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Author(s)	Abato, Jamael Cadampog
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Title of Doctoral Dissertation

Taxonomy and systematics of shallow-water and deep-sea nemerteans (phylum Nemertea) from the western Pacific region
(西太平洋の浅海及び深海ヒモムシ（紐形動物門）の分類学・体系学的研究)

Nemerteans, or ribbon worms (phylum Nemertea) are soft-bodied, non-truly-segmented worms, characterized by an eversible proboscis housed within a fluid-filled rhytocoel. To date, the phylum is composed of 1,355 named species distributed in the world's oceans, with most of the 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. In the western Pacific region, where ribbon-worm diversity studies have been active for several decades, new species are still being discovered and given formal taxonomy—with species mostly collected from Japanese and Russian waters, some from Australian, Chinese, Philippine, Thai, and Vietnamese waters. Species data from Japanese waters indicated that up to 2017, ~130 species have been documented. Since then, new species are being discovered and formally named almost yearly—indicating that even in intensively surveyed marine habitats, a plethora of undiscovered species is still waiting to be revealed and formally named. This observation is also true with other regions of the western Pacific and even in other oceans of the world. Meanwhile, only six species of nemerteans were reported from the Philippine marine waters from 1942–2020, despite being part of the Coral Triangle where marine biodiversity is expected to be high. This very small species data does not actually mean low nemertean diversity in the country but a reflection of the taxon being understudied due to the absence of taxonomist specializing on this group and being neglected for having no known economic value and human importance for the Filipinos. Most of the named nemerteans to date are shallow-water species, obtained from depths not less than 1000 m. Only a few species were given formal taxonomy from deep-sea habitats (≥ 1000 m deep) due to the expensiveness and difficulties in accessing deep-sea habitats. When accessed, the soft-bodied and fragile benthic nemerteans are usually broken or fragmented after being recovered from the trawled material rendering them not suitable for species description. This makes most deep-sea nemerteans unidentified with potentially new species left undescribed. Most of the named deep-sea nemerteans were obtained from the western Pacific, specifically in Russian deep-sea waters. It was very recently when deep-sea explorations in the Costa Rica Margin revealed seven deep-sea species in the Eastern Pacific, six of which were named as new and one named species of the Vema Fracture Zone in the Atlantic Ocean. In Japanese waters, only two species of deep-sea nemerteans were documented, the pelagic *Dinonemertes shinkaii* Kajihara & Lindsay, 2010 from the Japan Trench and the benthic *Genurikonemertes obesa* Hookabe, Koeda, Fujiwara, Tsuchida & Ueshima, 2022 from the Nishi-Shinchito Ridge. Since 2013 to present, only 13 species of benthic nemerteans were named from deep-sea habitats world-wide.

The taxonomy and systematics of phylum Nemertea are often challenging and problematic as species identification is mostly based on limited external characters of the worms such as size, shape, position of the mouth and proboscis pore, number and pattern of the 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. This method made subsequent species identification difficult particularly when tissue sections are not made from an unknown species. To address this challenge, modern nemertean taxonomists adopted the histology-free or turbotaxonomy approach in naming nemerteans. This technique of ribbon-worm description and identification requires detailed examination of the worm's external features and generating cytochrome *c*-oxidase subunit I (COI), histone H3, 16S rRNA (ribosomal RNA), 18S rRNA, and 28S rRNA sequences, with tissue sectioning being no longer mandatory (except when it is highly needed). The COI sequence is often used for molecular species identification using barcode sequences deposited publicly such as in GenBank while in combination with the rest of the gene markers can be used to study phylogenetic relationships within the phylum. Additionally, molecular taxonomy and systematics of the phylum are used to address taxonomic problems

and often used to reflect common ancestry in those problematic groups leading to revisions of some problematic genera. Lastly, molecular taxonomy has been used to reveal the occurrence of cryptic species commonly observed in the phylum. This dissertation aims to investigate further the diversity of nemerteans in the western Pacific region, focusing on shallow-water species in the Philippines and both shallow-water and deep-sea species in the Japanese waters. The taxonomy and systematics of the nemertean species considered in this study were investigated using the integrative approach—combining both the morphological and molecular taxonomy.

The results obtained from this study indicated the documentation of 42 Japanese nemertean species distributed in 10 known genera and 20 Philippine species in nine known genera. Of the collected species, about 23 Japanese species and 10 Philippine species were unidentified, most if not all, are potentially new species. New shallow-water species in known Hoplonemertea genera obtained from the western Pacific region were established using this approach (i.e., species in *Nemertovema* Chernyshev & Polyakova, 2018, *Oerstedia* Quatrefages, 1864, *Tetranemertes* Chernyshev, 1993, and *Tetrastemma* Ehrenberg, 1831). A new deep-sea species in the region is also established placed in a new genus *Profundonemertes* gen. nov. Known hoplonemerteans and pilidiophorans documented for the first time in Japanese and Philippine waters were reported in this study, updating their global distribution.

Seven new shallow-water species of Japanese 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. were established in this dissertation while two new species records in two genera—i) *Oerstedia phoresiae* (Kulikova, 1987) and ii) *Tetrastemma phaeobasisae* Kulikova, 1987 were reported. Also, a new combination is proposed in this study when the then *Emplectonema kandai* Kato 1939 is transferred to the genus *Tetranemertes* resulting in *Tetranemertes kandai* (Kato, 1939) comb. nov. Additionally, two new species in *Tetranemertes*—i) *Tetranemertes* sp. AHK-2024-1 and ii) *Tetranemertes* sp. AHK-2024-2 were reported and left undescribed in this dissertation due to the lack of the data on the morphology of the stylet-basis, which is important for the identification of this genus. Two new deep-sea species in Hoplonemertea were established from the Japan Trench (JT) and the Kuril-Kamchatka Trench (KKT) (i.e., under the Japanese territory)—i) *Nemertovema kojimai* sp. nov. and ii) *Profundonemertes hakuhoae* gen. et sp. nov. In addition, a new species record for the deep-sea *Nemertovema norenburgi* Chernyshev & Polyakova, 2019 is reported while leaving three potentially new hoplonemertean species undescribed due to their fragmented condition—i) Monostilifera sp. KH-2024-01, ii) Monostilifera sp. KH-2024-02, and iii) *Nemertovema* sp. KH-2024-01. Some known, newly reported, and unidentified deep-sea polystiliferous hoplonemerteans from the JT and KKT were reported in this study—i) *Dinonemertes arctica* (new record), ii) *Dinonemertes shinkaii*, iii) *Nectonemertes* cf. *mirabilis*, iv) *Phallanemertes murrayi* (new record), v) *Protopelagonemertes beebei*; vi) *Pelagica* sp. KH-2024-01; vii) *Pelagica* sp. KH-2024-02, and viii) *Reptantia* sp. KH-2024-01. Species data on deep-sea pilidiophorans and palaeonemerteans resulted to the discovery of seven pilidiophorans (one known valenciniid, new record while the rest were unidentified lineids)—*Baseodiscus profundus* Kajihara, Abukawa & Chernyshev, 2022 (new record) and Lineidae sp. KH-2024-01—Lineidae sp. KH-2024-06 and nine undescribed palaeonemerteans—Carininidae sp. KH-2024-01 and Tubulanidae sp. KH-2024-01—Tubulanidae sp. KH-2024-08. The intertidal nemertean faunal survey in the Philippines led to the discovery of three known monostiliferous hoplonemerteans, one of which newly recorded in the country—i) *Diplomma serpentinum* (Stimpson, 1855); ii) *Prosadenoporus olympiae* Kajihara, 2020, and iii) *Prosadenoporus spectaculus* (Yamaoka, 1940) (new record); six known species of pilidiophorans, five of which were newly reported—i) *Baseodiscus hemprichii* (Ehrenberg, 1831) (new record) ii) *Baseodiscus jonasii* Strand, Hjeltgren & Sundberg, 2005 (new record), iii) *Baseodiscus quinquelineatus* (Quoy & Gaimard, 1833), iv) *Dushia nigra* (Stimpson, 1855) species complex (new record), v) *Eousia verticivaria* (Gibson, 1990) (new record), and vi) *Quasilineus pulcherrimus* Gibson, 1981 (new record); documented four undescribed monostiliferous hoplonemerteans—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 undescribed reptant polystiliferans—i) *Drepanophoridae* sp. PH24-01 and ii) *Drepanophoridae* sp. PH24-02; one undescribed valenciniid—*Baseodiscus* sp. PH24-01; and three undescribed lineids—i) Lineidae sp. PH23-01, ii) Lineidae sp. PH23-02, and iii) *Notospermus* sp. PH24-03.

This study indicated that the diversity of the phylum Nemertea in the western Pacific region remains to be characterized despite being extensively studied. In the Japanese waters, both shallow-water and deep-sea unnamed species remain completely unexplored as evidenced by new species being constantly established from this region while the actual and expected high diversity of the phylum in the Philippines remain poorly documented and studied, making this research area in the Philippines worth investigating.