	MN211401	MN211451	MN205515	MN205472	Chernyshev & Polyakova (2019)
<i>Geonemertes pelaensis</i> DNA102574	JF277610	JF293017	HQ856887	HQ848592	JF277736	Andrade et al. (2012)
<i>Gononemertes parasita</i> DNA105583	JF277606	JF293014	HQ856889	HQ848607	JF277745	Andrade et al. (2012)
<i>Gononemertes</i> sp. IZ 45558	KP270855	KP270805	KP270830	KP270878	–	Kvist et al. (2015)
<i>Gononemertes</i> sp. SokhoBio 1-11	MN211499	MN211404	MN211454	MN205518	MN205475	Chernyshev & Polyakova (2019)

<i>Gononemertes</i> sp. SokhoBio 6-8	MN211498	MN211403	MN211453	MN205517	MN205474	Chernyshev & Polyakova (2019)
<i>Gurjanovella littoralis</i>	MZ231134	MZ231196	MZ231286	MZ216518	MZ216589	Chernyshev et al. (2021)
<i>Gurjanovella</i> sp. 11DS	MF512054	MF512082	MF512107	MF512123	MF512149	Chernyshev & Polyakova (2018a)
<i>Malacobdella grossa</i> DNA105592	JF277614	JF293015	HQ856882	HQ848591	JF277700	Andrade et al. (2012)
Monostilifera Kurambio1 4-3	MN211504	MN211410	MN211460	–	MN205482	Chernyshev & Polyakova (2019)
Monostilifera KuramBio2 82	–	MN211405	MN211455	–	MN205476	Chernyshev & Polyakova (2019)
Monostilifera sp. B15	OQ075750	–	–	OQ075687	–	Ellison et al. (2024)
Monostilifera sp. CB141_18_05	OK073513	–	–	OK081796	–	Maslakova et al. (2022)
Monostilifera sp. CB151_18_01	OK073396	–	–	OK081532	–	Maslakova et al. (2022)
Monostilifera sp. CB151_18_02	OK073332	–	–	OK081397	–	Maslakova et al. (2022)
Monostilifera sp. IZ 45641	KP270857	KP270807	KP270832	KP270880	–	Kvist et al. (2015)
<i>Nectonemertes</i> cf. <i>mirabilis</i>	–	MF512081	MF512106	MF512122	MF512148	Chernyshev & Polyakova (2018b)
<i>Nectonemertes mirabilis</i>	AJ436815	–	AJ436870	AJ436925	AJ436973	Tholleson & Norenburg (2003)
<i>Emplectonema buergeri</i>	JF277616	JF293066	HQ856880	HQ848600	JF277697	Andrade et al. (2012)
Nemertea sp. IZ 132533	KF935475	KF935308	KF935364	KF935524	KF935419	Kvist et al. (2014)
Nemertea sp. IZ 1449052	–	–	–	OK340102	–	Osborn et al. (unpubl.)
<i>Nemertopsis bivittata</i>	JF277609	JF293021	HQ856877	HQ848608	JF277701	Andrade et al. (2012)
<i>Nemertovema hadalis</i>	KY296891	KY296895	KY296903	KY296912	KY296921	Chernyshev & Polyakova (2018b)
<i>Nemertovema norenburgi</i> KB2hop33	MN211507	MN211413	MN211463	MN205524	MN205485	Chernyshev & Polyakova (2019)
<i>Nemertovema norenburgi</i> KB2hop32	MN211506	MN211412	MN211462	MN205523	MN205484	Chernyshev & Polyakova (2019)

<i>Nipponemertes arenaria</i>	MN211491	MN211394	MN211444	MN205509	MN205466	Chernyshev & Polyakova (2019)
<i>Nipponemertes cf. ogumai</i>	–	–	–	ON357614	–	Hookabe et al. (2022a)
<i>Nipponemertes pulchra</i>	JF277625	JF293012	HQ856871	HQ848597	JF277704	Andrade et al. (2012)
<i>Oerstedia oculata</i>	MN211495	MN211398	MN211448	MN205512	MN205469	Chernyshev & Polyakova (2019)
<i>Oerstedia phoresiae</i>	MN211496	MN211399	MN211449	MN205513	MN205470	Chernyshev & Polyakova (2019)
<i>Oerstedia</i> sp. IZ 132743	KF935488	KF935320	KF935376	KF935536	KF935432	Kvist et al. (2014)
<i>Pantinonemertes californiensis</i>	EF157585	–	–	EF157597	–	Maslakova & Norenburg (2008)
<i>Paradrepanophorus crassus</i>	JF277628	JF293008	HQ856867	HQ848603	JF277711	Andrade et al. (2012)
<i>Paranemertes peregrina</i>	MN211509	MN211415	MN211465	MN205526	–	Chernyshev & Polyakova (2019)
<i>Pelagonemertes</i> sp. 545	AJ436814	–	AJ436869	AJ436924	AJ436972	Tholleson & Norenburg (2003)
<i>Phallonemertes murrayi</i>	AJ436816	–	AJ436871	AJ436926	AJ436974	Tholleson & Norenburg (2003)
<i>Polystilifera</i> sp. DNA100544	JF277626	JF293055	HQ856869	HQ848632	JF277712	Andrade et al. (2012)
<i>Proamphiporus crandalli</i>	MN211505	MN211411	MN211461	MN205522	MN205483	Chernyshev & Polyakova (2019)
<i>Proamphiporus rectangulus</i>	–	–	–	KC812587	–	Strand et al. (2014)
<i>Prosadenoporus floridensis</i>	EF157584	–	–	EF157596	–	Maslakova & Norenburg (2008)
<i>Prosadenoporus winsori</i>	EF157581	–	–	EF157594	–	Maslakova & Norenburg (2008)
<i>Prosorhochmus americanus</i>	JF277619	JF293023	HQ856879	HQ848595	JF277698	Andrade et al. (2012)
<i>Prostoma eilhardi</i>	JF277620	JF293027	HQ856875	HQ848594	JF277695	Andrade et al. (2012)
<i>Protopelagonemertes beebei</i>	JF277629	JF293028	HQ856873	HQ848602	JF277752	Andrade et al. (2012)

<i>Protopelagonemertes beebei</i>	–	–	–	LC565011	–	Kajihara & Yamaguchi (2020)
<i>Psammaamphiporus elongatus</i>	JF277622	JF293026	HQ856874	HQ848609	JF277702	Andrade et al. (2012)
<i>Pseudotetrastemma</i> sp.	MN211501	MN211407	MN211457	MN205520	–	Chernyshev & Polyakova (2019)
<i>Quasitetrastemma stimpsoni</i>	KP270862	KP270812	KP270837	KP270885	MN205481	Kvist et al. (2014); Chernyshev & Polyakova (2019)
Reptant SokhoBio 1-9	MN211485	MN211387	MN211438	MN205505	MN205459	Chernyshev & Polyakova (2019)
Reptant sp. 281	AJ436818	–	AJ436873	AJ436928	AJ436976	Tholleson & Norenburg (2003)
Reptant sp. 481	AJ436819	–	AJ436819	AJ436929	AJ436977	Tholleson & Norenburg (2003)
Reptant sp. IZ 132526	KF935472	KF935305	KF935361	KF935522	KF935416	Kvist et al. (2014)
Reptantia KuramBio2 19	MN211487	MN211390	MN211440	MN205507	MN205462	Chernyshev & Polyakova (2019)
Reptantia Kurambio2 28	MN211486	MN211388	–	MN205506	MN205460	Chernyshev & Polyakova (2019)
Reptantia KuramBio2 41	–	MN211389	MN211439	–	MN205461	Chernyshev & Polyakova (2019)
Reptantia sp. IZ 133024	KF935473	KF935306	KF935362	KF935523	KF935417	Kvist et al. (2014)
Reptantia sp. IZ 45643	KP270853	KP270803	KP270828	KP270876	MN205458	Chernyshev & Polyakova (2019); Kvist et al. (2015)
<i>Sacconemertopsis belogurovi</i>	KP270861	KP270811	KP270836	KP270884	MN205479	Chernyshev & Polyakova (2019); Kvist et al. (2015)
<i>Sacconmertediae</i> sp. IZ 45649	KP270860	KP270810	KP270835	KP270883	–	Kvist et al. (2015)
<i>Tetrastemma elegans</i>	MZ231156	MZ231222	MZ231312	MZ216543	MZ216614	Chernyshev et al. (2021)
<i>Tetrastemma pseudocoronatum</i>	MN211502	MN211408	MN211458	MN205521	MN205478	Chernyshev & Polyakova (2019)

<i>Tetrastemma vittigerum</i>	MZ231192	MZ231272	MZ231362	MZ216585	MZ216663	Chernyshev et al. (2021)
<i>Tetrastemma wilsoni</i>	AJ436811	–	AJ436866	AJ436921	AJ436969	Thollessen & Norenburg (2003)
<i>Tortus tokmakovae</i>	MN211510	MN211416	MN211466	MN205527	MN205487	Chernyshev & Polyakova (2019)
<i>Uniporus alisae</i>	MF512053	MF512080	MF512105	MF512121	MF512147	Chernyshev & Polyakova (2018b)
<i>Vieitezia luzmurubeae</i>	JF277607	HQ443428	HQ856890	HQ443426	JF277746	Andrade et al. (2012)
<i>Zygonemertes</i> sp.	MN211503	MN211409	MN211459	–	MN205480	Chernyshev & Polyakova (2019)

Table 13. Sequenced species, specimen (catalogue number), and GenBank accession number of the sequences generated in the present study.

Species	Catalogue number	GenBank accession numbers				
		16S	18S	28S	COI	H3
Palaeonemertea						
Carininidae sp. KH-2024-01	ICHUM 8886	PQ516018	PQ516044	PQ529171	PQ510706	PQ530422
Carininidae sp. KH-2024-01	ICHUM 8887	PQ516019	PQ516045	–	PQ510704	PQ530423
Carininidae sp. KH-2024-01	ICHUM 8888	PQ516020	PQ516046	PQ529173	PQ510705	PQ530424
Tubulanidae sp. KH-2024-01	ICHUM 8897	PQ516021	–	PQ529172	PQ510707	PQ530426
Tubulanidae sp. KH-2024-01	ICHUM 8898	–	–	–	PQ510708	PQ530425
Tubulanidae sp. KH-2024-01	ICHUM 8899	–	–	–	PQ510709	PQ530427
Tubulanidae sp. KH-2024-02	ICHUM 8895	PQ516022		PQ529176	PQ510710	PQ530428
Tubulanidae sp. KH-2024-03	ICHUM 8894	PQ516023	PQ516047	–	PQ510711	PQ530429
Tubulanidae sp. KH-2024-04	ICHUM 8889	PQ516025	–	–	PQ510714	PQ530430
Tubulanidae sp. KH-2024-05	ICHUM 8892	PQ516024	–	–	PQ510712	PQ530431
Tubulanidae sp. KH-2024-06	ICHUM 8893	PQ516027	–	–	PQ510713	PQ530432
Tubulanidae sp. KH-2024-07	ICHUM 8890	PQ516026	–	–	PQ510715	PQ530433
Tubulanidae sp. KH-2024-08	ICHUM 8891	PQ516030	–	PQ611748	PQ510716	PQ530434
Pilidiophora						
<i>Baseodiscus profundus</i>	ICHUM 8885	PQ516028	PQ516049	PQ529165	PQ512831	PQ530410
Lineidae sp. KH-2024-01	ICHUM 8874	PQ516031	–	PQ529167	PQ512832	PQ530411
Lineidae sp. KH-2024-01	ICHUM 8875	PQ516029	–	–	PQ512834	PQ530412
Lineidae sp. KH-2024-02	ICHUM 8876	PQ516032	–	PQ529169	PQ512833	PQ530413
Lineidae sp. KH-2024-02	ICHUM 8877	PQ516033	–	PQ529170	PQ512835	PQ530414
Lineidae sp. KH-2024-03	ICHUM 8878	PQ516035	–	–	PQ512836	PQ530415
Lineidae sp. KH-2024-04	ICHUM 8879	PQ516034	PQ516048	–	PQ512837	PQ530416
Lineidae sp. KH-2024-04	ICHUM 8880	PQ516037	–	–	PQ512838	PQ530417
Lineidae sp. KH-2024-04	ICHUM 8881	PQ516038	–	–	PQ512839	PQ530418

Lineidae sp. KH-2024-04	ICHUM 8883	PQ516036	–	–	PQ512840	PQ530419
Lineidae sp. KH-2024-05	ICHUM 8882	PQ516039	–	–	PQ512841	PQ530420
Lineidae sp. KH-2024-06	ICHUM 8884	PQ516040	PQ516050	PQ529175	PQ512842	PQ530421
Hoplonemertea						
<i>Dinonemertes arctica</i>	ICHUM 8863	–	–	–	PQ505184	PQ530387
<i>Dinonemertes arctica</i>	ICHUM 8864	PQ516008	–	PQ529154	PQ505186	PQ530388
<i>Dinonemertes arctica</i>	ICHUM 8865	–	–	PQ529156	PQ505185	PQ530389
<i>Dinonemertes arctica</i>	ICHUM 8900	–	–	–	PQ505187	–
<i>Dinonemertes shinkaii</i>	ICHUM 8866	PQ516009	–	PQ529155	PQ505188	PQ530390
Monostilifera sp. KH-2024-01	ICHUM 8852	PQ516011	PQ516043	PQ529157	PQ505189	PQ530391
Monostilifera sp. KH-2024-02	ICHUM 8853	–	–	PQ529159	PQ505190	PQ530392
<i>Nectonemertes cf. mirabilis</i>	ICHUM 8867	–	–	–	PQ505191	PQ530393
<i>Nemertovema kojimai</i> sp. nov.	ICHUM 8857	PQ516010	–	–	PQ505192	PQ530394
<i>Nemertovema norenburgi</i>	ICHUM 8856	PQ516012	PQ516041	PQ529158	PQ505193	PQ530395
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8858	PQ516013	PQ516042	PQ529161	PQ561265	PQ530396
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8859	–	–	–	PQ505195	PQ530397
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8939	–	–	–	PQ561266	PQ530398
<i>Pelagica</i> sp. KH-2024-01	ICHUM 8860	PQ516015	–	–	PQ505194	PQ530399
<i>Pelagica</i> sp. KH-2024-02	ICHUM 8871	–	–	–	PQ505196	PQ530400
<i>Phallonemertes murrayi</i>	ICHUM 8869	–	–	–	PQ505245	PQ530402
<i>Phallonemertes murrayi</i>	ICHUM 8868	–	–	–	PQ505197	PQ530401
<i>Phallonemertes murrayi</i>	ICHUM 8870	PQ516014	–	PQ611731	PQ505246	PQ530403
<i>Profundonemertes hakuhoae</i> gen. et sp. nov.	ICHUM 8854	–	–	–	PQ505247	PQ530404
<i>Profundonemertes hakuhoae</i> gen. et sp. nov.	ICHUM 8855	–	–	–	PQ505248	PQ530405
<i>Protopelagonemertes beebei</i>	ICHUM 8872	–	–	PQ529164	PQ505249	PQ530406
<i>Protopelagonemertes beebei</i>	ICHUM 8873	–	–	–	PQ505250	PQ530407
Reptantia sp. KH-2024-01	ICHUM 8861	PQ516016	–	PQ529163	PQ505251	PQ530408
Reptantia sp. KH-2024-01	ICHUM 8862	PQ516017	–	PQ529166	PQ505252	PQ530409

Table 14. The main parameters of model-based inferred phylogenies.

Supermatrix	Subset	Best-fit model	Partition name	IQ-Tree model	RAxML model	MrBayes model	Harmonic mean	ASD	min ESS	PSRF
1. Palaeonemertea	subset 1	GTR + I + G	COI_1	GTR + I + G	GTR + G	GTR + I + G	-92228.4	0.002326	1251.47	1.000
	subset 2	TVM + I + G	COI_2	TVM I + G	GTR + G	GTR + I + G				
	subset 3	TVM + G	COI_3	TVM + G	GTR + G	GTR + G				
	subset 4	GTR + I + G	H3_1	GTR + I + G	GTR + G	GTR + I + G				
	subset 5	JC + I	H3_2	JC + I	GTR + G	F81 + I; F81				
	subset 6	TVMEF + G	H3_3	TVMEF + G	GTR + G	GTR + G				
	subset 7	GTR + I + G	16S	GTR + I + G	GTR + G	GTR + I + G				
	subset 8	TRN + I + G	18S	TRN + I + G	GTR + G	GTR + I + G				
	subset 9	TRN + I + G	28S	TRN + I + G	GTR + G	GTR + I + G				
2. Pilidiophora	subset 1	TRN + I + G	COI_1	TRN + I + G	GTR + G	GTR + I + G	-89948.51	0.004126	196.57	1.000
	subset 2	TVM + I + G	COI_2	TVM + I + G	GTR + G	GTR + I + G				
	subset 3	TRN + I + G	COI_3	TRN + I + G	GTR + G	GTR + I + G				
	subset 4	TVM + G	H3_1	TVM + G	GTR + G	GTR + G				
	subset 5	GTR + G	H3_2	GTR + G	GTR + G	GTR + G				

3. Hoplonemertea	subset 6	K80	H3_3	K80	GTR + G	K2P; SYM + I	-73645.92	0.003849	956.39	1.000
	subset 7	TVM + I + G	16S	TVM + I + G	GTR + G	GTR + I + G				
	subset 8	K80 + I + G	18S	K80 + I + G	GTR + G	GTR + I + G; SYM + I				
	subset 9	GTR + I + G	28S	GTR + I + G	GTR + G	GTR + I + G				
	subset 1	TIM + G	COI_1	TIM + G	GTR + G	GTR + G				
	subset 2	GTR + I + G	COI_2	GTR + I + G	GTR + G	GTR + I + G				
	subset 3	TVM + I + G	COI_3	TVM + I + G	GTR + G	GTR + I + G				
	subset 4	GTR + I + G	H3_1	GTR + I + G	GTR + G	GTR + I + G				
	subset 5	TVMEF + G	H3_2	TVMEF + G	GTR + G	GTR + I + G				
	subset 6	GTR + I + G	H3_3	GTR + I + G	GTR + G	GTR + G				
	subset 7	GTR + I + G	16S	GTR + I + G	GTR + G	GTR + I + G				
	subset 8	TRNEF + I + G	18S	TRNEF + I + G	GTR + G	GTR + I + G				
	subset 9	GTR + I + G	28S	GTR + I + G	GTR + G	GTR + I + G				

Results and systematic remarks

A total of 80 nemertean specimens were obtained from the research expeditions along the JT and the adjacent KKT onboard the R/V *Hakuho-maru* in October 2022 and September 2023. All specimens were collected from trawled material using the BT, the AGT, and from the inner nets attached to these trawls. No ribbon worm was collected using the EBS. The specimens retrieved from the inner nets were intact, consisting of 10 individuals—six species in Pelagica and one in Reptantia. Molecular analyses revealed that the 80 nemertean specimens represented 30 putative species, 21 of which are undescribed. Ribbon worms were collected from 24 of the 58 stations, at depths ranging from 2007 to 7654 m (Fig. 55; Table 15).

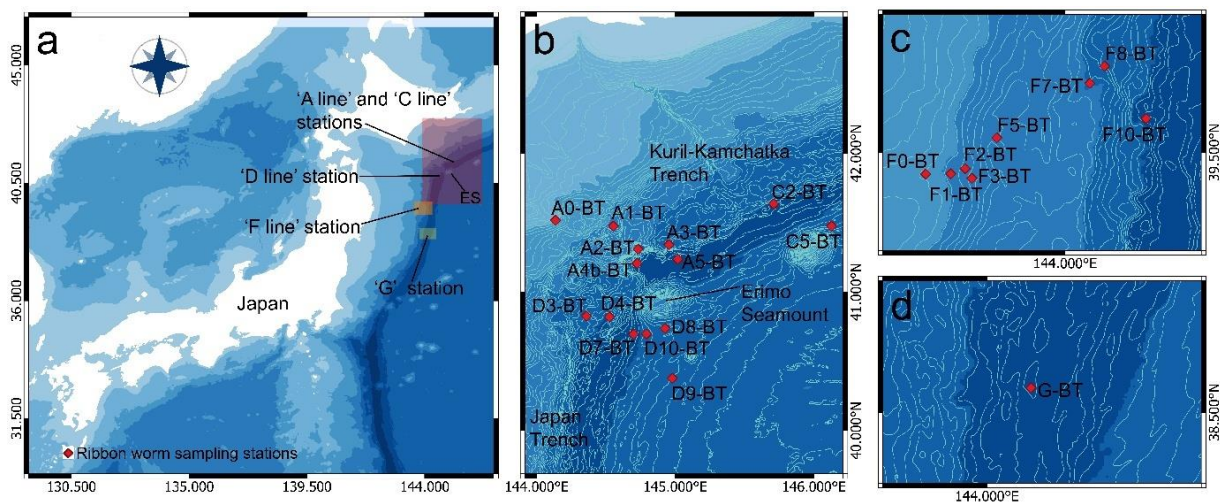


Figure 55. Sampling stations in the Japan Trench and Kuril-Kamchatka Trench where ribbon worms were collected (a–d). (a), general map highlighting the location of the five sampling stations along the Japan Trench (JT) and the Kuril-Kamchatka Trench (KKT); (b), map showing the detailed sampling stations in ‘A, C, and D lines’; (c), detailed sampling stations in ‘F line’; and (d), the ‘G station’. The sampling stations northeast the Erimo Seamount (ES) are situated in the KKT while stations to its south are in the JT.

Palaeonemertea

The species delimitation analysis using the ASAP method partitioned the palaeonemertean COI sequences into 10 subsets (putative species), with an ASAP score of 1.50, P-val(rank) of 0.788(2), and W(rank) of 0.0328(1). In contrast, the ABGD method produced 10 partitioning patterns, all suggesting nine groups (putative species). The best partition, partition 1, with nine groups, had the lowest prior maximal distance P-value of

0.001000. This partitioning result corresponded only to ASAP's second-best partitioning, which identified nine subsets (putative species) with an ASAP score of 3.00, P-val (rank) of (1), and W(rank) of 0.0160 (5). Applying the '3%-rule' in nemertean molecular taxonomy established by Sundberg et al. (2016), nine palaeonemertean species were recognized in the collection.

Tubulanidae Bürger, 1904

The palaeonemerteans collected along the JT and KKT were predominantly members of the family Tubulanidae. Eight species of tubulanids (six from the JT and two from the KKT) were obtained during the expeditions, and distributed across seven sampling stations (Table 15). All tubulanids were represented by fragmented specimens, except for one intact species, Tubulanidae sp. KH-2024-01, represented by specimen ICHUM 8899 (Fig. 56a–c), and collected at F14-BT station in the JT.

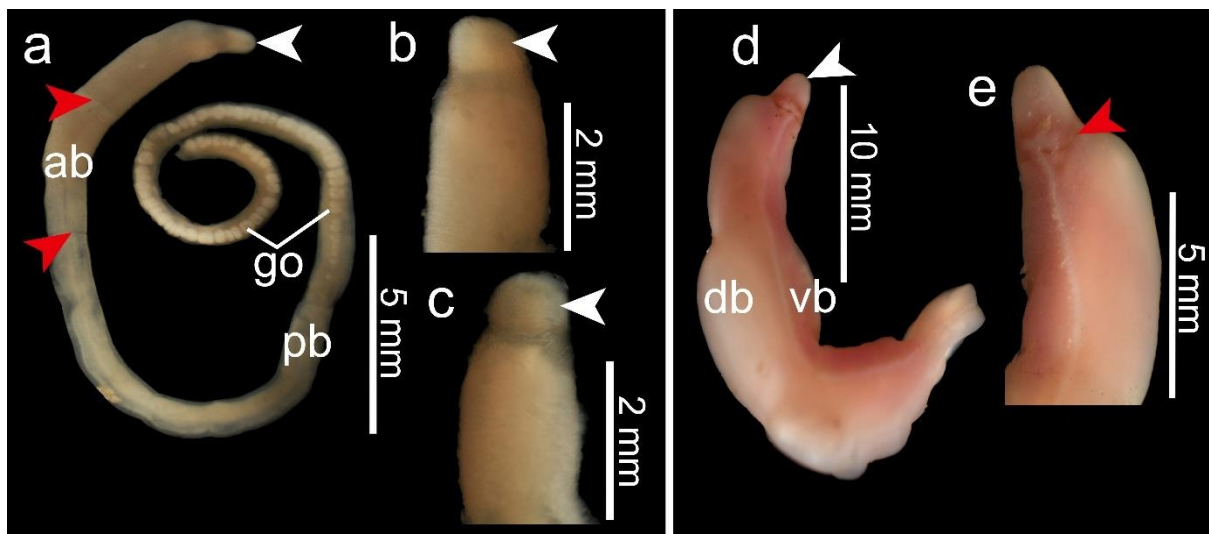


Figure 56. Some of the palaeonemerteans collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–e). (a–c), Tubulanidae sp. KH-2024-01, ICHUM 8899, from JT, (a), whole body at right-lateral view, (b), magnification of the anterior end at dorsal view, (c), same in b but at ventral view, white arrowheads pointed to the head; (d, e); Carininidae sp. KH-2024-01, ICHUM 8888, from KKT, (d), whole body at right-lateral view, (e), magnification of the anterior end at ventrolateral view, white arrowhead pointed to the head. Abbreviations: ab, anterior body; cf, cephalic furrow; go, gonads; pb, posterior body; tr, tubulanid ring.

Table 15. Species, specimen (catalogue number), location (coordinates and depths), type of gear, and sampling date of the 24 stations where ribbon worms were collected during the cruises KH-22-8 and KH-23-5 of the R/V *Hakuho-maru*.

Species	Catalogue number	Station ^a	Off-bottom location			Gear ^b	Date
			Latitude	Longitude	Depth (m)		
Palaeonemertea							
Carininidae sp. KH-2024-01	ICHUM 8886	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
Carininidae sp. KH-2024-01	ICHUM 8887	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
Carininidae sp. KH-2024-01	ICHUM 8888	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
Tubulanidae sp. KH-2024-01	ICHUM 8897	D3-BT	40°49.560'N	144°21.543'E	6211	AGT	15 October 2022
Tubulanidae sp. KH-2024-01	ICHUM 8898	D3-BT	40°49.560'N	144°21.543'E	6211	AGT	15 October 2022
Tubulanidae sp. KH-2024-01	ICHUM 8899	F14-BT	39°27.482'N	144°48.726'E	5798	BT	23 September 2023
Tubulanidae sp. KH-2024-02	ICHUM 8895	F2-BT	39°27.934'N	143°47.240'E	3890	BT	29 September 2023
Tubulanidae sp. KH-2024-03	ICHUM 8894	D7-BT	40°41.735'N	144°41.975'E	7040	AGT	13 October 2022
Tubulanidae sp. KH-2024-04	ICHUM 8889	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Tubulanidae sp. KH-2024-05	ICHUM 8892	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
Tubulanidae sp. KH-2024-06	ICHUM 8893	D3-BT	40°49.560'N	144°21.543'E	6211	AGT	15 October 2022
Tubulanidae sp. KH-2024-07	ICHUM 8890	D9-BT	40°22.690'N	144°58.790'E	5976	BT	14 September 2023
Tubulanidae sp. KH-2024-08	ICHUM 8891	F2-BT	39°27.934'N	143°47.240'E	3890	BT	29 September 2023
Pilidiophora							
<i>Baseodiscus profundus</i>	ICHUM 8885	A1-BT	41°28.533'N	144°33.273'E	4028	BT	2 October 2022
Lineidae sp. KH-2024-01	ICHUM 8874	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Lineidae sp. KH-2024-01	ICHUM 8875	F3-BT	39°26.687'N	143°48.111'E	4117	BT	28 September 2023
Lineidae sp. KH-2024-02	ICHUM 8876	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Lineidae sp. KH-2024-02	ICHUM 8877	F0-BT	39°27.240'N	143°42.189'E	3428	BT	27 September 2023
Lineidae sp. KH-2024-03	ICHUM 8878	F14-BT	39°27.482'N	144°48.726'E	5798	BT	23 September 2023
Lineidae sp. KH-2024-04	ICHUM 8879	D4-BT	40°49.211'N	144°31.565'E	6756	AGT	14 October 2022
Lineidae sp. KH-2024-04	ICHUM 8880	D7-BT	40°41.735'N	144°41.975'E	7040	AGT	13 October 2022

Lineidae sp. KH-2024-04	ICHUM 8881	C2-BT	41°38.096'N	145°42.631'E	6806	BT	20 September 2023
Lineidae sp. KH-2024-04	ICHUM 8883	F8-BT	39°41.023'N	144°05.056'E	5702	BT	26 September 2023
Lineidae sp. KH-2024-05	ICHUM 8882	F7-BT	39°38.847'N	144°03.148'E	5174	BT	26 September 2023
Lineidae sp. KH-2024-06	ICHUM 8884	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Hoplonemertea							
<i>Dinonemertes arctica</i>	ICHUM 8863	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
<i>Dinonemertes arctica</i>	ICHUM 8864	F10-BT	39°34.320'N	144°10.353'E	6871	AGT	25 September 2023
<i>Dinonemertes arctica</i>	ICHUM 8865	C2-BT	41°38.096'N	145°42.631'E	6806	BT	20 September 2023
<i>Dinonemertes arctica</i>	ICHUM 8900	D10-BT	40°41.807'N	144°47.595'E	6551	BT	15 September 2023
<i>Dinonemertes shinkaii</i>	ICHUM 8866	F1-BT	39°27.285'N	143°45.397'E	3685	BT	27 September 2023
Monostilifera sp. KH-2024-01	ICHUM 8852	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Monostilifera sp. KH-2024-02	ICHUM 8853	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
<i>Nectonemertes cf. mirabilis</i>	ICHUM 8867	F10-BT	39°34.320'N	144°10.353'E	6871	AGT	25 September 2023
<i>Nectonemertes cf. mirabilis</i>	ICHUM 8896	A5-BT	41°14.126'N	145°01.108'E	7185	AGT	6 October 2022
<i>Nemertovema kojimai</i> sp. nov.	ICHUM 8857	D3-BT	40°49.560'N	144°21.543'E	6211	AGT	15 October 2022
<i>Nemertovema norenburgi</i>	ICHUM 8856	A5-BT	41°14.126'N	145°01.108'E	7185	AGT	6 October 2022
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8858	D4-BT	40°49.211'N	144°31.565'E	6756	AGT	14 October 2022
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8859	D7-BT	40°41.735'N	144°41.975'E	7040	AGT	13 October 2022
<i>Nemertovema</i> sp. KH-2024-01	ICHUM 8939	D8-BT	40°44.208'N	144°55.613'E	6242	AGT	13 October 2022
<i>Pelagica</i> sp. KH-2024-01	ICHUM 8860	A3-BT	41°20.517'N	144°57.297'E	6131	BT	3 October 2022
<i>Pelagica</i> sp. KH-2024-02	ICHUM 8871	F5-BT	39°31.887'N	143°51.285'E	4565	BT	27 September 2023
<i>Phallonemertes murrayi</i>	ICHUM 8868	C5-BT	41°28.519'N	146°07.632'E	6183	BT	21 September 2023
<i>Phallonemertes murrayi</i>	ICHUM 8869	A2-BT	41°18.514'N	144°43.974'E	5013	BT	2 October 2022
<i>Phallonemertes murrayi</i>	ICHUM 8870	F14-BT	39°27.482'N	144°48.726'E	5798	BT	23 September 2023
<i>Profundonemertes hakuhoae</i> gen. et sp. nov.	ICHUM 8854	A4b-BT	41°12.354'N	144°43.439'E	6807	AGT	6 October 2022
<i>Profundonemertes hakuhoae</i> gen. et sp. nov.	ICHUM 8855	G-BT	38°32.980'N	144°05.381'E	7654	AGT	15 October 2022

<i>Protopelagonemertes beebei</i>	ICHUM 8872	F5-BT	39°31.887'N	143°51.285'E	4565	BT	27 September 2023
<i>Protopelagonemertes beebei</i>	ICHUM 8873	F8-BT	39°41.023'N	144°05.056'E	5702	BT	26 September 2023
Reptantia sp. KH-2024-01	ICHUM 8861	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022
Reptantia sp. KH-2024-01	ICHUM 8862	A0-BT	41°30.974'N	144°08.167'E	2007	BT	2 October 2022

^a Alpha-numeric station names beginning with 'A' were along a transect designated as 'A line' in the project, running perpendicular to the trench axis and 20–30 km northeast to the Erimo Seamount; 'C' were along a transect designated as 'C line', 99–138 km northeast to the seamount, thus, both were in the Kuril-Kamchatka Trench; 'D' were along a transect line designated as 'D line', 59–101 km southwest to the Erimo Seamount; 'F' were in a transect designated as 'F line', 180–215 km southwest to the seamount, both were in the Japan Trench; station G-BT was off Onagawa, about 270 km south-southwest to the Erimo Seamount, on the axis of the Japan Trench.

^b AGT, Agassiz trawl with a 3-m-width aperture; BT, beam trawl with a 4-m-width aperture.

This specimen measured approximately 34 mm in length, with the anterior body (around 5 mm from the tip) wider than the rest of the body and bearing the characteristic ‘tubulanid rings’ (Fig. 56a). Five fragments conspecific to *Tubulanidae* sp. KH-2024-01, with uncorrected pairwise COI *p*-distances of 0.3–0.9% (genetic distances are indicated by values in parentheses in the succeeding sentences unless otherwise noted), were obtained at D3-BT station in the JT. While other tubulanids collected along the JT and KKT were found only at one station, this species was recovered from two stations. *Tubulanidae* sp. KH-2024-01 represented by ICHUM 8897–8899 formed a fully supported subclade with two previously known species *i*) *Tubulanidae* sp. 33DS from the Sea of Okhotsk, and *Tubulanidae* sp. 14DS from near the Kuril Islands [33DS and 14DS differed by 2% COI *p*-distance, suggesting conspecificity], and *ii*) *Tubulanidae* Vema3 from the Vema Fracture Zone (VFZ) of the Atlantic Ocean (Fig. 57). The latter two species have been reported exclusively from the abyssal zone, whereas *Tubulanidae* sp. KH-2024-01 extended down into the upper hadal zone. *Tubulanidae* sp. KH-2024-02 represented by ICHUM 8895 from F2-BT station in the JT formed another fully supported subclade with two species: *i*) *Tubulanidae* KuramBio2 77/4 and *Tubulanidae* KuramBio2 77/1, both from the KKT [KuramBio2 77/4 and KuramBio2 77/1 were considered conspecific in Chernyshev & Polyakova (2019)] and *ii*) *Tubulanidae* Vema6 from the VFZ. These two tubulanid subclades, along with three other species—*i*) *Tubulanidae* KuramBio2 17 from the KKT, *ii*) *Tubulanidae* sp. KH-2024-03 from D7-BT station in the JT, *iii*) *Tubulanus* sp. E Arctica, and *iv*) *Tubulanus izuensis* Hookabe, Asai, Nakano, Kimura & Kajihara, 2020b off the Izu Peninsula, Shizuoka and the Sea of Kumano, Mie, Japan—formed the ‘deep-sea *Tubulanidae* clade’, with *Tubulanidae* sp. KH-2024-03 branching at the basal node of this clade (Fig. 57).

The phylogenetic tree (Fig. 57) further revealed that *Tubulanidae* sp. KH-2024-04 from A0-BT station in the KKT and *Tubulanus* sp. B Sakhalin were sister taxa with full support. A similar sister-taxon relationship was observed between *Tubulanidae* sp. KH-2024-05 from A3-BT station in the KKT and *Tubulanidae* sp. KH-2024-06 from D3-BT station in the JT. The four species of tubulanids collected from the JT (*Tubulanidae* spp. KH-2024-05–KH-2024-08), were not nested within the ‘deep-sea *Tubulanidae* clade’ despite being collected from abyssal to hadal depths. The phylogenetic position of these species within *Tubulanidae* remains unresolved.

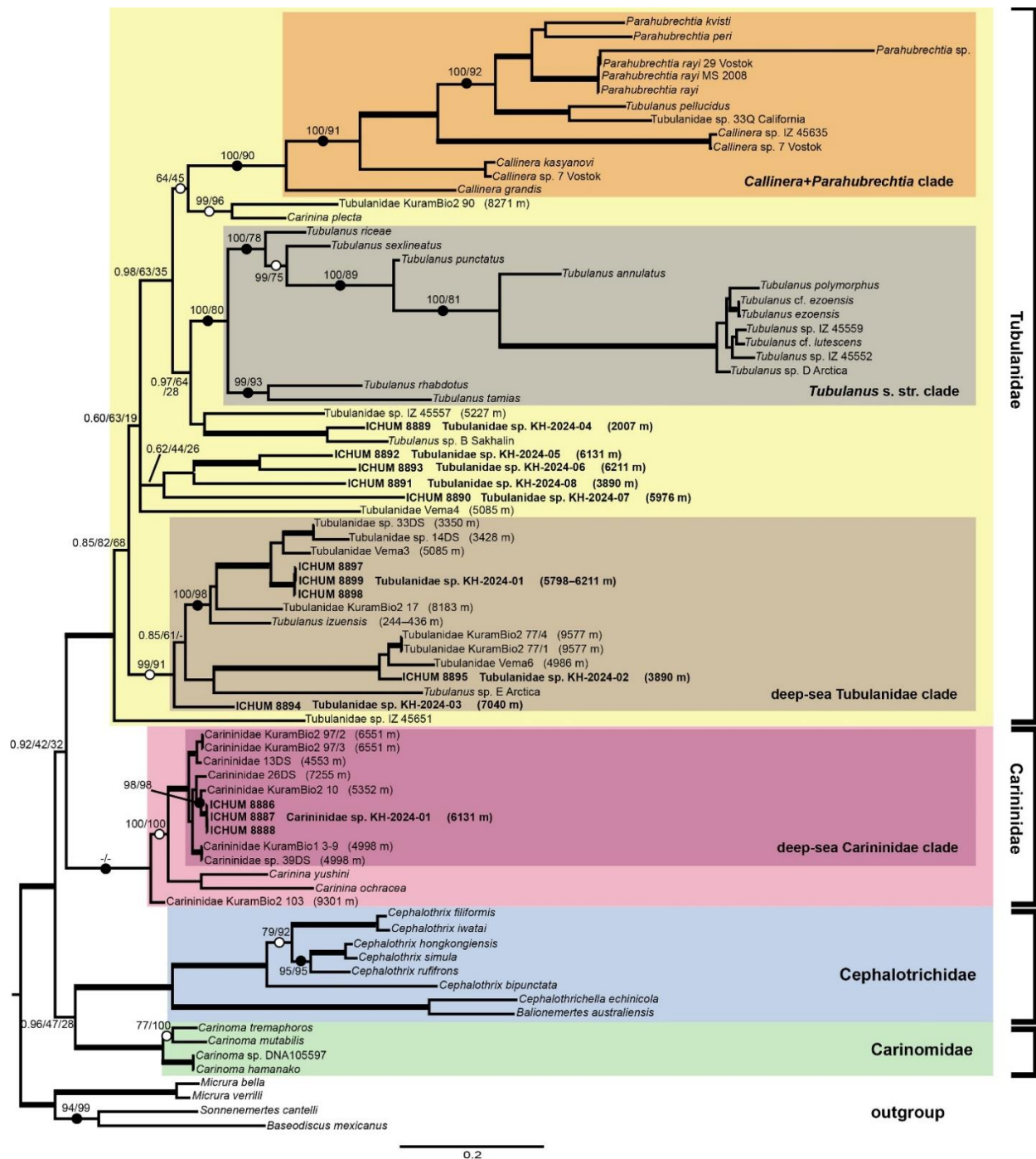


Figure 57. Rooted Bayesian phylogenetic tree for the Palaeonemertea supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAXML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support, 1.00 PP/100% UFBoot/100% BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones. Species in the present study are in bold. The habitat depths of deep-sea species are indicated in parentheses.

Carininidae Chernyshev, 2011

In the surveys, only one species of the family Carininidae (i.e., Carininidae sp. KH-2024-01 from A3-BT station in the KKT) was collected. Three specimens were obtained: two represented by body fragments, ICHUM 8886 and ICHUM 8872, and one intact specimen, ICHUM 8888 (Fig. 56d, e). These specimens were found to be conspecific (varying with 0.15–0.61%). Carininidae sp. KH-2024-01, ICHUM 8888, measured approximately 38 mm in length and 8 mm in width; its head was noticeably narrower than the body. This specimen exhibited light pink body coloration on the dorsal side, while the ventral side was darker except for the head and posterior end. Ventrally, it had a white vertical midline running from the ventral cephalic furrow down to the posterior end of the body. The ventral cephalic furrows were not continuous on the dorsal side.

The phylogenetic analysis (Fig. 57) indicated that Carininidae sp. KH-2024-01 was sister to the undescribed Carininidae KuramBio2 10, also collected in the KKT, with high support. The COI *p*-distance between the latter KKT species and Carininidae sp. ICHUM 8888 was 8.68%. Further DNA analysis demonstrated that the present carininid was distinct from other deep-sea carininids previously documented in the KKT—Carininidae KuramBio2 97/2 and Carininidae KuramBio2 97/3 (13.23–14%), Carininidae 26DS (13.37%), Carininidae KuramBio1 3-9 and Carininidae sp. 39DS (12.81–13.22%), and Carininidae KuramBio2 103 (20.96%). Although a COI sequence was not available for Carininidae 13DS, the tree clearly indicated that this previous KKT species was different from the present carininid.

The phylogenetic tree (Fig. 57) showed that all these KKT carininids formed the ‘deep-sea Carininidae clade’. Although the Bayesian tree did not include Carininidae KuramBio2 101 within this clade, this species was nested within the clade based on the ML trees generated in IQ-Tree and RAxML, albeit with low support (89 UFBoot and 63 BS, respectively).

Pilidiophora

The species delimitation analysis using the ASAP method best-partitioned the pilidiophoran COI sequences into six subsets (putative species), with an ASAP score of 2.00, P-val(rank) of 0.00344(1), and W(rank) of 0.0144(3). The ABGD method yielded nine partitioning patterns, each with seven groups (putative species), with the best

partitioning observed in partition 1, which had the lowest prior maximal distance P-value of 0.001000. The same partitioning was also indicated by the second-best partition generated through ASAP, with an ASAP score of 2.00, P-val(rank) of 0.160(2), and W(rank) of 0.015(2). In this case, seven pilidiophoran species were recognized in the collection following the nemertean COI intraspecific variation set by Sundberg et al. (2016).

Lineidae McIntosh, 1874

The pilidiophorans dominating the present samples were heteronemerteans in the family Lineidae, with five different species in each of the trench, distributed across nine stations (Table 15). Altogether, a total of six lineid species were found across the two trenches. Of the six species, two lineids were in good condition for future taxonomic study. One species, Lineidae sp. KH-2024-01 was represented by a nearly intact specimen, ICHUM 8874, (Fig. 58a–c), collected at A0-BT station in the KKT and by a worm fragment, ICHUM 8875, collected at F3-BT station in the JT. These two specimens belonged to a single species (0.2%). Lineidae sp. KH-2024-01, ICHUM 8874 was approximately 72 mm

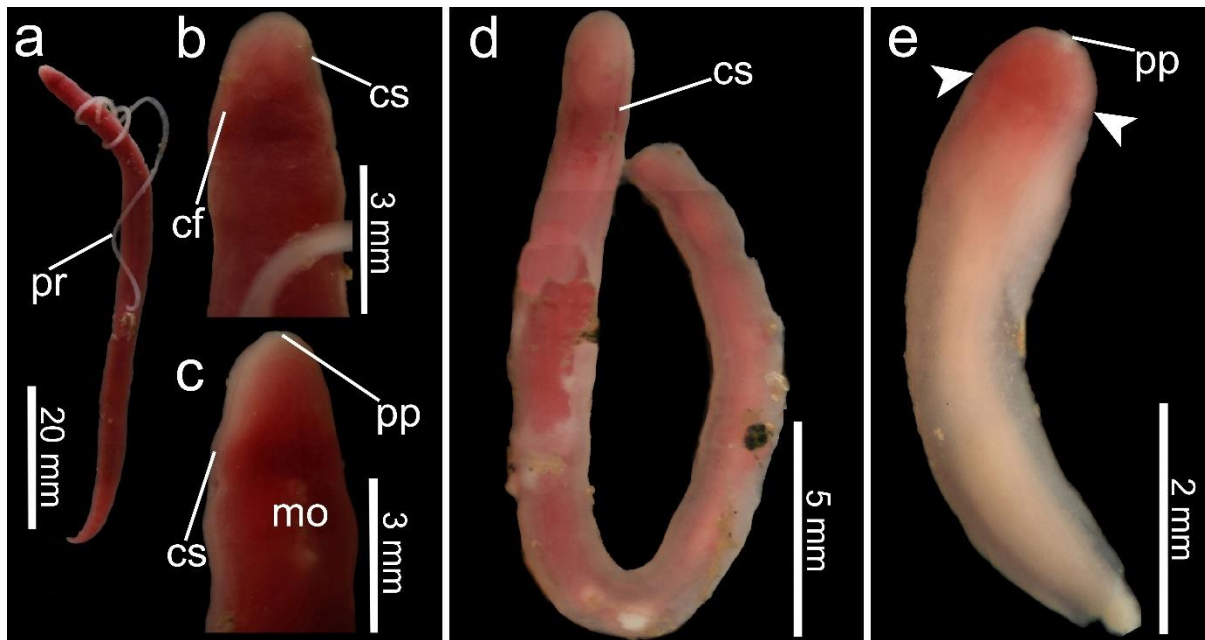


Figure 58. Some of the pilidiophorans collected along the Kuril-Kamchatka Trench (a–e). (a–c), Lineidae sp. KH-2024-01, ICHUM 8874, (a), whole body at dorsal view, (b), magnification of the anterior end at dorsal view, (c), same in b but at ventral view; (d), Lineidae sp. KH-2024-02, ICHUM 8876, anterior body fragment; (e) *Baseodiscus profundus*, ICHUM 8885, anterior body fragment, white arrowheads pointed to the thin cephalic furrow. Abbreviations: cf, cephalic furrow; cs, cephalic slit; mo, mouth; pp, proboscis pore; pr, proboscis.

in length and 4 mm in width, tapering posteriorly. It exhibited a uniformly pinkish color, with the anterolateral edges of the head on both sides, as well as the posterior end of the body, being lighter in shade. The tip of the head (Fig. 58b, c) was also paler on both sides. The mouth was about 2 mm in length, and the specimen had a pair of lateral cephalic slits (Fig. 58b, c) beginning from the tip of the head and extending to the level of the anterior edge of the mouth. Dorsally, a single cephalic furrow (Fig. 58b) was observed starting from the tip of the head and run diagonally downwards terminating at the left lateral edge where the cephalic slit started. A detailed examination of the dorsal body revealed fine, widely spaced horizontal thin bands (or lines) distributed from the anterior body to the posterior end; the bands (or lines) were more apparent posteriorly. This species was nested within Lineage G, sister to the unidentified Lineidae sp. Bering G06 from the Bering Sea, with low support (Fig. 59). These two species appeared to be the same (0.6%) based on 16S since no COI sequence was available for the Bering specimen.

Another species, Lineidae sp. KH-2024-02, ICHUM 8876 (Fig. 58d), was also collected at A0-BT station, KKT, and found to be conspecific with ICHUM 8877 from F0-BT station, JT (1.2%). ICHUM 8876 had lost the posterior end of its body; the size of the remaining specimen was approximately 27 mm in length and uniformly 2 mm in width. The body was entirely pinkish in color and had deep lateral cephalic slits running anteriorly to the tip of the head. This species formed a fully supported subclade with Lineidae sp. Bering G06 and Lineidae sp. KH-2024-01 in Lineage G (Fig. 59). These unidentified lineids formed a clade with *Micrura wilsoni* (Coe, 1904) and *Micrura purpurea* (Dalyell, 1853).

Lineidae sp. KH-2024-03 collected at F14-BT station in the JT, formed a fully supported subclade with Lineidae KuramBio2 85, Heteronemertea IZ 45556/KuramBio1, and Lineidae SokhoBio KB2het6, in the Sea of Okhotsk and in the KKT (Fig. 59). Further DNA analysis showed that these unidentified species were conspecific (0.5–0.9%) and were nested within Lineage D. This species appeared to be an abyssal nemertean, being consistently recovered from depths between 4469 m and 5798 m. Although its distribution along the JT was limited, the species seemed to be more widely distributed in the KKT and in adjacent waters.

Three lineid species from the collection were nested within Lineage B. One species, Lineidae sp. KH-2024-04 was represented by four conspecific specimens (0 to



Figure 59. Rooted Bayesian phylogenetic tree for the Pilidiophora supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAxML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support, 1.00 PP/100% UFBoot/100% BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones. Species in the present study are in bold. The habitat depths of deep-sea species are indicated in parenthesis.

0.3%): ICHUM 8881, from C2-BT station, KKT; ICHUM 8879 from D4-BT station, JT; ICHUM 8880 from D7-BT station, JT; and ICHUM 8883 from F8-BT station, JT. Additionally, the

unidentified Lineidae KuramBio1 12-5 from the KKT, also belonged to this species (0–1.0%). Among the collected lineids, this abysso-hadal species had the widest distribution range, being present at three JT stations and one KKT station. Another species within Lineage B, Lineidae sp. KH-2024-05 from F7-BT station, JT, showed a sister-taxon relationship with the former species (9.6–9.7%). These two unidentified lineids formed a fully supported subclade with Lineidae sp. Antarctica 16 (Fig. 59). Lastly, Lineidae sp. KH-2024-06 from A0-BT station, KKT, exhibited a sister-taxon relationship with *Micrura* sp. IZ 132532 (16.3%) from Panama within Lineage B.

Valenciniidae Hubrecht, 1879

The only valenciniid heteronemertean documented in this study was obtained at A1-BT station, KKT, represented by ICHUM 8885 (Fig. 58e), an anterior body fragment of 66 mm in length and 1.0 mm in width. The specimen, identified as *Baseodiscus profundus* Kajihara, Abukawa, & Chernyshev, 2022a had a dark rose (or pink) head and a pale rose body; dorsally, it displayed a thin transverse cephalic furrow without secondary furrows and lacked eyes. These characteristics were consistent with the original description of *B. profundus* (Kajihara et al. 2022a). Its identification as *B. profundus* was further confirmed by the phylogenetic analysis (Fig. 59). Both DNA and morphological evidence confirmed the presence of this species in the KKT, marking its first recorded occurrence in the trench and in Japanese waters. *Baseodiscus profundus* was only known from its type locality in the Sea of Okhotsk, at depths of 3300–3306 m (Kajihara et al. 2022a). This new record from the KKT extended its known distribution southeast of the type locality and to a greater depth (4028 m).

Hoplonemertea

The species delimitation analysis using the ASAP method best-partitioned the hoplonemertean COI sequences into 13 subsets (putative species) with an ASAP score of 2.00, P-val(rank) of 0.0113(1), and W(rank) of 0.00713(3). The ABGD method grouped the sequences into nine partitions, each suggesting 13 groups (putative species), with the best partitioning observed in partition 1, which had the lowest prior maximal distance P-value of 0.001000. Based on the ‘3%-rule’ intraspecific genetic variation set by Sundberg et al. (2016), there were 14 distinct COI sequences in the hoplonemertean

dataset. This result (i.e., having 14 putative species) corresponded to the third best-partition generated through ASAP method, with an ASAP score of 4.50, P-val(rank) of 0.0776 (7), and W(rank) of 0.00112(2)). Here, 14 hoplonemertean species were recognized in the collection.

Polystilifera Brinkmann, 1917

Five known and two unidentified species in *Pelagica* Brinkmann, 1917 were collected along the two trenches, distributed in 11 stations (Table 15). These species were i) *Dinonemertes arctica* Korotkevich, 1977; ii) *Dinonemertes shinkaii* Kajihara & Lindsay, 2010; iii) *Nectonermertes* cf. *mirabilis* Verrill, 1892; iv) *Phallonemertes murrayi* (Brinkmann, 1912); v) *Protopelagonemertes beebei* Coe, 1936; vi) *Pelagica* sp. KH-2024-01; and vii) *Pelagica* sp. KH-2024-02.

Reddish, transparent, and macerated worm fragments with noticeable intestinal diverticula were collected along the JT and KKT. These pelagic specimens were found at A3-BT and C2-BT stations in the KKT, and at D10-BT and F10-BT stations in the JT. All specimens belonged to the same species (0–0.35%). A MegaBLAST search using COI sequences from these specimens showed 99.24–99.39% sequence identity with GenBank accession number FJ602542, identified as *Dinonemertes arctica* by Bucklin et al. (2010) from off the northern coast of Alaska in the Arctic Ocean. The COI *p*-distance between the present material and the Arctic Ocean specimen ranged from 0.6 to 0.87%. The phylogenetic analysis (Fig. 60) showed that the JT and KKT *D. arctica* formed a fully supported clade with the Arctic Ocean specimen. Based on the identification by Bucklin et al. (2010), my molecular analysis confirmed this as the first record of *D. arctica* in Japanese waters. Previously, this species was only known from the Arctic Ocean (Korotkevich 1977; Bucklin et al. 2010). The findings of this species in the JT and KKT expanded its habitat range to the Pacific Ocean.

ICHUM 8866, identified herein as *Dinonemertes shinkaii*, was collected at F1-BT station in the JT, approximately 70 km from the type locality. In the living state, the specimen was 54 mm in length and 17 mm in width (Fig. 61a, b). It had 35 pairs of intestinal diverticula, compared to 25 pairs in the holotype (Kajihara and Lindsay, 2010), a morphological difference I interpret as intraspecific variation. All other characteris-



Figure 60. Rooted Bayesian phylogenetic tree for the Hoplonemertea supermatrix based on concatenated sequences of five gene markers (16S, 18S, 28S, COI, and H3). Numbers at each branch represent support values, posterior probability (PP)/IQ-Tree maximum-likelihood ultrafast bootstrap value (UFBoot)/RAXML maximum-likelihood bootstrap value (BS). Thickened branches indicate full support, 1.00 PP/100% UFBoot/100% BS. Closed circles indicate 1.00 PP, open circles indicate 0.99 PP. Support values were omitted except for those major, deeper nodes and those fully and highly supported ones.

tics of ICHUM 8866 matched the original description of the species. The phylogenetic analysis (Fig. 60) indicated that *D. arctica* and *D. shrinkaii* were sister taxa.

Two intact pelagic nemerteans, identified as *Nectonemertes* cf. *mirabilis* (Fig. 61c–f), ICHUM 8867 and ICHUM 8896, were collected at F10-BT, JT, and A5-BT, KKT stations, respectively. Of the two, ICHUM 8867 was sequenced and confirmed to be the same species as those previously collected in the Kuril Islands (Chernyshev & Polyakova 2018b) and off the Pacific coast of North America (Thollessen & Norenburg 2003) (0–0.46%). I followed Chernyshev & Polyakova (2018b) in refraining from applying a definitive species name to my specimens, considering the possibility that the Pacific population may represent *Nectonemertes pelagica* Cravens & Heath, 1906, rather than *N. mirabilis* s.str., originally established based on Atlantic material (Verrill 1892). I regarded *Nectonemertes japonica* Foshay, 1912 and *Nectonemertes major* Korotkevich, 1955 as synonymous with at least *N. pelagica*, if not *N. mirabilis*. The external features of ICHUM 8867 and ICHUM 8896 were consistent with the descriptions of *N. mirabilis* (Coe 1926; Kajihara 2017; Chernyshev & Polyakova 2018b). ICHUM 8867 (Fig. 61c, d), a female without tentacles, measured 20 mm in length and 1.7 mm in width, while ICHUM 8896 (Fig. 61e, f), a male with tentacles, measured 26 mm in length and 2.7 mm in width. In the living state, both specimens exhibited deep orange coloration dorsally and ventrally, with the brain visible as a red mass in the head, prominent in dorsal view; both had caudal fins. *Nectonemertes* cf. *mirabilis* (i.e., the Pacific species) has been previously reported in the North Pacific (i.e., Japan—Okinawa, Russia-Okhotsk Sea, Kuril Islands and Kamchatka, Bering Sea, Gulf of Alaska, USA, Mexico); and South Pacific (next to Peru) (Roe & Norenburg 1999; Thollessen & Norenburg 2003; Kajihara 2017; Chernyshev & Polyakova 2018b, 2019).

Three intact pelagic nemerteans (COI *p*-distance up to 0.61%) were collected at A2-BT and C5-BT stations, KKT, and F14-BT station, JT. These specimens were found to be conspecific (0.3%) with the pelagic nemertean identified as *P. murrayi* off the Pacific coast of North America (Thollessen & Norenburg, 2003). All three specimens were female: ICHUM 8868 (Fig. 61g, h) measured 48 mm in length and 7 mm in width, with 26 pairs of intestinal diverticula and ovaries alternating in all intestinal diverticula; ICHUM 8869 measured 36 mm in length and 6 mm in width, with 38 pairs of intestinal diverticula and ovaries alternating from the 3rd to 27th intestinal diverticula; and ICHUM 8870

measured 38 mm in length and 5 mm in width, with 35 pairs of intestinal diverticula and ovaries alternating from the 1st to 32nd intestinal diverticula. In the living state, the speci-

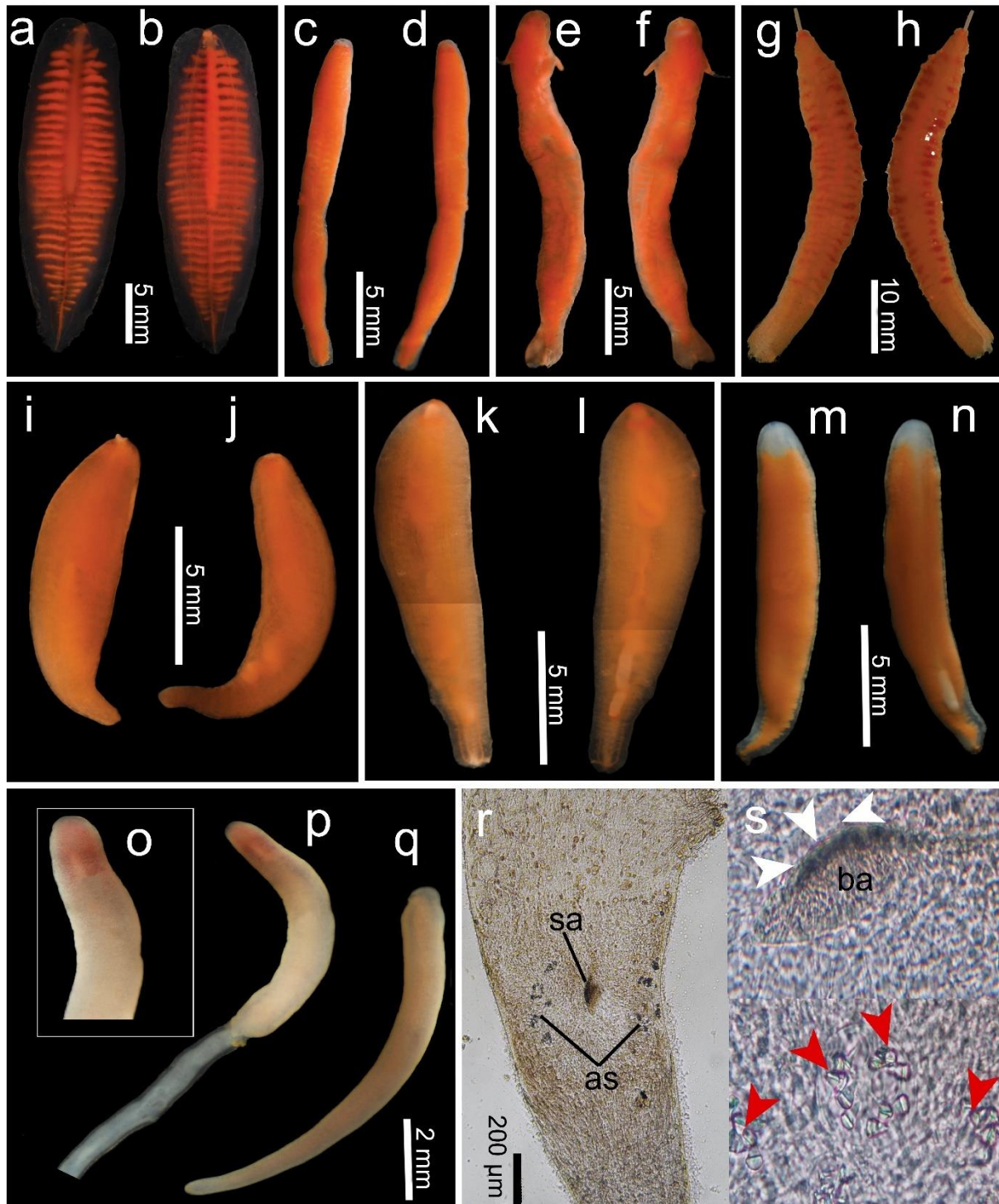


Figure 61. Some of the polystiliferous hoplonemertean collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–s). (a, b), *Dinonemertes shinkaii*, ICHUM 8866, from JT, (a), dorsal and (b), ventral views; (c, d), *Nectonemertes* cf. *mirabilis*, ICHUM 8867, female, from JT, (c), dorsal and (d) ventral views; (e, f), *N. cf. mirabilis*, ICHUM 8896, male, from KKT, (e), dorsal and (f), ventral views; (g, h),

Phallonemertes murrayi, ICHUM 8868, female, from KKT, (g), dorsal and (h) ventral views; (i, j), *Protopelagonemertes beebei*, ICHUM 8872, from JT, (i), dorsal and (j), ventral views; (k, l), *Pelagica* sp. KH-2024-01, ICHUM 8860, from KKT, (k), dorsal and (l), ventral views; (m, n), *Pelagica* sp. KH-2024-02, ICHUM 8871, from JT, (m), dorsal and (n), ventral views; (o–s), *Reptantia* sp. KH-2024-01, ICHUM 8862, from KKT, (o), magnification of the anterior body, (p), anterior body fragment, (q), posterior body fragment, (r), proboscis region containing the stylet apparatus at squeezed preparation, (s), same in r but central (pointed by white arrowheads) and accessory stylets (pointed by red arrowheads). Abbreviations: as, accessory stylets; ba, basis; sa, stylet apparatus.

mens displayed color variation, with the first two being deep orange and the latter showing a lighter hue. The brain appeared as an orange mass near the tip of the head, most prominent in ventral view. The lateral nerve cords were visible ventrally, extending to the posterior end of the body; all specimens had caudal fins. *Phallonemertes murrayi*, currently a single member of the genus, has been previously known to be distributed in the North Atlantic and off the Pacific coast of North America (Coe 1945; Gibson 1995; Roe & Norenburg 1999; Tholleson & Norenburg 2003). The phylogenetic tree (Fig. 60) showed that *P. murrayi* was sister to the clade formed by *P. beebei* and the unidentified Pelagic sp. KH-2024-02 from the JT. This finding indicated the first report of *P. murrayi* in Japanese waters.

Two conspecific (0%) pelagic nemerteans (Fig. 61i, j) that morphologically resembled *Protopelagonemertes beebei* (Kajihara et al. 2011; Kajihara & Yamaguchi 2020) were collected at F5-BT and F8-BT stations, JT. Generally, the worms were uniformly orange in the living state, with a cross-sectionally rounded body, tapering at the head and tail. The intestinal diverticula were not visible from the exterior; tentacles or fins were absent. ICHUM 8872 was 10–12 mm in length and 3 mm in width, while ICHUM 8873 was 26–35 mm in length and 5 mm in width. Their identity as *P. beebei* was further confirmed molecularly when the JT specimens showed conspecificity with the specimens identified as *P. beebei* (0%) from Sagami Bay and Hokkaido, Japan (Kajihara et al. 2011; Kajihara & Yamaguchi 2020). However, these four *P. beebei* specimens were found to be molecularly different (17.5%) from GenBank accession number HQ848602 from the specimen MCZ DNA106132, identified as *P. beebei* by Andrade et al. (2012). The latter COI sequence was probably contaminated or mislabeled. *Protopelagonemertes beebei* was previously recorded in the Northwest Atlantic, Northeast Pacific, and

Northwest Pacific and lived in the bathyal to hadal zones of the deep ocean (Coe 1936, 1954; Kajihara et al. 2011; Kajihara & Yamaguchi, 2020).

An unidentified pelagic nemertean, *Pelagica* sp. KH-2024-01 (Fig. 61k, l), represented by ICHUM 8860, was collected at A3-BT station, KKT. In the living state, the specimen measured 14 mm in length and 4 mm in width, with a uniform orange coloration. It was opaque except for the slightly transparent posterior end. The brain was visible externally as a reddish mass, most prominent ventrally. The lateral nerves were noticeable ventrally, especially towards the posterior, extending down to the caudal fin, other internal body structures noticeable ventrally. *Pelagica* sp. KH-2024-01 bore a superficial resemblance to *P. beebei*, though it differed from the latter species in several aspects: it was cross-sectionally less-round, brain mass visible in the anterior body, and with caudal fins. A MegaBLAST search using the COI barcode sequence of ICHUM 8860 revealed a close genetic similarity to *D. arctica*, with 91.44% sequence identity. The phylogenetic analysis (Fig. 60) revealed that *Pelagica* sp. KH-2024-01 was sister to the clade formed by species of the genera *Pelagonemertes* Moseley, 1875, *Dinonemertes* Laidlaw, 1906, *Phallonemertes* Brinkmann, 1917, and *Protopelagonemertes* Brinkmann, 1917.

Another unidentified pelagic nemertean (Fig. 61m, n), *Pelagica* sp. KH-2024-02, represented by ICHUM 8871, was collected from F5-BT station, JT. The specimen was 14 mm in length and 3 mm in width in living condition. The body was transparent. The internal body structures appeared as orange masses visible from both dorsally and ventrally. The head was opaque, white in color. It had caudal fins. The phylogenetic tree (Fig. 60) indicated that this species was sister to a clade formed by *P. beebei* and the specimen identified as *P. beebei* in Andrade et al. (2012).

Lastly, four specimens representing an unidentified reptant polystiliferan were obtained at A0-BT station, KKT. All specimens belonged to a single species (0%), *Reptantia* sp. KH-2024-01. Two specimens, ICHUM 8861–8862 were included in the phylogenetic analysis. ICHUM 8862 (Fig. 61o–s) was 21 mm in length and 1.5 mm in width in the living state. The body narrows towards its posterior end. Dorsally, the head was pale rose in color. The body had no notable pigment, uniformly opaque white. The body surface was dotted with minute white grains. The phylogenetic tree (Fig. 60) indicated

that Reptantia sp. KH-2024-01 was sister to an unidentified reptantian from the Sea of Okhotsk, Reptant SokhoBio 1-9 (5.4%).

Monostilifera Brinkmann, 1917

A total of six species in Monostilifera were recorded in the survey. Three species were documented along the JT and four in the KKT, with one species shared between the two trenches (Table 15). Five of the species belonged in Amphiporina Chernyshev & Polyakova, 2019 emend. Kajihara, 2021: i) Monostilifera sp. KH-2024-01, KKT; ii) Monostilifera sp. KH-2024-02, KKT; iii) *Nemertovema* sp. KH-2024-01, JP; iv) *Nemertovema norenburgi*, KKT); and v) *Nemertovema kojimai* sp. nov., JP; while one new species, *Profundonemertes hakuhoae* gen. et sp. nov., JP and KKT, belonged in Oerstediiina Chernyshev & Polyakova, 2019 emend. Kajihara, 2021.

Monostilifera sp. KH-2024-01, represented a single specimen, ICHUM 8852, collected at A0-BT station, KKT, was a pinkish and cross-sectionally round worm fragment, with minute white granules all over the epidermis giving it a ‘powdery’ texture. It was 19 mm in length and 5 mm in width. The phylogenetic analysis (Fig. 60) indicated that Monostilifera sp. KH-2024-01 formed a highly supported clade with the unidentified Monostilifera spp. from the east coast of the Pacific Ocean.

Monostilifera sp. KH-2024-02, represented by an anterior body fragment, ICHUM 8853 (Fig. 62m–o), was also recovered at A0-BT station. This worm fragment was cross-sectionally round, and pale reddish in color. It was anteriorly narrowed, ending with an opaque white tip, with the head not demarcated from the body. The cephalic furrow encircled the head. The fragment was 13 mm in length and 2 mm in width. The dark and long stylet basis was 905 µm in length and 157 µm in width, broad at the posterior end. Although the central stylet appeared broken (Fig. 62o), a single stylet pouch was found with two, short accessory stylets, 156 µm in length. This undescribed species was nested within Amphiporina although its phylogenetic position within the infraorder remained unresolved. (Fig. 60).

A white, opaque worm fragment, with a semi-translucent body wall, 20 mm in length and 4 mm in width (Fig. 62p) was collected at A5-BT station, KKT. This fragment appeared to be *N. norenburgi*, as a COI MegaBLAST search resulted in an identity match

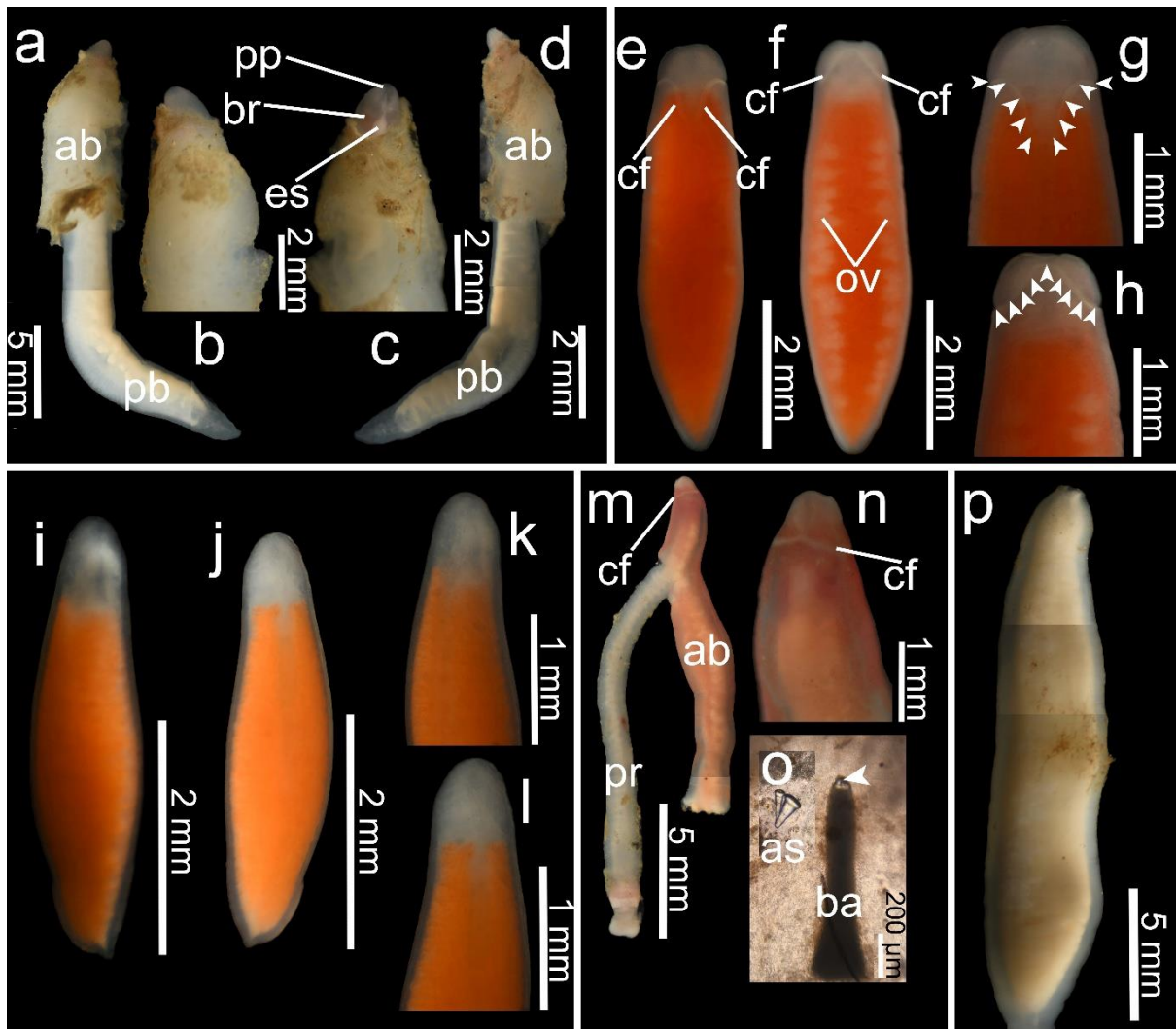


Figure 62. Some of the monostiliferous hoplonemertean specimens collected along the Japan Trench (JT) and the adjacent Kuril-Kamchatka Trench (KKT) (a–p). (a–d), *Nemertovema kojimai* sp. nov., ICHUM 8857, from KKT, (a), whole body at right-lateral view, (b), magnification of the anterior end at right-lateral view, (c), same in b but at ventrolateral view, (d), same in a but at left-lateral view, posterior body covering detached; (e–h), *Profundonemertes hakuhoae* gen. et sp. nov., ICHUM 8854, from KKT, (e), whole body at dorsal view, (f), same in e but at ventral view, (g), magnification of the anterior end at dorsal view, white arrowheads trace the shape of the cephalic furrows, (h), same in g but at ventral view; (i–l), *P. hakuhoae* gen. et sp. nov., ICHUM 8855, from JT, (i), whole body at dorsal view, (j), same in i but at ventral view, (k), magnification of the anterior end at dorsal view, (l), same in k but at ventral view, cephalic furrows not visible since epidermis of the specimen has been detached from the body; (m–o), *Monostilifera* sp. KH-2024-02, ICHUM 8853, from KKT, (m), anterior body fragment with released proboscis at lateral view, (n), magnification of the anterior end at lateral view, (o), stylet apparatus, white arrowhead pointed to the broken central stylet; (p), *Nemertovema norenburgi*, ICHUM 8856, body fragment, from KKT. Abbreviations: ab, anterior body; as, accessory stylets; ba, basis; br, brain; cf, cephalic furrow; es, esophagus; ov, ovary; pb, posterior body; pp, proboscis pore; pr, proboscis.

of 99.54–99.70% with the specimens KBhop 32 and KBhop 33 of *N. norenburgi* also in the KKT. The COI *p*-distance between these three specimens of *N. norenburgi* was 0.5% (Table 16), which further confirmed conspecificity. My study represented a second record of *N. norenburgi* after its original description from the KKT at depths of 8221–8271 m (Chernyshev & Polayakova 2018a).

Two specimens obtained in the JT, one represented by a transparent worm fragment, 4 mm in length and 0.3 mm in width, with orange intestinal material, obtained at D4-BT station, the other, a small, white, opaque fragment, 3.3 mm in length and 0.6 mm in width collected at D7-BT station. These specimens were considered to belong to a single species (1.0%). A MegaBLAST search of the COI of the one collected at D4-BT station, ICHUM 8858, showed that it was closely related to *N. norenburgi* (90.47–90.80% identity), based on available COI sequences from two specimens (GenBank accession numbers, MN205523 and MN205524) while the one collected at D7-BT, ICHUM 8859, showed 89.45–89.62% identity to the same species. The phylogenetic analysis (Fig. 60) revealed that specimens ICHUM 8858 and 8859 were nested within the genus *Nemertovema* Chernyshev & Polyakova, 2018a, and sister to the clade formed by *N. hadalis*, *N. norenburgi*, and *N. kojimai* sp. nov. The genetic distance analysis using the COI of this species, denoted as *Nemertovema* sp. KH-2024-01, with the rest of the genus congeners confirmed that it is a unique member of the genus (Table 16). Thus, *Nemertovema* sp. KH-2024-01 represented an undescribed species of the genus in the JT. Another *Nemertovema* fragment, ICHUM 8939 was obtained at D8-BT station, JT. This

Table 16. Uncorrected COI *p*-distance (%) between the described and undescribed *Nemertovema* congeners. *Nemertovema* with *p*-distance $\geq 3\%$ were treated as different species.

Species and specimen	1	2	3	4	5	6	7	8
1. <i>Nemertovema hadalis</i>								
2. <i>Nemertovema kojimai</i> sp. nov. ^a	7.5							
3. <i>Nemertovema norenburgi</i> ^b	8.1	4.8						
4. <i>Nemertovema norenburgi</i> ^c	8.5	5.2	0.5					
5. <i>Nemertovema norenburgi</i> ^d	8.3	5.2	0.5	0.3				
6. <i>Nemertovema</i> sp. KH-2024-01 ^e	10.8	10.4	9.2	9.5	9.5			
7. <i>Nemertovema</i> sp. KH-2024-01 ^f	12.2	11.6	10.4	10.6	10.6	1.0		
8. <i>Nemertovema</i> sp. KH-2024-01 ^g	13.4	13.0	11.2	11.5	11.5	1.9	2.5	

^aICHUM 8867, ^bKB2hop32, ^cKB2hop33, ^dICHUM 8856, ^eICHUM 8858, ^fICHUM 8859, ^gICHUM 8939

fragment was conspecific with ICHUM 8858-8859 (1.9–2.5%) (Table 16). *Nemertovema* sp. KH-2024-01 was left undescribed in the study since the recovered specimens were too imperfect, rendering them not suitable for species description.

Order Monostilifera Brinkmann, 1917

Suborder Eumonostilifera Chernyshev, 2003

Family Amphiporidae Örsted, 1843

Genus *Nemertovema* Chernyshev & Polyakova, 2018a

***Nemertovema kojimai* sp. nov.** Abato & Kajihara (Fig. 62a–d).

urn:lsid:zoobank.org:act:27569AC8-9596-4341-834F-96FB905F9A9F.

Material examined. Holotype, ICHUM 8857, extracted genomic DNA, collected along the Japan Trench, AGT, on Oct. 15, 2022, station D3-BT, 40°49.560'N, 144°21.543'E, 6211 m.

Etymology. The specific epithet is a noun in the genitive case, attributed to Prof. Shigeaki Kojima of the University of Tokyo, being the primary research investigator of the R/V *Hakuho-maru* research expeditions along the Japan Trench.

Type locality and distribution. At present the species is known only from the Japan Trench hadal zone, station D3-BT.

Sequences. Sequences generated from *Nemertovema kojimai* sp. nov. were deposited in GenBank with accession numbers shown in Table 13.

Description. *External features.* In the living state, the body was white in color, opaque, cross-sectionally round in shape (skin/epidermal covering at the posterior body detached) (Fig. 62a, d); 6.2 mm in length and 4 mm in width on its anterior region where skin was intact, and 1 mm in width on its posterior region where skin was detached. The head was not demarcated from the body and with narrowed anterior end. No visible furrows were observed. No sign of anterior body pigmentation.

Taxonomic remarks. The new species was placed in the genus *Nemertovema* mainly based on DNA and phylogenetic data, being distinct from the current two described species of the genus, *N. hadalis* (7.5%) and *N. norenburgi* (4.8–5.2%) (Table 16). The phylogenetic analysis (Fig. 60) showed that *N. kojimai* sp. nov. and *N. norenburgi* were fully supported sister taxa and were closely related to *N. hadalis*. The described species of *Nemertovema* were all white in color, opaque, without body pigmentation, and no cephalic furrows, making them impossible to discriminate using external morphology alone and without determining their COI barcode sequence. Compared to another known hadal monostiliferan, *Abyssonemertes kajiharai* Chernyshev & Polyakova, 2018a, to which the body form of the new species was similar, the latter had no reddish pigmentation on the tip of the head.

Order Monostilifera Brinkmann, 1917

Suborder Eumonostilifera Chernyshev, 2003

Family Oerstidiidae Chernyshev, 1993

Genus *Profundonemertes* gen. nov. Abato & Kajihara.

Type species. *Profundonemertes hakuhoae* sp. nov. (Fig. 62e–l), fixed by original designation.

Diagnosis. Small monostiliferous hoplonemerteans not more than 5 mm in length; ground body semi-translucent, uniformly pigmented except semi-translucent head; eyes absent; a single ‘V’-shaped cephalic furrow present; large cerebral organs present; cephalic glands enormous, extending posterior to the brain; cephalic blood vessels protrude into the rhynchocoel; rhynchocoel wall two-layered; acidophilic submuscular glands ventrally present; lateral nerve without accessory nerve; mid-dorsal blood vessel present, not forming vascular plug; proboscis anterior chamber notably short.

Etymology. The generic name, feminine in gender, is a combination of the Latin *profundus* meaning ‘deep’ and the Greek *Nemertes* meaning the ‘nereid of unerring’, one of the marine-nymph daughters of *Nereus* and *Doris* in Greek mythology. Together, the

genus *Profundonemertes* means deep-sea nymphs or nemerteans inhabiting the hadal zone of the deep ocean. The specific epithet *hakuhoae*, a noun in the genitive case, was taken from the R/V *Hakuho-maru*, which was used to explore the deep-sea nemerteans along the JT and the adjacent KKT.

Remarks. *Profundonemertes* gen. nov. is erected as a new genus based on combined external morphological and histological features and on its phylogenetic distinctiveness within Oerstedidae. The sister taxon relation and exact phylogenetic position of the new genus within the family were not fully resolved in the present analysis.

***Profundonemertes hakuhoae* sp. nov.** Abato & Kajihara (Fig. 62e–l).

urn:lsid:zoobank.org:act:FFC480EA-62D7-4703-8BCF-814316274B55.

Material examined. Holotype, ICHUM 8854 (Fig. 62e–h), extracted genomic DNA and transverse sections (eight slides), collected along the Kuril-Kamchatka Trench, AGT, on Oct. 6, 2022, station A4b-BT, 41°12.354'N, 144°43.439'E, 6807 m. Paratype, ICHUM 8855 (Fig. 62i–l), extracted genomic DNA and transverse sections (6 slides), collected along the Japan Trench, AGT, on Oct. 15, 2022, station G-BT, 38°32.980'N, 144°05.381'E, 7654 m.

Type locality and distribution. At present the species is known only from the Japanese waters, hadal zone, in the KKT, station A4b-BT and JT, station G-BT.

Sequences. Sequences generated from *Profundonemertes hakuhoae* gen. et sp. nov. were deposited in GenBank with accession numbers shown in Table 13.

Description. *External features.* Holotype. Body at living state was about 5 mm in length, 1.4 mm in width; dorso-ventrally flat, anterior end narrowed than mid-body, posterior end tapered; ground body semi-translucent in color, with reddish-orange pigmentation immediately after the colorless or semi-translucent head (Fig. 62e–h); a single pair of deep cephalic furrow encircled the head, dorsally, the furrows from the lateral edges of the head run then converged backwards resembling a posteriorly-open 'V' (Fig. 62e, g),

ventrally, the furrows from the lateral edges of the head run then converged anteriorly immediately below the tip of the head, resembling a broad 'Λ' (Fig. 62f, h); mature gonads (i.e., ovaries) were visible in the ventral body (Fig. 62f, h); no ocelli; stylet apparatus not observed.

Histology based on the holotype.

Body wall and musculature. Composed of ciliated epidermis, connective tissue basement membrane, outer circular muscle layer and inner circular muscle (Fig. 63b–f). Acidophilic subdermal glands notably present starting from the pre-cerebral region extending posterior to the brain, volume decreases to the hindgut down to the succeeding region (Fig. 63b–g).

Proboscis apparatus. Rhynchocoel wall with two very thin muscle layers (outer circular, inner longitudinal) (Fig. 63e–i). Rhynchocoel extended to the posterior end of the body. With two accessory stylet pouches (Fig. 63f). Proboscis with inner circular and outer longitudinal muscle layers (Fig. 63d–f); anterior chamber significantly short. Number of proboscis nerves indiscernible (Fig. 63e–f).

Alimentary canal. Combined proboscis and esophagus opening sub-terminal.

Blood vascular system. Mid-dorsal blood vessel present, extremely flattened, situated laterally (Fig. 63h). With lateral cephalic blood vessels protruding into the rhynchocoel (Fig. 63e).

Nervous system. Lateral nerve with a single neuropil, no accessory lateral nerve (Fig. 63g–i).

Apical organ and cephalic glands. Basophilic gland lobules enormous, extend posterior to the brain (Fig. 63a–f).

Sense organs. A pair of cerebral organ present, reaches the brain, terminates at the ventral cerebral commissure, cerebral canal simple, unforked, opens ventro-laterally into the ventral cephalic furrows. No eyes (Fig. 63c, d).

Reproductive system. Sexes are separate; mature female; ovaries were arranged in ventro-lateral row, do not regularly alternate in the lateral intestinal diverticula; anterior-most ovaries appear near the posterior end of the pylorus; each ovary contains up to 10 primary oocytes (Fig. 63h, i).

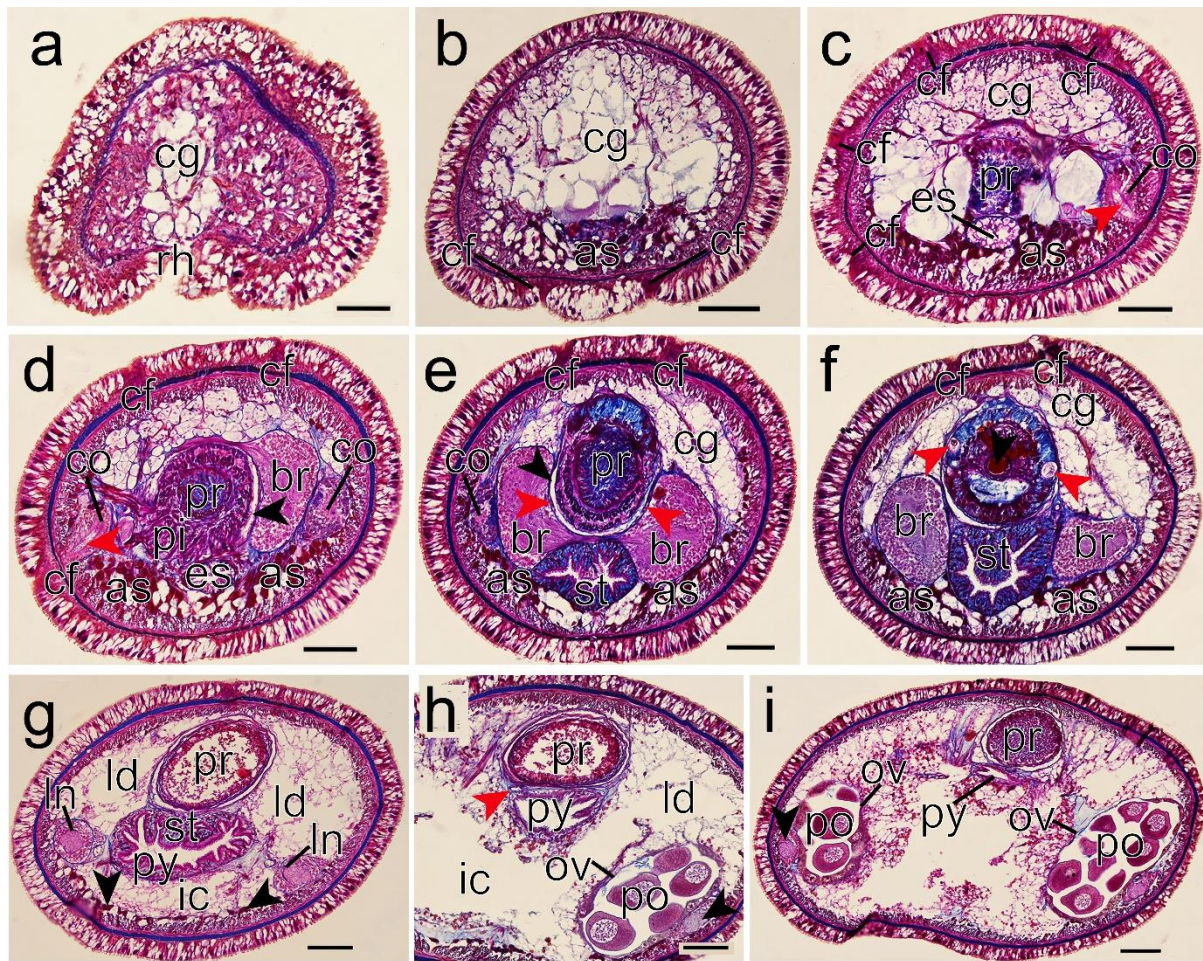


Figure 63. *Profundonemertes hakuhoae* gen. et sp. nov., holotype, ICHUM 8854, transverse histological sections (a–i). (a), anterior end of the head; (b, c), pre-cerebral region, red arrowhead pointed to the cerebral organ canal; (d), proboscis insertion, red arrowhead pointed to the cerebral organ canal, black arrowhead to the rhynchocoel; (e), midgut region, black arrowhead pointed to the rhynchocoel, red arrowheads on the cephalic blood vessels protruding into the rhynchocoel; (f), proboscis armature, black arrowhead pointed on the circular stylet basis, red arrowheads on the accessory stylet pouches; (g), stomach-pyloric junction; (h), mid-dorsal blood vessel (pointed by red arrowhead) in the posterior pyloric region; (i), ovaries containing primary oocytes. Abbreviations: as, acidophilic sub-dermal glands; br, brain; cg, cephalic gland lobules; cf, cephalic furrow; co, cerebral organ; es, esophagus; ic, intestinal cecum; ld, lateral diverticulum of the intestinal cecum; ln, lateral nerve; ov, ovary; pi, proboscis insertion, po, primary oocytes; pr, proboscis; py, pylorus; rh, rhynchostomodaeum. Scale bars: a–i = 100 μ m.

External features. Paratype. Body at living state was 4 mm in length, 1 mm in width; dorso-ventrally flat, narrowed at both ends, ground body semi-translucent, with bright orange pigmentation, head colorless on both sides; no ocelli; cephalic furrows not apparent; stylet apparatus not observed; ovaries not apparent from the body (Fig. 62i–l).

Taxonomic remarks. ICHUM 8854 and ICHUM 8855 differed in their external body features—the former had a reddish-orange ground body color; the characteristic cephalic furrows; and its matured ovaries visible from the ventral body as pale-yellow masses while the latter had a bright orange ground body color, no cephalic furrows and no visible gonads on the ventral side. Histological sections revealed that both specimens were female. ICHUM 8854 have ripened ovaries with cavities occupied by primary oocytes which had undergone active cell division while ICHUM 8855 had smaller oocytes located on the periphery of the unripened ovary, thought to be the reason why the ovaries in ICHUM 8855 were not visible at the ventral view. The absence of the characteristic cephalic furrows in ICHUM 8855 was due to the damaged and removed epidermis of the worm confirmed by the histological sections of the specimen. Nonetheless, both specimens showed the characteristic enormous acidophilic subdermal glands. The COI sequence from both specimens further confirmed conspecificity (0.54%), despite the observed difference in external features and a great habitat distance (301 km apart).

Profundonemertes hakuhoae's unique external feature was the construction of its cephalic furrows. Having a deep, mid-dorsal 'V'-shaped cephalic furrows had been recently reported among members of the genus *Proamphiporus* Chernyshev & Polayakova, 2019. A closed mid-dorsal 'V'-shaped furrows, a 'Λ'-shaped mid-ventral one, and an additional transverse furrow immediately above the anterior tip of the ventral furrows was observed in *P. crandalli* Chernyshev & Polyakova, 2019. *Proamphiporus kaimeiae* Hookabe, Tsuchida, Fujiwara & Kajihara, 2020a also displayed a closed mid-dorsal 'V'-shaped furrows which was continuous ventrally as a transverse line in front of the head (Hookabe et al. 2020a). The same mid-dorsal 'V'-shaped furrows (this time, non-closed) was observed in *P. rectangulus* (Strand, Herrera-Bachiller, Nygren & Kåneby, 2014), also continuous ventrally as a transverse line ("furrows pale, deep, and very obvious encircling head meeting in rather steep 'V'-shape dorsally"). The discovery of the mid-dorsal 'V'-shaped furrows in *P. hakuhoae* sp. nov. indicated that this kind of cephalic furrows were not unique to *Proamphiporus*. The mid-dorsal 'V'-shaped cephalic furrows in the new species was like that of *P. rectangulus* in that the furrows open posteriorly. However, the way these furrows were formed ventrally was unique to the new genus and was not observed in any member of the genus *Proamphiporus*. Additionally, the phylogenetic analysis (Fig. 60) showed that the new species in the new genus was not

closely related to *Proamphiporus*, the former being nested within Oerstedina while the latter in Amphiporiina within Eumonostilifera

Diversity of deep-sea nemerteans

The number of the collected nemertean specimens in the present study (i.e., 80) was equal to the ones collected in the Costa Rica margin (CRM) (Sagorny et al. 2022), bigger than the ones collected in the VFZ of the Atlantic Ocean (about 30–33 specimens) in 2014–2015 (Chernyshev & Polyakova, 2018a), but smaller compared to the specimens collected in the abyssal plain adjacent to the KKT (129–133 specimens) during the KuramBiol expedition in 2012 (Chernyshev et al. 2015), in the Kuril Basin of the Sea of Okhotsk and in the Pacific Ocean between the Bussol Trait and the KKT (90 specimens) in 2015 (Chernyshev & Polyakova 2018b), and along the KKT (123 specimens) in 2016 (Chernyshev & Polyakova 2019).

Of the 30 nemertean species collected along the JT and the KKT, nine species were bathyal (i.e., three in Palaeonemertea, two in Pilidiophora, and four in Hoplonemertea); seven species were abyssal (i.e., one in Paleonemertea, four in Pilidiophora, and two in Hoplonemertea); and 14 were hadal (i.e., five in Palaeonemertea, one in Pilidiophora, and eight in Hoplonemertea). The study showed that the palaeonemertean and hoplonemertean species were unique at every depth zone of the trenches. However, this was not observed in all pilidiophorans, as one species was found to be distributed both in the abyssal and hadal zones. Species in Hoplonemertea were the most common along the JT and KKT (14 species), followed by Palaeonemertea (nine species), and Pilidiophora (seven species). Additionally, the present study revealed that 23 out of the 30 collected nemertean species were undescribed, most of which could likely be placed in new genera. Two of the undescribed species were established as new species in this study, one of which was placed in a new genus. All these findings were in accordance with the results of the previous nemertean deep-sea surveys in the CRM, KKT, and VFZ, which had shown that: *i*) species composition at various vertical zones of the deep ocean were distinct; *ii*) palaeonemerteans and hoplonemerteans were abundantly and diversely represented in deep-sea; and *iii*) deep-sea species were mostly undescribed species (Chernyshev et al. 2015; Chernyshev & Polyakova 2018a, 2018b, 2019; Sagorny et al.

2022). The DNA sequence data obtained from the 30 nemertean species documented in the study were deposited in GenBank, increasing the available sequence information for deep-sea nemerteans from the world's oceans.

Palaeonemertean diversity

All deep-sea palaeonemerteans so far documented were members of the family Carininidae Chernyshev, 2011 and Tubulanidae Bürger, 1904, usually, new to science (Chernyshev & Polyakova 2018b, 2019; Chernyshev 2020). This was the same observation with the present palaeonemertean collection. The current study documented eight undescribed species of deep-sea tubulanids and one carininiid. The data on tubulanid species diversity was surprisingly high compared to the ones observed in the CRM (Sagorny et al. 2022) and the VFZ (Chernyshev & Polyakova 2018a) while almost comparable to those previously documented in the KKT (Chernyshev & Polyakova 2018b, 2019). Interestingly, the palaeonemerteans documented in the present study had never been recorded in previous deep-sea nemertean studies at least based on sequence data. Although each of the JT, KKT, and VFZ harbored unique species of tubulanids, species from these deep-sea habitats were evolutionary closely related. The tubulanids known to date from the CRM were closely related not to deep-sea species but to shallow-water ones. Including the barcode sequence data of the current study, at least 18 species of deep-sea tubulanids are currently known in the Atlantic and Pacific Oceans (Chernyshev & Polyakova 2019; Sagorny et al. 2022). The members of the family Cephalotrichidae McIntosh, 1874 (except for *Cephalothrix iwatai* Chernyshev, 2013 from Sea of Japan) and Carinomidae Bergendal, 1900 appeared to be very rare in deep-sea environments, and were not documented in all deep-sea surveys. Although the family Carininidae was predominantly composed of shallow-water species, this group was assumed to be quite diverse in greater depths (Chernyshev & Polyakova 2019). A total of eight undescribed deep-sea carininids have already been documented in the last decade. In contrast, at least three species of the genus *Carinina* Hubrecht, 1885 have been found in shallow waters of the North Pacific (Kajihara 2006; Kvist et al. 2015; Chernyshev et al. 2021), despite more than a hundred years of nemertean biodiversity studies in this region.

Comparing the number of species documented on the southern side of the Erimo Seamount (i.e., along the JT) and on its northern side (i.e., along the KKT, present data),

six species in Palaeonemertea were collected in the former while only three species were obtained on the latter, with no species common between the two trenches. The absence of species overlap was similarly observed when JT palaeonemertean barcode sequences were compared to those coming from the species previously collected in the KKT and from the other deep-sea habitats situated on the northern side of the seamount (Chernyshev & Polyakova 2018b, 2019). The number of JT palaeonemertean species data gained through the R/V *Hakuho-maru* expeditions were comparable to the previous data gathered from the northern side of the seamount obtained from several and more intensive deep-sea expeditions (Chernyshev & Polyakova 2018b, 2019). This may imply that future rigorous ribbon-worm surveys along the JT alone could reveal more undescribed palaeonemerteans, perhaps more diverse than in the KKT.

Pilidiophoran diversity

Previous nemertean surveys showed that most deep-sea pilidiophorans were heteronemerteans in the family Lineidae McIntosh, 1874 with a few representatives from the family Valenciniidae Hubrecht, 1879 (Chernyshev & Polyakova 2018b). This pattern was also observed using the current heteronemertean species data wherein six species in Lineidae and one species in Valenciniidae were collected. The number of deep-sea lineid species collected in the present study was comparable to the ones documented previously in the KKT (Chernyshev & Polyakova 2019), and more diverse compared to the VFZ, where this group was not documented (Chernyshev & Polyakova 2018a), and to the CRM, where only one species was collected (Sagorny et al. 2022).

The present species data for Pilidiophora indicated an equal number of species between the two sides of the Erimo Seamount (i.e., five species each along the JT and the KKT). However, four of the five species were shared between the two sides of the seamount: *i*) Lineidae sp. KH-2024-01, ICHUM 8875, JT and Lineidae sp. KH-2024-01, ICHUM 8874, KKT; *ii*) Lineidae sp. KH-2024-02, ICHUM 8877, JT and Lineidae sp. KH-2024-02, ICHUM 8876, KKT; *iii*) Lineidae sp. KH-2024-04, ICHUM 8879, 8880, 8883, JT and Lineidae sp. KH-2024-04, ICHUM 8881, KKT; and *iv*) Lineidae sp. KH-2024-03, ICHUM 8878, JT with Lineidae KuramBio2 85 (Chernyshev & Polyakova 2019). Only the pilidiophoran valenciniid *B. profundus* was unique to the northern side of the Erimo

Seamount while the undescribed Lineidae sp. KH-2024-05, ICHUM 8882, was unique to its southern side.

The collected JT and KKT lineids were nested within the Lineages B, D, and G of the family (Chernyshev & Polyakova 2023). The two undescribed JT and KKT lineids in Lineage G could probably belong to the same genus with a low interspecific COI *p*-distance of 8.7–9.3%. Similarly, the interspecies genetic distance between members of Lineage B was 8.8–20.2%. In comparison, interspecific COI *p*-distance between members of Lineage H, which could be treated as a single genus, was 10–19% while the COI interspecific variation in the genus *Maculaura* Hiebert & Maslakova, 2015 was 12.9–18%. Like most deep-sea nemerteans lacking distinctive external morphological characters (Chernyshev et al. 2015), COI barcode sequences for deep-sea lineids (as with the tubulanids) are significant to confirm their species identity. Despite being frequently collected from the deep, immediate species establishment for Pilidiophora (also for Palaeonemertea) is hampered by specimens usually being not in good condition for species description, the non-applicability of turbotaxonomy for these unicolored, non-patterned groups, and the necessity to generate tissue sections and provide internal morphology description to supplement the scarce external features of the worms for species identification. Prior to this study, there were only two heteronemerteans known from the deep-sea habitat of the Northwest Pacific, the lineid *Micrura bathyalis* Chernyshev, 2013 collected in the Sea of Japan and the valenciniid *Sonnenemertes cantelli* Chernyshev, Abukawa & Kajihara, 2015 in the Sea of Okhotsk and KKT, the latter was the only known valenciniid in the KKT not until the discovery of *B. profundus* in the current study. Previous sequence data also showed that there were only four distinct deep-sea lineids along the KKT (Chernyshev & Polyakova 2019). This number was increased to nine using the lineid collections in the present study.

The majority of the deep-sea heteronemerteans reported herein were species different from the ones previously recorded in the KKT and VFZ, indicating a high diversity of this group in the two trenches of the Northwest Pacific, although the two showed a number of species overlap.

Hoplonemertean diversity

In previous deep-sea nemertean surveys, monostiliferous hoplonemerteans were highly represented in terms of quantity and number of different species than polystiliferans (Chernyshev & Polyakova 2018a, 2019; Sagorny et al. 2022). The present study documented six different species of monostiliferans and eight species of polystiliferans. There were fewer undescribed species in Polystilifera than in Monostilifera (i.e., three compared to five) in the present species data.

Six species in Polystilifera were documented on the northern side of the Erimo Seamount while five species to the southern side. In the present study, two polystiliferous hoplonemerteans appeared to be unique on each side of the seamount: *i)* *D. shinkaii* and *ii)* *Pelagica* sp. KH-2024-02 along the JT and *i)* *Pelagica* sp. KH-2024-01 and *ii)* *Reptantia* sp. KH-2024-01 in the KKT.

The results of the phylogenetic analysis for Hoplonemertea were consistent and in accordance with what was previously established that *Reptantia* Brinkmann, 1917 was not monophyletic (Chernyshev & Polayakova 2019; Sagorny et al. 2022). Including the data from the current study, three clades of reptant polystiliferans were found to be closely related to *Pelagica* (Fig. 60). The taxonomy of *Reptantia* was known to be problematic for most species were established based on single specimens which were inappropriately fixed leading to potentially inaccurate information with regards to internal anatomy. Additionally, most of these species lack DNA sequence data. The lack of point of comparison, both morphology and molecular, makes the identification of recently discovered reptantians difficult.

Most of the monostiliferous hoplonemerteans discovered from the deep oceans in the last decade were new species placed in new genera. The phylogenetic studies on these deep-sea nemerteans showed relationships with shallow-water species (Chernyshev & Polyakova 2018a, 2018b, 2019; Hookabe et al. 2022a; Sagorny et al. 2022; Chernyshev & Kuznetsov 2024). The previous findings of deep-sea hoplonemerteans resulted to the establishment of two genera in Amphiporina: *i)* *Nemertovema*, *ii)* *Proamphiporus* (Chernyshev & Polyakova 2018a, 2019); five genera in Oerstedina: *i)* *Abyssonemertes*, *ii)* *Alvinonemertes* Sagorny, Döhren, Rouse & Tilic, 2022, *iii)* *Chernyshevia* Sagorny, Döhren, Rouse & Tilic, 2022, *iv)* *Galathenemertes* Chernyshev & Polyakova, 2019, and *v)* *Genrokunemertes* Hookabe, Koeda, Fujiwara, Tsuchida &

Ueshima, 2022 (Chernyshev & Polyakova 2018a, 2019; Hookabe et al. 2022a; Sagorny et al. 2022). From these, only the genus *Alvinonemertes* was not monotypic. With the addition of the genus herein established, *Profundonemertes* gen. nov., it appeared that most deep-sea hoplonemerteans were nested within Oerstidiina of Eumonostilifera.

Four species in Monostilifera were documented on each side of the Erimo Seamount with only one species shared between the JT and KKT, *P. hakuhoae* gen. et sp. nov. Two members of the genus *Nemertovema* were distributed along the JT: i) *Nemertovema kojimai* sp. nov. and ii) *Nemertovema* sp. KH-2024-01. The three species unique to the KKT were: i) Monostilifera sp. KH-2024-01, ii) Monostilifera sp. KH-2024-02, and iii) *N. norenburgi*.

The findings of *N. kojimai* sp. nov. and the other undescribed one in the JT and *N. norenburgi* in the KKT confirmed that *Nemertovema* is a true deep-sea genus with congeners so far consistently obtained from the hadal zone. This study also showed a highly supported subclade within Amphiporina formed by several undescribed monostiliferans from the American coast and Northwest Pacific (Fig. 60).

Conclusions

In this study I provided the first information on the taxonomic diversity of deep-sea nemerteans along the JT and updated the species diversity in the KKT. Based on the collected specimens and on the DNA sequence data generated from the collection, unique and mostly undescribed deep-sea nemerteans inhabit the two trenches. The current data also indicated that most of the deep-sea species along the trenches were found in the abyssal and hadal zones of the deep ocean. Interestingly, benthic species appeared to be restricted in the hadal depths and were never distributed in the abyssal or bathyal depths. Because of the methods used to collect deep-sea benthic ribbon worms, describing these species traditionally to reveal the true diversity of the phylum in the deep ocean remains a challenge. Therefore, generating DNA sequences from deep-sea species, whether from intact or fragmented ones, is so far the most effective way to investigate the species diversity of ribbon worms from the deep oceans of the world. The nucleotide sequences generated from the deep-sea samples could be used to identify their evolutionary relationships with the more commonly studied shallow-water species and reveal patterns of distribution.

Additionally, applying taxonomic names to the vast undescribed deep-sea ribbon worms is important for proper diversity estimations and communications among scientists and should not be hindered by the complex internal structure examination of the species. Equally, combining DNA sequence data with detailed external morphology information accompanied by high-quality photographs of the species, known as the integrative and turbotaxonomic approach, which has been practiced by many nemertean researchers, when possible, should be similarly applied to deep-sea species, which in most cases are represented only by single specimens in sampled material. This approach could accelerate the description of the highly diverse deep-sea nemerteans providing scientists with a more realistic estimates of their diversity.

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Chapter 5. Summary

This dissertation has updated the diversity of ribbon worms in the western Pacific region by investigating nemertean species from Japanese waters—both shallow- and deep-water species and from the Philippine intertidal zone.

The faunal surveys and taxonomic studies on Philippine nemerteans resulted in the discovery of three known species of monostiliferous hoplonemerteans in two genera, one of which was newly recorded in the country—*i*) *Diplomma serpentinum*; *ii*) *Prosadenoporus olympiae*, and *iii*) *Prosadenoporus spectaculus* (new record); six known species of pilidiophorans in four genera, four of which were newly reported—*i*) *Baseodiscus hemprichii* (new record) *ii*) *Baseodiscus jonasii* (new record), *iii*) *Baseodiscus quinquelineatus*, *iv*) *Dushia nigra* species complex (new record), *v*) *Eousia verticivaria* (new record), and *vi*) *Quasilineus pulcherrimus* (new record); documented four unidentified (potentially undescribed) monostiliferous hoplonemerteans in three genera—*i*) *Ototyphlonemertes* sp. PH24-01, *ii*) *Prosadenoporus* sp. PH23-01, *iii*) *Prosadenoporus* sp. PH23-02, and *iv*) *Tetrastemma* sp. PH24-01; re-discovered one known reptant polystiliferan—*Coella gloriae*; documented two unidentified (also potentially undescribed) reptant polystiliferans in one family—*i*) Drepanophoridae sp. PH24-01 and *ii*) Drepanophoridae sp. PH24-02; one unidentified and potentially undescribed valenciniid—*Baseodiscus* sp. PH24-01; and five unidentified (potentially undescribed) lineids in one family and one genus—*i*) Lineidae sp. PH23-01, *ii*) Lineidae sp. PH23-02, *iii*) Lineidae sp. PH24-01, *iv*) Lineidae sp. PH24-02, and *v*) *Notospermus* sp. PH24-03.

This is also the first comprehensive work on nemertean diversity in the Philippines. The present nemertean species data presented in this dissertation has significantly increased the named species of the phylum from eight to 14. The results of the faunal surveys shortly conducted in 2023 and 2024 showed that a highly diverse phylum can be uncovered from the Philippine waters if researchers will begin working on this taxon. The species data herein presented showed that species in Hoplonemertea and Pilidiophora are almost equally represented in terms of species diversity in the intertidal zones of the Visayas and Mindanao, Philippines—10 species in the former and 12 in the latter.

The study on the Philippine nemertean diversity resulted in the discovery of two members of the *Dushia nigra* species complex, one member dominating the other, two members of the Lineidae sp. PH23-02 and two members of *Quasilineus pulcherrimus*—all indicating the frequent occurrence of cryptic species in the phylum and at the same time can be used to evaluate the usefulness of genetic-distance analyses to delineate such species. The Philippine species data also indicated that *Notospermus* sp. PH24-03 is a highly polymorphic species along with Lineidae sp. PH23-01. The *Prosadenoporus* study using the Philippine species indicated that the known congeners distributed in the Philippines such as *P. spectaculus*, *P. olympiae*, *P.* sp. PH23-01 and *P.* sp. PH23-02 cannot be reliably identified based on morphology alone, except for the latter species. There are individuals of *P. spectaculus* that do not evidently display the characteristic spectacle on the head, making them appear like *P. olympiae* and *P.* sp. PH23-01. All of these taxonomic observations imply the necessity of DNA sequence data and the implementation of integrative approach for the correct and reliable taxonomic and systematics studies of ribbon worms.

The taxonomic studies of the phylum in Japanese waters resulted in the establishment of seven new shallow-water species of monostiliferous hoplonemerteans in three genera—i) *Oerstedia fuscosparsa* sp. nov., ii) *Oerstedia pseudoculata* sp. nov., iii) *Oerstedia rugosa* sp. nov., iv) *Oerstedia viridifusca* sp. nov., v) *Tetrastemma parallelus* sp. nov., vi) *Tetrastemma luteum* sp. nov., and vii) *Tetranemertes aurantia* sp. nov.; reported two new species records in two genera—i) *Oerstedia phoresiae* and ii) *Tetrastemma phaeobasisae*; proposed one new combination—*Tetranemertes kandai* comb. nov.; and reported two undescribed species of *Tetranemertes*—i) *Tetranemertes* sp. AHK-2024-1 and ii) *Tetranemertes* sp. AHK-2024-2.

The study of the phylum's diversity in Japanese deep-sea waters led to the establishment of two new species of monostiliferous hoplonemerteans in two genera, one of which was placed in a new genus—i) *Nemertovema kojimai* sp. nov. and ii) *Profundonemertes hakuhoae* gen. et sp. nov.; reported one new species record—*Nemertovema norenburgi*; discovered three undescribed monostiliferans—i) Monostilifera sp. KH-2024-01, ii) Monostilifera sp. KH-2024-02, and iii) *Nemertovema* sp. KH-2024-01; documented five known species of pelagic polystiliferous hoplonemerteans in four genera, two of which represented new species distribution record—i)

Dinonemertes arctica (new record), *ii*) *Dinonemertes shinkaii*, *iii*) *Nectonemertes* cf. *mirabilis*, *iv*) *Phallonemertes murrayi* (new record), *v*) *Protopelagonemertes beebei*; discovered two undescribed species in one suborder—*i*) *Pelagica* sp. KH-2024-01 and *ii*) *Pelagica* sp. KH-2024-02; discovered one undescribed species of reptant polystiliferan—*Reptantia* sp. KH-2024-01; reported one new distribution record of a valenciniid—*Baseodiscus profundus*; discovered six undescribed species of pilidiophorans in one family—*i*) *Lineidae* sp. KH-2024-01, *ii*) *Lineidae* sp. KH-2024-02, *iii*) *Lineidae* sp. KH-2024-03, *iv*) *Lineidae* sp. KH-2024-04, *v*) *Lineidae* sp. KH-2024-05, and *vi*) *Lineidae* sp. KH-2024-06; nine undescribed palaeonemerteans in two families—*i*) *Carininidae* sp. KH-2024-01, *ii*) *Tubulanidae* sp. KH-2024-01, *iii*) *Tubulanidae* sp. KH-2024-02, *iv*) *Tubulanidae* sp. KH-2024-03, *v*) *Tubulanidae* sp. KH-2024-04, *vi*) *Tubulanidae* sp. KH-2024-05, *vii*) *Tubulanidae* sp. KH-2024-06, *viii*) *Tubulanidae* sp. KH-2024-07, and *ix*) *Tubulanidae* sp. KH-2024-08.

The taxonomic and systematic studies of the genus *Oerstedia* updated both the diversity and taxonomy of the banded and non-banded congeners in the Japanese shallow waters. Using molecular taxonomy and phylogenetics, the identity of a polymorphic *Oerstedia* previously identified and recognized by many as *Oerstedia oculata* has been settled by establishing it as a new species based on morphological and molecular data. The taxonomic study on the polymorphic Japanese *Oerstedia* indicated that molecular taxonomy is indeed indispensable not just for species establishment but also for congener identification. Species identification solely based on morphology is not reliable when dealing with highly polymorphic species in the genus.

The study on *Tetranemertes* increased the genus' diversity by describing one new species, reporting two undescribed ones, and transferring to it one species previously in *Emplectonema*. The previously identified *Emplectonema kandai* has been transferred to the genus *Tetranemertes* based on morphological features shared by this species with *Tetranemertes* congeners, resulting in a new combination. The taxonomic study on *Tetranemertes* also led to the proposal that the genus could be a junior synonym of *Ommatoplea* and that the currently recognized *Emplectonema ophiocéphala* could be a *Tetranemertes*. It is also reiterated in this work that combined morphological features such as *i*) filiform body shape, *ii*) diamond-shaped head, *iii*) the ocelli arranged in four rows, *iv*) a single ventral 'Λ'-shaped cephalic furrow, *v*) very short rhynchocoel and

proboscis, and *vi*) bilobed to deeply forked central stylet basis could be used to identify nemerteans to the genus *Tetranemertes*.

This work also provided the first comprehensive documentation of the diversity of deep-sea nemerteans in the Japan Trench and a portion of the Kuril-Kamchatka Trench within the Japanese territory. The deep-sea species data reported in this study serves as the baseline information of the phylum's diversity in Japanese deep-sea habitats. The 21 undescribed deep-sea species documented in this study are potentially new to science and mostly unique from the already known deep-sea species worldwide. Sequences from the 30 nemertean species collected from the two trenches were deposited in GenBank and increased publicly available DNA sequences from deep-sea representatives. This work has increased the named deep-sea nemerteans by establishing two species, one placed in a new genus. Deep-sea species data from the study showed that benthic monostiliferous hoplonemertean and palaeonemertean species from the two adjacent trenches were generally unique with no species overlap. This observation seemed to be different from pilidiophorans from which several species overlap between the two trenches were observed.

In total, this taxonomic and systematics studies of nemerteans from the western Pacific region dealt with 22 species of nemerteans in nine known genera were dealt with using specimens from the Philippines, of which 12 are highly likely undescribed species, which will be given formal taxonomy in the future. The nemertean studies in Japanese waters dealt with 42 species of ribbon worms distributed in 10 known genera, of which 23 undescribed species are potentially new, which can be given formal taxonomic names when specimens of good condition are collected in the future.