PHYLOGENETIC POSITION OF *ARCTOTANAIS* IN THE SUBORDER TANAIDOMORPHA (PERACARIDA: TANAIDACEA)

Keiichi Kakui, Norio Kobayashi, and Hiroshi Kajihara

(KK, correspondence, k_kakui@mail.goo.ne.jp; HK) Department of Natural History Sciences, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan;

(NK, kobayashi-norio@spu.ac.jp) Center for University-wide Education, School of Health and Social Services, Saitama Prefectural University, Koshigaya 343-8540, Japan

ABSTRACT

Lack of an ischium on pereopods 1–6 was previously considered a synapomorphy for the tanaidacean superfamily Tanaoidea, although descriptions of *Arctotanais alascensis*

(Richardson, 1899), the sole species in *Arctotanais*, indicated presence or absence of the ischium. To resolve this ambiguity, we examined newly collected specimens of *A. alascensis* (including males, which had not previously been described) from Hokkaido, Japan, using light and scanning electron microscopy. We also conducted molecular phylogenetic analyses based on partial sequences of the 18S rRNA gene to examine the phylogenetic position of *A. alascensis*. Here we describe in detail the morphology of the male of *A. alascensis*, which proved to be similar to that of the females. This species bears an ischium on pereopods 1–6, which contradicts the current diagnoses of Tanaoidea and Tanaidae, although other synapomorphies remain valid. Molecular phylogenetic analyses strongly supported the placement of *A. alascensis* in Tanaoidea, and consequently we amended the diagnoses for Tanaoidea and Tanaidae to include either presence or absence of the ischium on the pereopods.

KEY WORDS: *Arctotanais*, ischium, molecular phylogeny, Tanaidacea, 18S rRNA

DOI:

INTRODUCTION

The monofamilial superfamily Tanaoidea Dana, 1849 contains 19 genera (Shiino, 1978; Sieg, 1980; Bamber, 2005; Bamber and Boxshall, 2006; Edgar, 2008). It is distinguished from the other extant tanaidomorph superfamilies, Paratanaoidea and Neotanaoidea (see Kakui et al., in press), by several synapomorphies suggested by Sieg (1980) (character states in non-tanaoid tanaidomorphs are indicated in parentheses): 1) pleon with three pairs of pleopods (with zero or five pairs); 2) all pereopods without ischium (with ring-shaped ischium); 3) marsupium consisting of sack-like oöstegites (consisting of flat oöstegites); 4) uropod uniramous (uni- or biramous); and 5) claw of pereopods 4–6 having spiniform setae (lacking spiniform setae). In addition, pleonites 4 and 5 in Tanaoidea, when present, are always narrower than pleonites 1–3; this feature has not been reported from other tanaidomorphs.

Arctotanais alascensis (Richardson, 1899) is the sole member of the tanaoid genus *Arctotanais*, described as *Tanais alascensis* based on specimens collected in "Kyska Harbor," Alaska, without providing detailed descriptions of most appendages, including the mouthparts and pereopods. Kussakin and Tzareva (1974) provided a supplemental description based on specimens from the Kurile Islands. Sieg (1980) established the new genus *Arctotanais* for this species and redescribed *A. alascensis* based on Richardson's (1899) type specimens. Sieg (1983) regarded *Tanais alascensis* sensu Kussakin and Tzareva (1974) as conspecific with *A. alascensis* sensu Sieg (1980).

Kussakin and Tzareva (1974) stated, "the ischium in all seven pairs of pereopods (= cheliped and pereopods 1–6) is very short, not well developed" [KK's translation]. Ignoring this statement in his redescription, Sieg (1980) noted from Richardson's (1899) type material that the cheliped and pereopods 1–6 lack an ischium, and included this absence among the synapomophies for Tanaoidea (and thus automatically also for Tanaidae).

If the pereopods in *A. alascensis* actually have an ischium, this would partly contradict the current diagnoses of both the superfamily and the family, and raise doubts concerning this species' affiliation, since the ischium is present in members of both the superfamilies Paratanaoidea and Neotanaoidea. Elucidating the phylogenetic position of *A. alascensis* among Tanaidomorpha is thus relevant to the superfamily/family diagnoses.

In 2009 and 2010, we collected several fresh specimens of *A. alascensis* from Hokkaido Island, northern Japan; these included males, which had not been previously described. Here we report the results of scanning electron microscope (SEM) examinations to determine whether the pereopodal ischium is present in *A. alascensis* and four other tanaid species, describe males of *A. alascensis*, and present the results of a molecular phylogenetic analysis to determine the position of *A. alascensis* within Tanaidomorpha.

MATERIAL AND METHODS

Material

Table 1 lists the specimens used in this study. SEM observations were made on one species each for all five tanaoid genera available in Japan; three unnamed species (*Hexapleomera* sp., *Sinelobus* sp. 1, and *Zeuxo* sp. 1) are identical to those in Kakui et al. (in press), and were collected from the same sites as in that study. We included *Kalliapseudes* sp., the apseudomorph closest to tanaidomorphs in phylogenetic analyses by Kakui et al. (in press), as an out-group taxon in molecular phylogenetic analyses.

Morphological Observations

Dissections were carried out with chemically sharpened tungsten wire needles under a Nikon SMZ 1500 microscope. Appendages were mounted on glass slides in glycerin and observed with an Olympus BX51 microscope. Drawings were made with Adobe Illustrator ver. 10 from draft line drawings made with a camera lucida and/or from digital images taken with a digital camera system (Hiruta and Kakui, 2010). Selected specimens were treated with hexamethyldisilazane (Nation, 1983) and observed at 10, 15, or 25 kV accelerating voltage with a Hitachi S-3000N scanning electron microscope. Some of the SEM images were processed by using GIMP 2.6.7. Morphological terminology is as defined in Larsen (2003). Measurements were made axially: dorsally on the body, antennae, and uropods; laterally on pereopods and pleopods. Material has been deposited in the Zoological Institute, Faculty of

Science, Hokkaido University, Japan (ZIHU).

Molecular Phylogenetic Analyses

We sequenced part of the 18S rRNA gene for *A. alascensis*. Methods for DNA extraction, DNA amplification, and sequencing were as described in Kakui et al. (in press). Table 2 lists the primers used for PCR and cycle sequences.

Nucleotide sequence pre-alignments were performed with Clustal W (Thompson et al., 1994) in MEGA 5 (Tamura et al., in press), with the default settings: gap opening cost = 15, gap extension cost = 6.66, and transition weight = 0.5. The pre-aligned data were then realigned by eye according to the secondary structure of the gene predicted with CentroidFold (Sato et al., 2009), and all indels were removed from the data set. To determine whether nucleotide composition bias occurred among taxa, χ^2 goodness-of-fit tests were performed on the sequence data. Aligned data were then imported into MEGA 5 for minimum evolution (ME) analysis, and into PAUP*4.0b (Swofford, 2002) for the maximum parsimony (MP) and maximum likelihood (ML) analyses.

ME trees were constructed by using maximum composite likelihood (Tamura et al., 2004), assuming homogeneity of base composition among lineages. ME trees were searched by closest neighbor interchange (CNI; Nei and Kumar, 2000) at a search level of 1. Bootstrap values (Felsenstein, 1985) for ME tree were determined from 10,000 pseudo-replicates.

Unweighted MP trees were obtained through 1000 heuristic search replicates, with starting trees generated by random sequence addition, followed by the tree bisection reconnection (TBR) branch swapping. Bootstrap values for MP tree were determined from 1000 pseudo-replicates, for each of which an MP tree was obtained through 100 heuristic search replicates with random sequence addition and TBR branch swapping.

ML trees were obtained by TBR branch swapping, starting with a topology given by neighbor joining (NJ; Saitou and Nei, 1987). Parameters for the ML analysis were selected on the basis of the Akaike information criterion (AIC; Akaike, 1974) in jModelTest (Posada, 2008). Bootstrap values for the ML trees were calculated from 1000 pseudo-replicates analyzed by nearest neighbor interchange (NNI) searches, with the starting topology given by an NJ tree.

A Bayesian analysis (BA) was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Parameters for this analysis were selected by the Bayesian information criterion (BIC; Schwarz, 1978) test implemented in jModelTest. A Markov-Chain Monte-Carlo (MCMC) search was performed with four chains, each of which was run for 500,000 generations. Trees were sampled every 100th generation. Topological convergence diagnostics for BA were performed with Tracer 1.5 (Rambaut and Drummond, 2009). The first 125,000 generations were discarded as burn-in. A consensus of sampled trees was computed, and the posterior probability for each interior branch was obtained to assess the robustness of the inferred relationships.

RESULTS

SEM Observation of the Ischium on the Pereopods

Arctotanais alascensis possesses an ischium on all of the pereopods (Fig. 1A, B); the ischium is short, crescent shaped, and lacks a dorsal portion. In *Hexapleomera* sp., *Sinelobus* sp. 1, *Tanais tinhauae* Bamber and Bird, 1997, and *Zeuxo* sp. 1 (Fig. 1C–F), all pereopods lack an ischium.

Description of the Male of *Arctotanais alascensis* (Richardson, 1899)

Figs. 2–5

[Synonymy prior to this study is listed in Sieg (1983).]

Material examined.—Non-types: ZIHU-3949, 1 male (6.96 mm), dissected, most appendages mounted on glass slides, and remains preserved in glass vial; Pekin-no-hana, Shiretoko, Hokkaido, Japan (44°16′17.64″N, 145°21′56.22″E), rocky shore, intertidal zone, 12 May 2009. ZIHU-3950, 1 male (8.82 mm), dissected, most appendages mounted on glass slides, and remains preserved in glass vial; Aidomari, Shiretoko, Hokkaido, Japan (44°11′29.34″N, 145°19′43.92″E), rocky shore, intertidal zone, 13 May 2009. ZIHU-3951, 1 female (5.65 mm), dissected, several appendages mounted on glass slides or SEM stubs, and remains preserved in glass vial; Muroran, Hokkaido, Japan (42°18′18.80″N, 140°59′18.40″E), intertidal zone, 12 April 2009. ZIHU-3970, 1 female with rudimentary oöstegite (5.49 mm), intact, preserved in glass vial; Oshoro, Hokkaido, Japan (43°12′46.23″N, 140°51′29.03″E), intertidal zone, 3 April 2010. ZIHU-3971, 1 female with rudimentary oöstegite (6.03 mm), intact, preserved in glass vial, same data as for ZIHU-3970. ZIHU-3972, 1 individual (2.89 mm), exoskeleton only, used for DNA extraction, almost same locality as for ZIHU-3970, 21 September 2008. ZIHU-3949 and -3950 were collected by Seiji Goshima, -3951 and -3972 by Keiichi Kakui, and -3970 and -3971 by Taiki Yoshihara.

Description of male.—Body (Fig. 2A, a1, B) cylindrical, slightly flattened dorsoventrally, 3.76 times as long as wide. Red-brown pigmentation covers the body, as illustrated, forming two transverse stripes on each pereonite; pigmentation retained in ethanol.

Cephalothorax about one-fifth total body length, 1.2 times as wide as long, almost triangular in dorsal view, with 3 pairs of lateral simple setae near eyes, 1 pair of rows of simple setae in dorsolateral region. Eyes well defined, black. Anterior edge obtuse triangular in outline in dorsal view, not extending to distal margin of eye lobe. Posterior end swollen laterally. Sclerite with 11 lateral simple setae.

Pereonites all transversely oblong-rectangular, with several pairs of dorsolateral simple setae; pereonite 1 shortest among pereonites, with 5 pairs of dorsal simple setae; pereonites 2 and 3 shorter than succeeding pereonites, with 1 pair of clumps of simple setae in dorsal region; pereonites 4 and 5 subequal in length, setation like that of pereonite 2; pereonite 6 shorter than pereonites 4 and 5, with 1 pair of genital cones and setation like that of pereonite 2.

Pleon length 0.32 times total body length, with 4 pleonites and 1 pleotelson. Pleonites all wider than long, oblong rectangular in dorsal view, with 1 pair of rows of simple setae on dorsolateral margin; pleonites 1–3 as wide as pereon, with 1 pair of clumps of simple setae in dorsal region, rows of plumose setae (in only anterior half of pleonite 3) on lateral margin, and 1 pair of pleopods; pleonite 4 narrower and shorter than preceding ones. Pleotelson wider than long, gradually tapering posteriorly, narrower than pleonites 1–3 but wider than pleonite 4, with simple setae as illustrated.

Antennule (Fig. 3A, a1) with 5 articles, length 0.96 times cephalothorax length. Article 1 length 0.55 times cephalothorax length, with 20 outer and 10 inner simple setae, and several broom setae. Article 2 length 0.36 times article 1 length, with 12 outer distal and 10 inner distal simple setae and 5 broom setae. Article 3 as long as article 2, with 6 outer distal and 4 inner distal simple setae and 2 broom setae. Article 4 length 0.1 times article 3 length, with 5 medial and 4 distal simple setae and 2 aesthetascs. Article 5 very short, with 2 simple setae, 1 broom seta, and 26 aesthetascs at tip.

Antenna (Fig. 3B, b1) with 7 articles, thin, as long as antennule. Article 1 naked. Article 2 longest, with 16 simple setae. Article 3 with 1 simple seta. Article 4 with 4 distal simple setae. Article 5 with 4 distal simple and 3 broom setae. Articles 6 and 7 with 30 setae in total (the origin of setae from article 6 or 7 was obscure and remains uncertain). Article 7 minute.

Labrum (Fig. 3C) rectangular in shape, slightly expanding along lateral and distal margins, carpeted with fine setae.

Mandibles (Fig. 3D, E, d1, e1) with well-developed molar process, naked, lacking corrugation in masticatory region. Left mandible (Fig. 3D, d1) with smooth incisor; lacinia mobilis small, tuberculate on distal margin. Right mandible (Fig. 3E, e1) with incisor slightly concave on distal margin; lacinia mobilis with 5 teeth; 2 setae present at insertion on lacinia mobilis.

Labium (Fig. 3F, f1) with inner and outer lobes setulate on distal margin; labial process carpeted with fine setae, and with an elaborate projection on distal margin.

Maxillule (Fig. 3G, g1) with palp having 2 articles, the distal one bearing 10 setulate hook-tipped setae; endite with 9 distal spiniform setae, and cluster of fine setae in distal region.

Maxilla (Fig. 3H) oval, with 1 seta.

Maxilliped (Fig. 3I, J) with coxa bearing 2 simple setae. Basis widest anteriorly, with 4 distal simple setae. Endite with 1 distal projection covered by fine setae; distal margin with 2 short and 2 long distal simple setae; outer margin serrate. Palp article 1 with 2 inner ventral and 1 outer ventral simple setae; article 2 with 1 outer distal and 13 inner simple setae and 6 inner biserrate setae; article 3 inner margin with 7 dorsal, 13 marginal, and 4 ventral simple setae; article 4 with 1 outer subdistal and 24 inner simple setae, 4 dorso-distal pinnate setae, and 1 dorso-distal setulate seta.

Epignath (Fig. 3K) with kidney-shaped lobe; terminal seta setulate.

Cheliped (Fig. 4A, a1, B) basis as long as wide, bearing 4 ventro-subdistal simple setae. Ischium semicircular in shape, naked (cf. Fig. 4a1). Merus triangular, with 4 outer and 5 ventro-subdistal simple setae. Carpus 1.50 times as long as wide, outer proximal portion partly covering basis, with 1 dorsal, 12 dorso-distal, and 13 ventral simple setae, and 1 ventro-medial process. Propodus longer than carpus. Propodal palm with 6 outer and 1 inner simple setae at insertion of dactylus. Fixed finger with 8 ventral and 2 inner distal simple setae; cutting surface with 14 simple setae, and 1 dorsal expansion bearing several teeth. Dactylus longer than fixed finger, gradually curving ventrally; cutting surface with 1 ventro-proximal projection and row of lamellae.

Pereopod 1 (Fig. 4C, c1) length 0.32 times body length. Coxa with weak dorsal projection, bearing 4 simple setae and 1 circumplumose seta (Fig. 4c1). Basis length 0.42 times pereopod 1 length, cylindrical, slightly arched; dorso-proximal region with 4 simple and 1 broom setae. Ischium semicircular in shape, with 3 ventro-distal simple setae. Merus with 3 dorso-distal and 4 ventro-distal simple setae. Carpus shorter than merus, with 6 dorso-distal, 3 inner, and 6 ventro-distal simple setae. Propodus longer than carpus, with 2 dorso-distal and 10 ventral simple setae and 1 dorsal broom seta. Dactylus and unguis shorter than propodus, falciform. Dactylus with 1 dorsal seta. Unguis length 0.46 times dactylus length.

Pereopod 2 (Fig. 4D) length 0.93 times pereopod 1 length; otherwise similar to pereopod 1, except that coxa lacks dorsal projection and circumplumose setae, carpus has 1 dorso-distal and 1 ventro-distal spiniform setae but lacks inner simple setae, and the numbers of simple and broom setae are different.

Pereopod 3 (Fig. 4E) shorter than pereopod 2, but otherwise similar to pereopod 2, except that merus has 1 ventro-distal spiniform seta, carpus has 2 ventro-distal spiniform setae, and the numbers of simple and broom setae are different.

Pereopod 4 (Fig. 4F) longer than pereopod 3. Coxa short, with 2 simple setae. Basis with several minute setae and broom setae. Ischium like that of pereopod 3. Merus with 3 dorso-distal, 3 inner, and 4 ventro-distal simple setae, and 2 ventro-distal spiniform setae.

Carpus almost half as long as merus; outer and inner regions each with 3 simple and 6 spiniform setae. Propodus longer than carpus, with 4 ventral simple setae and 1 dorsal broom seta; outer region with 1 dorso-distal pinnate and 1 ventro-distal setulate setae; inner region with 1 dorso-distal setulate seta. Dactylus and unguis fused to form claw. Claw shorter than propodus, with 5 outer and 5 inner spiniform setae, and ventro-proximal serration.

Pereopod 5 (Fig. 4G) slightly longer than pereopod 4. Coxa short, with 1 simple seta. Basis and ischium like those of pereopod 3. Merus with 5 dorso-distal and 5 ventral simple setae, and 2 ventro-distal spiniform setae. Carpus, propodus, and dactylus and unguis like those of pereopod 4.

Pereopod 6 (Fig. 4H, h1–3) slightly longer than pereopod 5. Coxa short, naked. Basis, ischium, and merus like those of pereopod 5. Carpus almost half as long as merus; outer and inner regions each with 3 (inner) or 2 (outer) dorso-distal simple setae and 7 ventral spiniform setae. Propodus longer than carpus, with 4 ventral simple setae, 1 dorso-distal broom seta, and 6 dorso-distal serrate setae (Fig. 4h2); outer region with 1 dorso-distal pinnate seta (Fig. 4h3) and 1 ventro-distal setulate seta; inner region with 2 dorso-distal pinnate setae. Dactylus and unguis like that of pereopod 5.

Pleopods (Fig. 4I, i1) biramous, 3 pairs, all similar in shape; pleopod 3 smaller than others. Basal article with 11–15 outer plumose setae and 1 inner expansion bearing 2–4 plumose setae. Exopod comprises 1 article, with 46–48 outer plumose setae. Endopod comprises 1 article, with 27–29 outer and 1 inner plumose setae, and 1 distal distally hooked plumose seta (Fig. 4i1).

Uropod (Fig. 4J, j1) length 0.14 times total body length. Basal article with 5 distal simple setae. Endopod with 6 articles; article 1 naked; articles 2–6 with 9, 9, 11, 9, and 9 distal simple setae, respectively; articles 2–6 each with 2 broom setae.

Female. Generally similar to male; as in males, each of the chelipeds and all pereopods bears a semicircular ischium (Figs. 1A, B, 5A–D).

Remarks.—Our specimens of *A. alascensis* all have an ischium on the cheliped and all pereopods, as reported by Kussakin and Tzareva (1974). Among Tanaidacea, a chelipedal ischium has previously been reported or observed in members of Neotanaidae (e.g., Gardiner, 1975: fig. 4A) and in two species of *Tanais*: *T. dulongii* (Audouin, 1826) sensu Lauterbach (1970) and *T. tinhauae* (Lauterbach, 1970: p. 127, figs. 25 and 27; K. Kakui, unpublished data). The shape of the chelipedal ischium in our specimens of *A. alascensis* (Fig. 5A–D) is similar to that in *Tanais*, i.e., semicircular and lacking a ventral portion (see Fig. 5B). The pereopodal ischium in our material is crescent-shaped and lacks a dorsal portion (Fig. 1A, B), which differs from the ring-shaped ischium in other tanaidaceans.

Our specimens comprise the first record of males for *A. alascensis*. Except for having one pair of genital cones, males differ little from females. Kussakin and Tzareva (1974) and

Sieg (1980) reported differences among individuals (variation they reported is indicated in parentheses) in morphological characters, including the carapace width/length ratio (1.2–1.35 or 1.4–1.5); the number of spiniform setae on the maxillular endite (7–13); the number of spiniform setae on the carpus of pereopod 4 (7–13); and the number of articles of the uropodal endopod (5 or 6). Our specimens showed individual differences, and in some cases differences from the descriptions by Kussakin and Tzareva (1974) and Sieg (1980), in characters including the number of setae on appendages, e.g., maxillipedal endite, maxillipedal palp, right mandible, maxilla, and pleopodal basal article. Among specimens previously reported and in this study, there is a broad range in body length (3.5–8.82 mm), and the differences mentioned above might include ontogenetic changes, intraspecific variation, and possibly sexual dimorphism.

Arctotanais alascensis was originally collected in "Kyska Harbor," Alaska (Richardson, 1899). This place name probably refers to what is commonly spelled as "Kiska" Harbor, Kiska Island, western Aleutian Islands, and Sieg (1980) appears to have erroneously located this site on his distribution map (Sieg, 1980: p.120, fig. 32). The currently known distribution of this species is the western Aleutian Islands, Kurile Islands, and Hokkaido Island (Richardson, 1899; Kussakin and Tzareva, 1974; this study) (Fig. 6), at depths of 0–48 m.

On the basis of this study, we amend the diagnosis of *Arctotanais* as follows.

Pleon consists of 4 pleonites and 1 pleotelson. Pleonites lack transverse dorsal row of plumose setae. Antennules with 5 articles; bases abutting; article 1 at least 2.5 times length of article 2; article 5 tiny, with numerous aesthetascs. Antenna with 7 articles; article 2 longer than wide. Maxillule endite with 7–13 spiniform setae; palp with several long setae. Labium outer lobe with terminal process. Maxilliped endite with distal projection covered by fine setae. Cheliped with rudimentary ischium. All pereopods with semicircular ischium. Pereopod 1 coxa with weak dorsal projection bearing 1 circumplumose seta. Pereopod 6 propodus without ventro-distal row of “flattened denticulate setae” (Edgar, 2008) in inner region. Pleopod basis with inner expansion, bearing 2–4 plumose setae; endopod with only 1 plumose seta on inner margin. Uropodal endopod with 5 or 6 articles; terminal article not reduced. Sexes similar.

Molecular Phylogeny

The 18S sequence we determined for *A. alascensis* was 1791 bp long (DDBJ/EMBL/GenBank accession number AB622810). The aligned data set was 1520 bp long after elimination of indels, with characteristics given in Table 3; no compositional heterogeneity was detected. The optimal models found for the ML and BA analyses are shown in Table 4.

Optimality values for the MP, ME, and ML analyses were: a single MP tree was obtained (tree length = 1799, CI = 0.663, RI = 0.789); ME-score = 1.12862; –ln L = 9519.18385. The MP and ML trees are shown in Fig. 7; the ME and BA trees were identical in topology to the MP tree and are not shown. All the analyses (Fig. 7A, B) strongly supported Clades 1, 8, and 14 (corresponding to Paratanaoidea, Tanaoidea, and Neotanaoidea, respectively). Clade 8 including *A. alascensis* occurred in all four trees. In the MP, ME, and BA trees (Fig. 7A), *A. alascensis* was the basal taxon in Clade 8, comprising the sister group to the other tanaoids (Clade 9). In contrast, in the ML tree (Fig. 7B), *Sinelobus* (Clade 10) was the basal taxon in Clade 8, being the sister group to Clade 16 containing *A. alascensis* (basal) and the other tanaoids (Clade 11).

DISCUSSION

Morphological observations showed that *A. alascensis* bears an ischium on pereopods 1–6, which contradicts the current diagnosis of the superfamily, while sharing the other synapomorphies for tanaoids: 1) the pleon has three pairs of pleopods, 2) the uropod is uniramous, 3) the claw of pereopods 4–6 has spiniform setae, and 4) pleonite 4 is narrower than pleonites 1–3. All four molecular phylogenetic analyses strongly indicated that *A.*

alascensis belongs in Tanaoidea (Clade 8) (Fig. 7A, B), and thus it is necessary to amend the superfamilial diagnosis.

The amended diagnosis for Tanaoidea (also for Tanaidae) is as follows.

> Eyes well defined, black (absent in *Protanais*). Pleonites 4 and 5, when present, narrower than pleonites 1–3. Thoracic glands present. Antennule with 3–5 articles. Antenna with 6–8 articles. Lacinia mobilis present on left and right mandibles. Maxillule with 1 endite, bearing palp. Maxilla rudimentary, oval in shape. Maxilliped with coxa; maxillipedal coxae and bases not fused medially. Cheliped ischium present (in *Arctotanais* and several species of *Tanais*) or absent. Ischium on pereopods present (in *Arctotanais*) or absent. Dactylus and unguis of pereopods 4–6 forms claw; claw bears rows of spiniform setae. Pleopods 3 pairs. Uropod uniramous. Females with only 1 pair of sac-like oöstegites, arising from coxae of pereopod 4.

The absence of an ischium on all pereopods of tanaoids has been regarded as an apomorphy (Sieg, 1980) because peracarid pereopods generally bear an ischium, and most tanaidaceans also possess a ring-shaped ischium. Vonk and Schram (2007) reported that all members of the extinct tanaidomorph family Alavatanaidae have an ischium only on pereopod 1, i.e., pereopods 2–6 lack it; these authors suggested that this character relates to an intermediate stage between the superfamilies Tanaoidea and Paratanaoidea. Our study has shown that *A. alascensis* possesses an ischium on pereopods 1–6, but that the ischium lacks the dorsal portion. The shape of the ischium in *A. alascensis* is unique in Tanaidacea, and may represent a state of partial loss. These observations indicate that there are two different routes in evolution of the ischium within Tanaidacea: 1) a decrease in the number of pereopods bearing a typically shaped ischium, as in Alavatanaidae, and 2) modification of the shape of the ischium without a change in the number of ischia, as in *A. alascensis*.

Acknowledgements

We thank Dr. Seiji Goshima and Mr. Taiki Yoshihara of Hokkaido University for providing specimens of *Arctotanais alascensis*; Drs. Masanori Nonaka and Atsushi Kaneko of Okinawa Churaumi Aquarium for permitting us to collect specimens of *Hexapleomera* sp. in the aquarium; Dr. Yoshihiro Hayashi of Kochi Prefectural Fisheries Experimental Station for providing specimens of *Sinelobus* sp. 1; and Dr. Matthew Dick for editing the manuscript. This study was supported in part by a Grant-in-Aid (No. 22·1119) from the Japan Society for the Promotion of Science.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716-723.

Audouin, V. 1826. Explication sommaire des planches de Crustacés de l´Égypte et de la Syrie, pp. 77-98. In, J. C. Savigny, Déscription de l´Égypte, ou Recueil de Observations et des Recherches qui ont été Faites en Égypte Pendent l´Éxpéditioin de l´Armée Française. Histoire Naturelle (1. Auflage). Impériale, Paris.

Bamber, R. N. 2005. The tanaidaceans (Arthropoda: Crustacea: Peracarida: Tanaidacea) of Esperance, Western Australia, Australia, pp. 613-728. In, F. E. Wells, D. I. Walker, and G. A. Kendrick (eds.), The Marine Flora and Fauna of Esperance, Western Australia. Western Australian Museum, Perth.

———, and G. J. Bird. 1997. Peracarid crustaceans from Cape d´Aguilar and Hong Kong, II. Tanaidacea: Apseudomorpha, pp. 87-102. In, B. Morton (ed.), The Marine Flora and Fauna of Hong Kong and Southern China IV. Proceedings of the Eighth International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and southern China, Hong Kong, 2–20 April 1995. Hong Kong University Press, Hong Kong.

———, and G. A. Boxshall. 2006. A new genus and species of the Langitanainae (Crustacea: Peracarida: Tanaidae) bearing a new genus and species of nicothoid parasite (Crustacea: Copepoda: Siphonostomatoida: Nicothoidae) from the New Caledonia slope. Species Diversity 11: 137-148.

Dana, J. D. 1849. III. Zoology. 1. Conspectus Crustaceorum. Conspectus of the Crustacea of the exploring expedition. American Journal of Science and Arts, Series 2 8: 424-428.

Edgar, G. J. 2008. Shallow water Tanaidae (Crustacea: Tanaidacea) of Australia. Zootaxa 1836: 1-92.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-781.

Gardiner, L. F. 1975. The systematics, postmarsupial development, and ecology of the deep-sea family Neotanaidae (Crustacea: Tanaidacea). Smithsonian Contributions to Zoology 170: 1-265.

Hiruta, S. F., and K. Kakui. 2010. Digital illustration techniques using graphics software. TAXA, Proceedings of the Japanese Society of Systematic Zoology 29: 19-30. [In Japanese]

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

Ishimaru, S. 1985. A new species of *Leptochelia* (Crustacea, Tanaidacea) from Japan, with a redescription of *Leptochelia savignyi* (Krøyer, 1842). Publications of the Seto Marine Biological Laboratory 30: 241-267.

Kakui, K., H. Kajihara, and S. F. Mawatari. 2010. A new species of *Nesotanais* Shiino, 1968 (Crustacea, Tanaidacea) from Japan, with a key to species and a note on male chelipeds. ZooKeys 33: 1-17.

———, T. Katoh, S. F. Hiruta, N. Kobayashi, and H. Kajihara. in press. Molecular systematics of Tanaidacea (Crustacea: Peracarida) based on 18S sequence data, with an amendment of suborder/superfamily-level classification. Zoological Science.

Kussakin, G. O., and L. V. Tzareva. 1974. On the fauna of Tanaidacea (Crustacea, Tanaidacea) from the intertidal zone of the Kurile Islands, pp. 215-226. In, Symposium No.1: Fauna and Flora of the Near Shore Zone of the Kurile Islands. Academy of Sciences, USSR, Far Eastern Science Center, Institute of Marine Biology. [In Russian]

Larsen, K. 2001. Morphological and molecular investigation of polymorphism and cryptic species in tanaid crustaceans: implications for tanaid systematics and biodiversity estimates. Zoological Journal of the Linnean Society 131: 353-379.

———. 2003. Proposed new standardized anatomical terminology for the Tanaidacea (Peracarida). Journal of Crustacean Biology 23: 644-661.

Lauterbach, K-E. 1970. Der Cephalothorax von *Tanais cavolinii* Milne Edwards (Crustacea-Malacostraca). Ein Beitrag zur vergleichenden Anatomie und Phylogenie der Tanaidacea. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 87: 94-204.

Nation, J. L. 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Stain Technology 58: 347-351.

Nei, M., and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York. xiv+333 pp.

Posada, D. 2003. Using Modeltest and PAUP* to select a model of nucleotide substitution, pp. 6.5.1-6.5.14. In, A. D. Baxevanis, D. B. Davison, R. D. M. Page, G. A. Petsko, L. D. Stein, and G. D. Stormo (eds.), Current Protocols in Bioinformatics.

———. 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25: 1253-1256.

Rambaut, A., and A. J. Drummond. 2009. Tracer v1.5. http://tree.bio.ed.ac.uk/software/tracer/ [accessed on 8.XI.2010].

Richardson, H. 1899. Key to the isopods of the Pacific coast of North America, with descriptions of twenty-two new species. Proceedings of the United States National Museum 21: 815-869.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new methods for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Sato, K., M. Hamada, K. Asai, and T. Mituyama. 2009. CENTROIDFOLD: a web server for RNA secondary structure prediction. Nucleic Acids Research 37 Supplement 2: W277-W280.

Schwarz, G. 1978. Estimating the dimension of a model. The Annals of Statistics 6:

461-464.

Shiino, S. M. 1978. Tanaidacea collected by French Scientists on board the survey ship "Marion-Dufresne" in the regions around the Kuerguelen Islands and other subantarctic islands in 1972, '74, '75, '76. Science Report of Shima Marineland 5: 1-122.

Sieg, J. 1980. Taxonomische Monographie der Tanaidae Dana 1849 (Crustacea: Tanaidacea). Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 537: 1-267.

———. 1983. Crustaceorum Catalogus pars 6. Tanaidacea. Dr W. Junk Publishers, The Hague. ix+552 pp.

Spears, T., R. W. DeBry, L. G. Abele, and K. Chodyla. 2005. Peracarid monophyly and interordinal phylogeny inferred from nuclear small-subunit ribosomal DNA sequences (Crustacea: Malacostraca: Peracarida). Proceedings of the Biological Society of Washington 118: 117-157.

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland.

Tamura, K., M. Nei, and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America 101: 11030-11035.

———, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. In press. MEGA5:

Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences, pp. 57-86. In, R. M. Miura (ed.), Some mathematical questions in biology—DNA sequence analysis (Lectures on Mathematics in the Life Sciences 17. American Mathematical Society, USA.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position—specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Vonk, R., and F. R. Schram. 2007. Three new tanaid species (Crustacea, Peracarida, Tanaidacea) from the lower Cretaceous Álava amber in northern Spain. Journal of Paleontology 81: 1502-1509.

Yamaguchi, S., and K. Endo. 2003. Molecular phylogeny of Ostracoda (Crustacea) inferred from 18S ribosomal DNA sequences: implication for its origin and diversification. Marine Biology 143: 23-38.

RECEIVED: 8 April 2011

ACCEPTED: 21 May 2011

Tables

Table 1. Summary of specimens used in this study. [a]Specimens deposited in ZIHU were used for SEM observations. [b]1, Kakui et al. (in press); 2, This study; 3, Spears et al. (2005). [c]See description for ZIHU catalog numbers.

Taxa	ZIHU-[a]	Accession number	Source[b]
Suborder APSEUDOMORPHA			
Superfamily Apseudoidea			
Family Kalliapseudidae			
Kalliapseudes sp. (outgroup taxon)		AB618179	1
Suborder TANAIDOMORPHA			
Superfamily Tanaoidea			
Family Tanaidae			
Arctotanais alascensis (Richardson, 1899)	[c]	AB622810	2
Tanais tinhauae Bamber and Bird, 1997	4029	AB618190	1
Hexapleomera sp.	4030	AB618191	1
Sinelobus sp. 1	4031	AB618192	1
Sinelobus sp. 2		AB618193	1
Zeuxo sp. 1	4032	AB618194	1
Zeuxo sp. 2		AB618195	1

Superfamily Paratanaoidea		
Family Agathotanaidae		
Paranarthrura sp.	AB618196	1
Family Leptocheliidae		
Letochelia itoi Ishimaru, 1985	AB618197	1
Family Nototanaidae		
Nesotanais ryukyuensis Kakui et al., 2010	AB618198	1
Family Paratanaidae		
Paratanais sp.	AB618199	1
Paratanais malignus Larsen, 2001	AY781429.1	3
Family *incertae sedis*		
Chauliopleona sp.	AB618200	1
Metatanais sp.	AB618201	1
Superfamily Neotanaoidea		
Family Neotanaidae		
Neotanais sp. 1	AB618188	1
Neotanais sp. 2	AB618189	1

Table 2. List of PCR and cycle sequencing (CS) primers used in this study for the 18S rRNA gene.

Primer name	Reaction	Primer sequence (in 5'-3' direction)	Direction	Source
18S-a1F	PCR & CS	GGYGAAACCGYGAAWGGYTC	Forward	Kakui et al. (in press)
18S-F2	CS	CCTGAGAAACGGCTRCCACAT	Forward	Kakui et al. (in press)
18S-b4F	CS	TGCGGTTAAAAAGCTCGTAGTTG	Forward	Kakui et al. (in press)
18S-b4R	CS	TCCAACTACGAGCTTTTTAACC	Reverse	Kakui et al. (in press)
18S-F3	CS	GYGRTCAGATACCRCCSTAGTT	Forward	Yamaguchi and Endo (2003)
18S-b6F	CS	CCTGCGGCTTAATTTGACTC	Forward	Kakui et al. (in press)
18S-a6R	CS	AACGGCCATGCACCAC	Reverse	Kakui et al. (in press)
18S-b8R	CS	TCTAAGGGCATCACAGACCTG	Reverse	Kakui et al. (in press)
18S-b8F	CS	GGTCTGTGATGCCCTTAGATG	Forward	Kakui et al. (in press)
18S-a9R	PCR & CS	CCTTGTTACGACTTTTAGTTCC	Reverse	Kakui et al. (in press)

Table 3. Characteristics of the 18S sequence data set, after alignment and the removal of indels. TS, total number of sites; VS, number of variable sites; PIS, number of parsimony-informative sites.

TS	VS	PIS	Base frequencies (%)				Compositional heterogeneity	
			A	C	G	T	χ^2	*P*
1520	689	587	26.9	21.1	26.7	25.3	39.98 (df = 48)	0.964

Table 4. Optimal substitution models for the maximum-likelihood (ML) and Bayesian (BA) analyses of the 18S data set, selected by AIC and BIC in jModelTest, respectively. GTR, general time reversible model (Tavaré, 1986); TIM, transitional model (Posada, 2003); I, proportion of invariant sites; G, gamma distribution shape parameter.

Analysis	Model	I	G	Base frequencies	Rate matrix
ML	GTR+I+G	0.2660	0.3820	A = 0.2646	A-C = 0.7844
				C = 0.2077	A-G = 1.4652
				G = 0.2657	A-T = 0.9397
				T = 0.2620	C-G = 0.3446
					C-T = 2.7111
					G-T = 1.0000
BA	TIM3+I+G	0.2640	0.3810	A = 0.2707	AC = 0.5761
				C = 0.2077	AG = 1.5104
				G = 0.2596	AT = 1.0000
				T = 0.2619	CG = 0.5761
					CT = 2.7948
					GT = 1.0000

Figure captions

Fig. 1. S EM images showing pereopods with or without the ischium. A, *Arctotanais alascensis*, left pereopod 5, inner view; B, *A. alascensis*, left pereopod 4, ventral view; C, *Hexapleomera* sp., right pereopod 3; D, *Sinelobus* sp. 1, left pereopod 6; E, *Tanais tinhauae*, left pereopod 6; F, *Zeuxo* sp. 1, left pereopod 5. Abbreviations: b, basis; m, merus. Arrowheads indicate ischium. Scale bars: 50 μm.

Fig. 2. *Arctotanais alascensis*, non-type, male (6.96 mm), ZIHU-3949. A, body, dorsal view; a1, same, pleonites 3 and 4, and pleotelson, dorsal view; B, body, lateral view. Scale bar: 1 mm.

Fig. 3. *Arctotanais alascensis*, non-type, male (6.96 mm), ZIHU-3949. A, left antennule, ornamentation on articles 4 and 5 not shown; a1, same, articles 3 (distal portion), 4, and 5; B, left antenna, ornamentation on articles 6 and 7 not shown, b1, same, articles 6 (distal portion) and 7; C, labrum; D, left mandible; d1, same, inner view; E, right mandible; e1, same, incisor and lacinia mobilis, inner view; F, labium; f1, same, labial process, fine setae not shown; G, left maxillule; g1, same, distal portion of endite; H, right maxilla; I, entire left and right maxillipeds, except for coxa and palp, ventral view, several setae on left palp not shown; J, right maxillipedal palp, dorsal view; K, right epignath. Scale bars: A, B, I, K, 0.5 mm; a1, b1,

d1–g1, C–H, J, 0.25 mm.

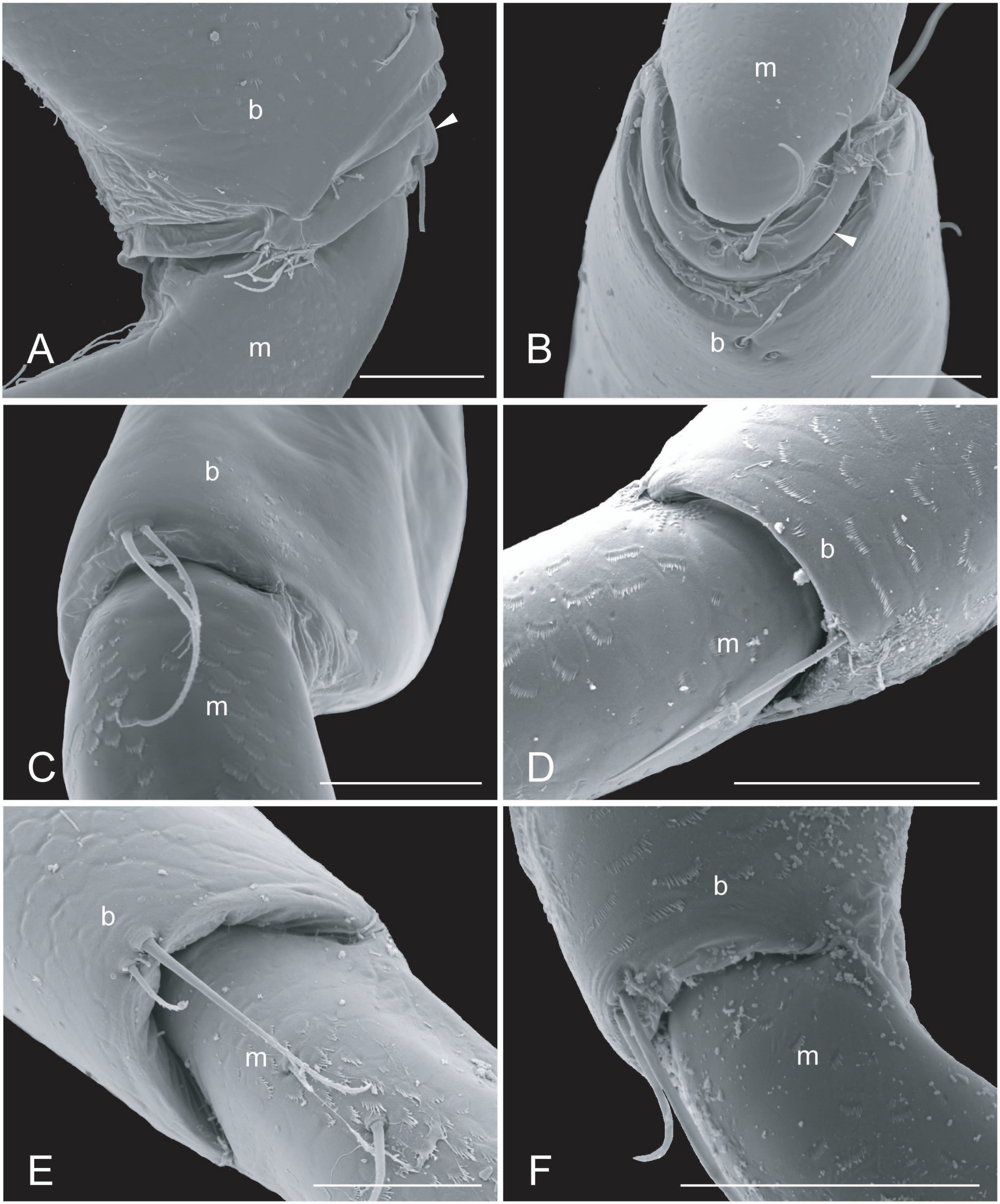
Fig. 4. *Arctotanais alascensis* (Richardson, 1899), non-type, male (6.96 mm), ZIHU-3949. A, right cheliped, outer view; a1, diagrammatic cross section of connection between basis and merus, with ischium indicated by heavy solid line (i, inner; d, dorsal); B, left cheliped, outer view, most setae not shown; C, right pereopod 1, outer view; c1, same, circumplumose seta; D–G, right pereopods 2–5, respectively, outer view; H, right pereopod 6, outer view; h1, same, claw, inner view, outer setae not shown; h2, same, serrate seta; h3, same, pinnate seta; I, right pleopod 1, ventral view, most setal ornamentation not shown; i1, same, distal end of distally hooked plumose seta of endopod; J, left uropod; j1, same, uropod-endopodal articles 5 (distal portion) and 6. Scale bars: A–J, j1, 0.5 mm; h1, i1, 0.25 mm; c1, 0.2 mm; h2, h3, 0.1 mm.

Fig. 5. *Arctotanais alascensis*, non-type, female (5.65 mm), ZIHU-3951, SEM images. A, right cheliped, dorsal view; B, right cheliped, ventral view; C, right cheliped, outer view; D, right cheliped, inner view. Abbreviations: b, basis; m, merus; c, carpus. Arrowheads indicate ischium. Scale bars: 0.1 mm.

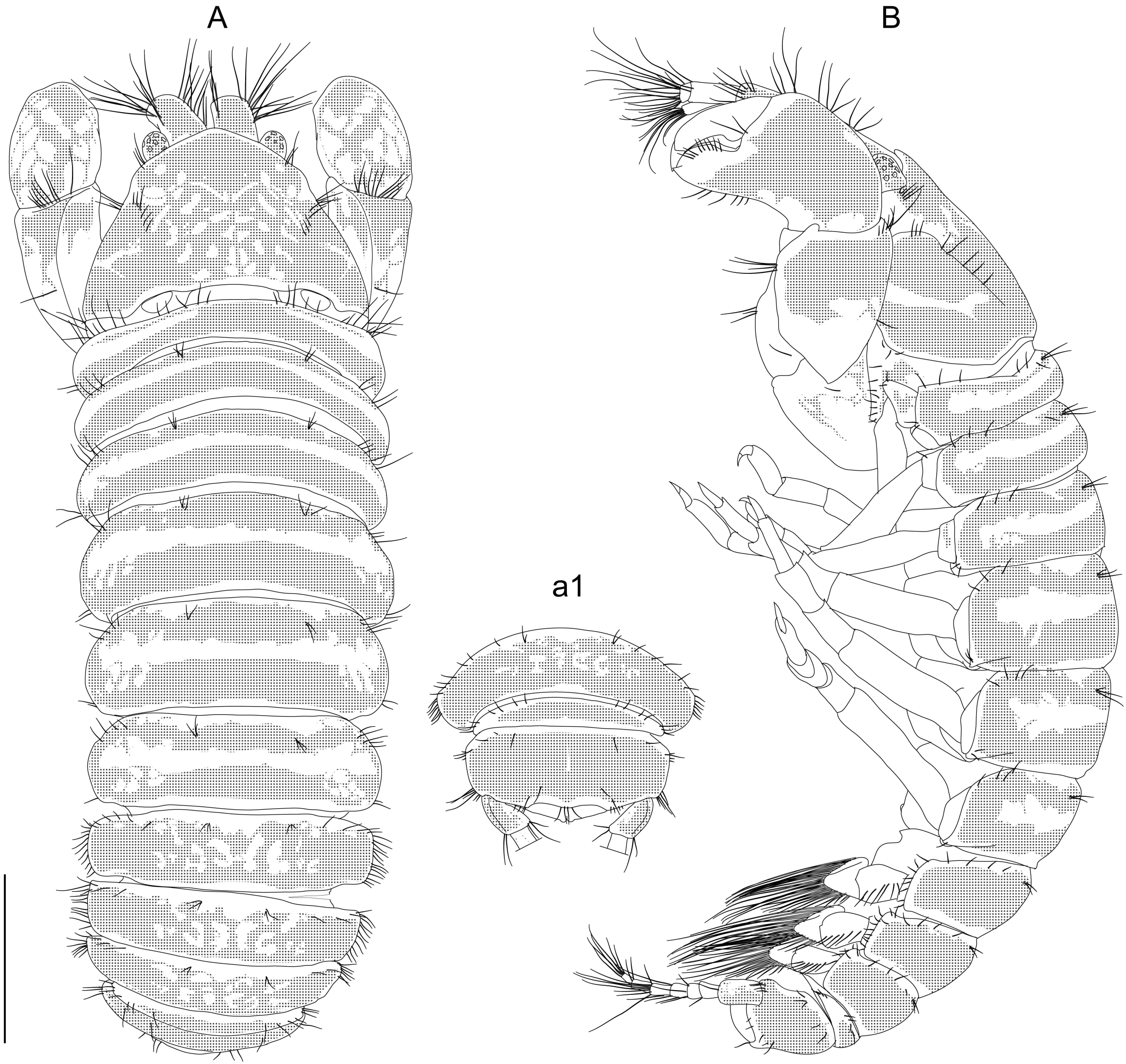
Fig. 6. Map showing the known distribution of *Arctotanais alascensis*. A, The apparently misidentified site interpreted by Sieg (1980) as Richardson’s (1899) locality “Kyska;” B,

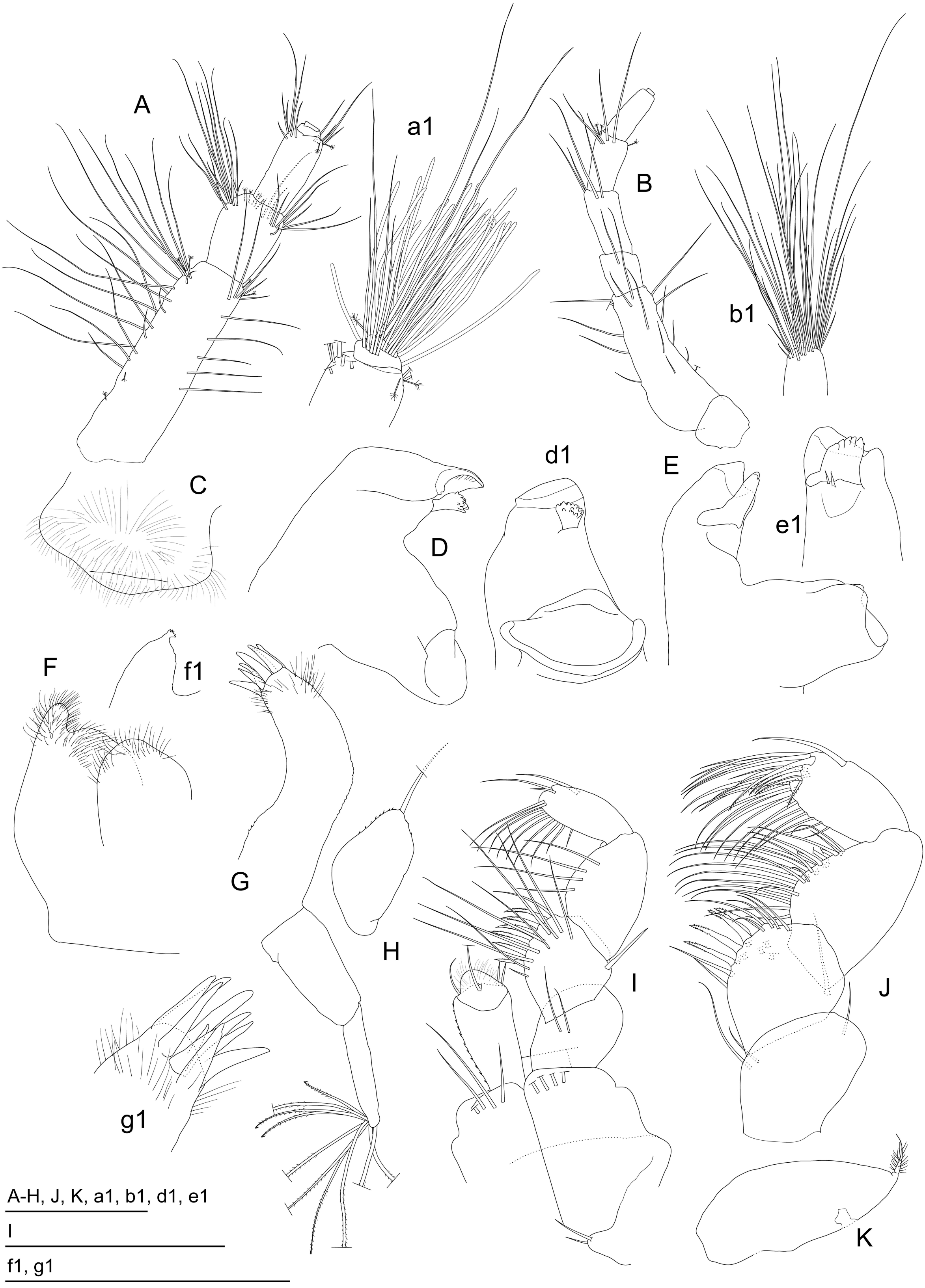
Kiska Island, the locality likely indicated by Richardson (1899) as "Kyska;" C, Simushir Island (Kussakin and Tzareva, 1974); D, Urup Island (op. cit.).; E, Iturup Island (op. cit.); F, Pekin-no-hana, Shiretoko (this study); G, Aidomari, Shiretoko (this study); H, Muroran (this study); I, Oshoro (this study).

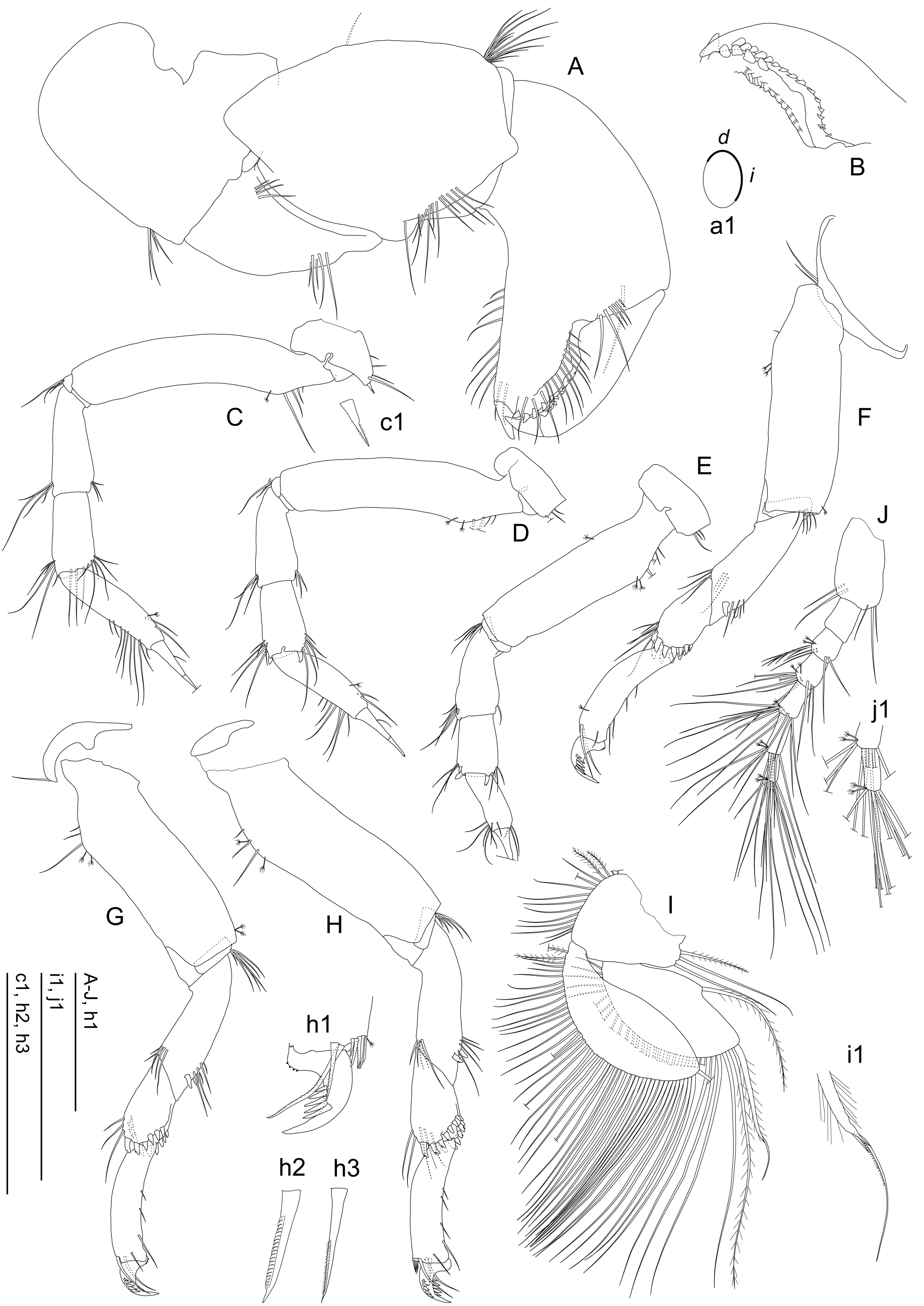
Fig. 7. Phylogenetic trees based on 18S sequence data. A, Maximum-parsimony tree (length = 1799), bootstrap values > 50% and the Bayesian posterior probability values > 0.90 are presented in the order MP/ME/BA; B, Maximum-likelihood tree based on the GTR+I+G substitution model (–lnL = 9519.18385), bootstrap values > 50% are shown. Numbers in squares indicate clades with a > 50% bootstrap value or > 0.90 Bayesian posterior probability value. (O.G.), out-group.

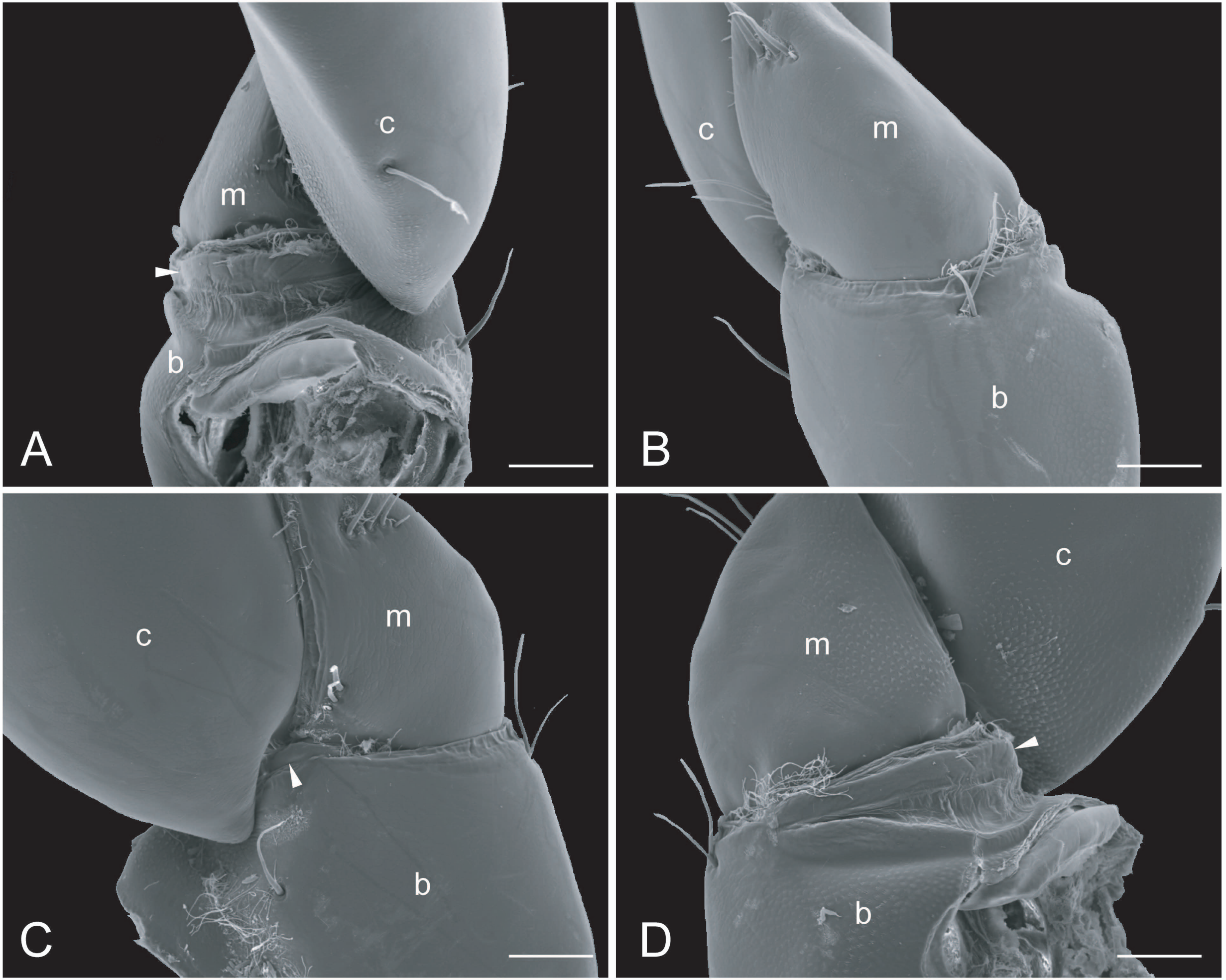


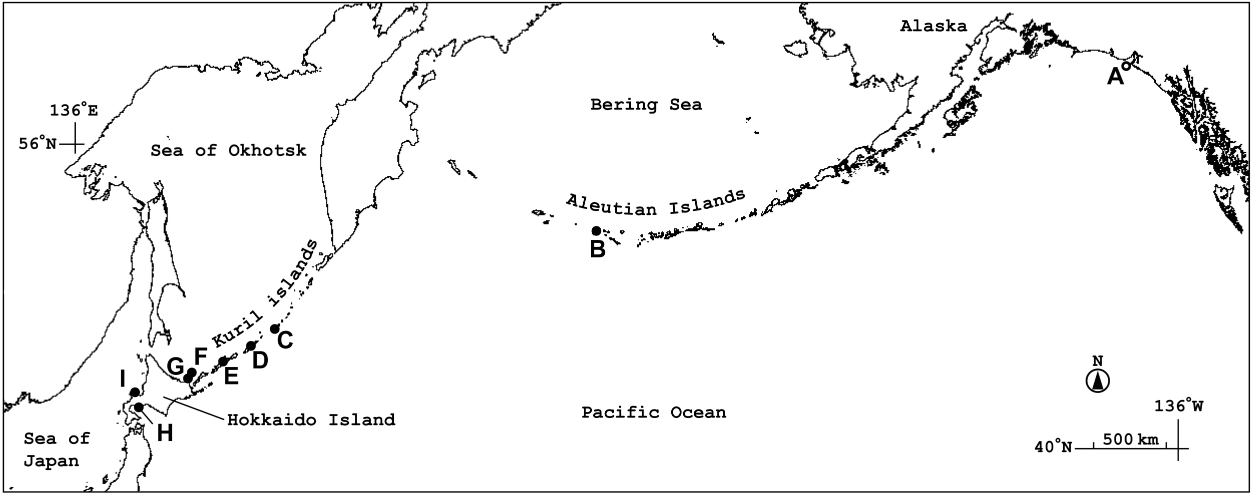
b
m
A
m
b
B
b
m
C
b
m
D
b
m
E
b
m
F

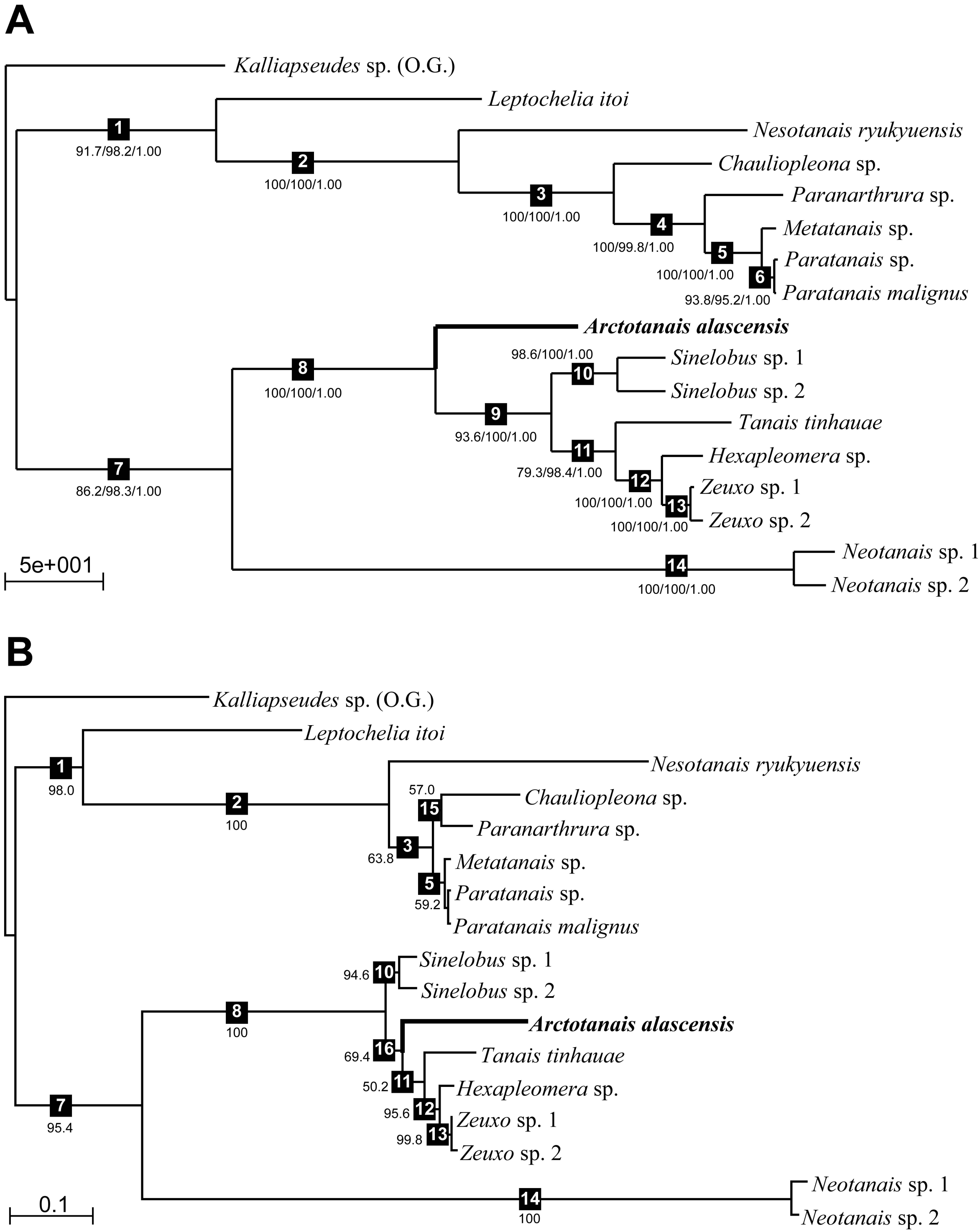

A
B
a1


A
a1
B
b1
C
D
d1
E
e1
F
f1
G
g1
H
I
J
K
A-H, J, K, a1, b1, d1, e1
I
f1, g1


A
B
d
i
a1
C
c1
D
E
F
J
j1
G
H
I
i1
h1
h2
h3
A-J, h1
i1, j1
c1, h2, h3


A
c
m
b
B
c
m
b
C
c
m
b
D
m
c
b


Alaska
A
Bering Sea
136°E
56°N
Sea of Okhotsk
Aleutian Islands
B
Kuril islands
C
D
E
F
G
I
H
Hokkaido Island
N
Pacific Ocean
136°W
Sea of
Japan
40°N
500 km


A
Kalliapseudes sp. (O.G.)
Leptochelia itoi
Nesotanais ryukyuensis
Chauliopleona sp.
Paranarthrura sp.
Metatanais sp.
Paratanais sp.
Paratanais malignus
Arctotanais alascensis
Sinelobus sp. 1
Sinelobus sp. 2
Tanais tinhauae
Hexapleomera sp.
Zeuxo sp. 1
Zeuxo sp. 2
Neotanais sp. 1
Neotanais sp. 2
1
91.7/98.2/1.00
2
100/100/1.00
3
100/100/1.00
4
100/99.8/1.00
5
100/100/1.00
6
93.8/95.2/1.00
7
86.2/98.3/1.00
8
100/100/1.00
9
93.6/100/1.00
10
98.6/100/1.00
11
79.3/98.4/1.00
12
100/100/1.00
13
100/100/1.00
14
100/100/1.00
5e+001
B
Kalliapseudes sp. (O.G.)
Leptochelia itoi
Nesotanais ryukyuensis
Chauliopleona sp.
Paranarthrura sp.
Metatanais sp.
Paratanais sp.
Paratanais malignus
Sinelobus sp. 1
Sinelobus sp. 2
Arctotanais alascensis
Tanais tinhauae
Hexapleomera sp.
Zeuxo sp. 1
Zeuxo sp. 2
Neotanais sp. 1
Neotanais sp. 2
1
98.0
2
100
15
57.0
3
63.8
5
59.2
10
94.6
8
100
16
69.4
11
50.2
12
95.6
13
99.8
7
95.4
14
100
0.1