



Revision of basal calanoid copepod families, with a description of a new species and genus of Pseudocyclopidae

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A newly discovered plesiomorphic genus and species of calanoid copepod (*Pinkertonius ambiguus* gen. et sp. nov.) taken with an epibenthic sledge from the flanks of the Chatham Rise, east of New Zealand, at a depth of about 900 m, could not be assigned to any known genus or family based on available diagnoses. A morphology-based cladistic analysis of all genera previously placed in the Epacteriscidae, Pseudocyclopidae, Ridgewayiidae, Boholinidae, and the new taxon is presented. The Pseudocyclopidae and Epacteriscidae are confirmed as monophyletic families, and the family names Ridgewayiidae and Boholinidae become synonyms of Pseudocyclopidae. There are no grounds upon which more than a single basal superfamily, the Pseudocyclopoidea, can be recognized. The superfamily Pseudocyclopoidea, and families Pseudocyclopidae, Epacteriscidae, and the new genus are diagnosed. *Pinkertonius ambiguus* gen. et sp. nov. is placed within the Pseudocyclopidae. Genetic data adds to the definition of the new taxon and confirms the basal position of the Pseudocyclopoidea in a revised Calanoida phylogeny. This phylogeny contributes to an improved resolution of the relationships among the Centropagoidea, Megacalanoidea, Bathypontioidea, Eucalanoidea, and Clausocalanoidea, as well as providing testable hypotheses for future work.

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INTRODUCTION

Over recent years a large number of genera and species have been added to the relatively plesiomorphic calanoid copepod families Epacteriscidae, Pseudocyclopidae, Ridgewayiidae, and Boholinidae (e.g. Jaume & Boxshall, 1995; Ohtsuka, Fosshagen & Putschakarn, 1999; Fosshagen, Boxshall & Iliffe, 2001; Boxshall & Jaume, 2003, 2012; Fosshagen & Iliffe, 2004, 2007; Suárez-Morales & Iliffe, 2007; Andronov, 2007). Also, Ferrari,

Chullasorn & Dahms (2011) interpreted the developmental characteristics of *Pseudocyclops schminkei* in a context of Pseudocyclopidae being the oldest extant family. It has become clear that relationships among the basal genera (Table 1) need to be revised (Andronov, 2007). Nearly all species within these families have been found in shallow benthopelagic or anchialine cave habitats, although *Stygoridgewayia* is found in fresh groundwater in north-west Australia and *Boholina* species in Indonesia were found in brackish water of low salinity (6 g kg⁻¹). But in 2007, Andronov (2007) discovered *Miheptneria abyssalis* in the central eastern Atlantic at a depth of 4500 m. His analysis led him to conclude

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Table 1. Taxa used as exemplars in the morphology-based cladistic analysis of the basal calanoid copepod families and sources of information. The sources of information are also the original description except for *Disseta palumbii* Giesbrecht, 1889, and where otherwise indicated

Family	Species	References
Heterorhabdidae Sars, 1902	<i>Disseta palumbii</i> Giesbrecht, 1889	Park, 2000
Epacteriscidae Fosshagen, 1973	<i>Azygonectes plumosus</i>	Fosshagen, Iliffe, 2007, 2004 (genus)
	<i>Balinella ornate</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Bofuriella vorata</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Bomburiella gigas</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Bunderia misophaga</i>	Jaume, Humphreys, 2001
	<i>Caiconectes antiquus</i>	Fosshagen, Iliffe, 2007
	<i>Cryptonectes brachyceratus</i>	Fosshagen, Iliffe, 2004
	<i>Edaxiella rubra</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Enantiosus bermudensis</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Enantronia canariensis</i>	Fosshagen, Boxshall, Iliffe, 2001 (♂ unknown)
	<i>Enantronoides bahamensis</i>	Fosshagen, Boxshall, Iliffe, 2001 (♀ unknown)
	<i>Erebonectes nesioticus</i>	Fosshagen, Iliffe, 1985
	<i>Erebonectoides macrochaetus</i>	(Fosshagen & Iliffe, 1994)
	<i>Epacteriscus rapax</i>	Fosshagen, 1973
	<i>Gloinella yagerae</i>	Fosshagen, Boxshall, Iliffe, 2001
	<i>Iboyella cubensis</i>	Boxshall, Jaume, 2003 (♀ unknown)
	<i>Minnonectes melodactylus</i>	Fosshagen, Iliffe, 2004, 2007
	<i>Oinella longiseta</i>	Fosshagen, Boxshall, Iliffe, 2001, Fosshagen, Iliffe, 2004 (♂)
Ridgewayiidae M.S. Wilson, 1958	<i>Badijella jalzici</i>	Krsinić, 2005
	<i>Brattstonia longicauda</i>	Fosshagen, Iliffe, 1991
	<i>Exumella mediterranea</i>	Jaume, Boxshall, 1995
	<i>Exuminella bucculenta</i>	Fosshagen, Iliffe, 1998
	<i>Hondurella verrucosa</i>	Suárez-Morales, Iliffe, 2007
	<i>Normancavia minuta</i>	Fosshagen, Iliffe, 2003
	<i>Placocalanus longicauda</i>	Ohtsuka, Fosshagen, Soh, 1996
	<i>Robpalmeria asymmetrica</i>	Fosshagen, Iliffe, 2003
	<i>Ridgewayia stygia</i>	Ohtsuka, Kase, Boxshall, 2000
	<i>Stargatia palmeri</i>	Fosshagen, Iliffe, 2003
	<i>Stygoridgewayia trispinosa</i>	Tang, Barron, Goater, 2008
Pseudocyclopidae Giesbrecht, 1893	<i>Miheptneria abyssalis</i>	Andronov, 2007 (♀ unknown)
	<i>Pseudocyclops ornaticauda</i>	Ohtsuka, Fosshagen, Putschakarn, 1999
Boholinidae Fosshagen, Iliffe, 1989	<i>Boholina parapurgata</i>	Boxshall, Jaume, 2012
New species	<i>Pinkertonius ambiguus</i> gen. et sp. nov.	Present description

that there should be just one family to encompass all these basal calanoids, the Pseudocyclopidae Giesbrecht, 1893. He assigned *Miheptneria* to the subfamily Epacteriscinae. Andronov further suggested that these genera can be assigned to two subfamilies, which he referred to as the Pseudocyclopinae Giesbrecht, 1893 and Epacteriscinae Fosshagen, 1973; however, he did not confirm the monophyletic status of either subfamily.

A newly discovered plesiomorphic genus and species was taken with an epibenthic sledge, at a depth of about 900 m, from the flanks of the Chatham Rise, east of

New Zealand. This copepod also could not be assigned to any known genus or family based on available diagnoses (e.g. Boxshall & Halsey, 2004). Therefore, we describe the genus and species and then present our cladistic analyses. All genera previously placed in the Epacteriscidae, Pseudocyclopidae, Ridgewayiidae, and Boholinidae were subjected to a morphology-based cladistic analysis, and Andronov's taxonomic conclusions were evaluated. The position of the new taxon within the Calanoida was evaluated based on genetic data. Thus, we place our new taxon within a new hierarchy and diagnose its taxa.

MATERIAL AND METHODS

SPECIMENS

Specimens of the new species were sorted from samples taken by the upper and lower nets of a Brenke sledge deployed on the flanks of the Chatham Rise, east of New Zealand, from RV *Tangaroa* voyage TAN1116 (NIWA, 2011) (Fig. 1). The Brenke sledge (Brenke, 2005) has an upper net (sampling 0.77–1.12 m above the sea floor) and a lower net (sampling 0–0.60 m above the sea floor) (Lörz, Kaiser & Bowden, 2013). Each net has a 500- μ m mesh and a cod end of 300- μ m mesh. This epibenthic sledge has an opening/closing mechanism and samples the biota on or above the sea floor. Animals were separated from sediment by elutriation. One-third of the sample was placed in 95% ethanol, one-third was placed in formalin, and one-third was frozen. It was from the ethanol-preserved and frozen samples that the current specimens were separated.

Sorted copepods were observed and drawn whole in water, and dissected parts were mounted in gum chloral (Pantin, 1964). Mounted specimens were observed using Nomarski differential interference contrast (DIC) microscopy, drawn using a drawing tube, and 'inked' digitally (Coleman, 2003). The system of morphological nomenclature is based on that of Huys & Boxshall (1991).

GENETIC METHODS

Specimens of the new species were selected for analysis as well as the pseudocyclopoid taxa (*Pseudocyclops juanibali* Figueroa, 2011 and *Pseudocyclops schminkei* Chullasorn, Ferrari & Dahms, 2010). These taxa were used to add to the description of the new taxon and, along with additional species from GenBank, to improve the resolution of the current gene-based phylogeny (Blanco-Bercial, Bradford-Grieve & Bucklin, 2011) (Appendix S1). DNA extractions were carried out using the E.Z.N.A.® Mollusc DNA Kit (OMEGA), following the procedure indicated for arthropods. All polymerase chain reactions (PCRs) were carried out as described in Blanco-Bercial *et al.* (2011), with the exception of PCRs for 18S, in which the primers 18Sf and 18Sr (Huys *et al.*, 2006) were used because they proved to be more efficient at amplifying this superfamily.

Alignments of the four genes were carried out separately in MAFFT 7 (Katoh & Standley, 2013), under the L-INS-i option (Appendix S2). Partitioned phylogenetic analyses for the alignment of the four genes were carried out using maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were computed using RAxML 7.5.5 (Stamatakis, 2006), under the GTRGAMMA option, with a completely random starting tree and 10 000 bootstrap replicates. The BI analysis was carried out using the MPI (parallel) version

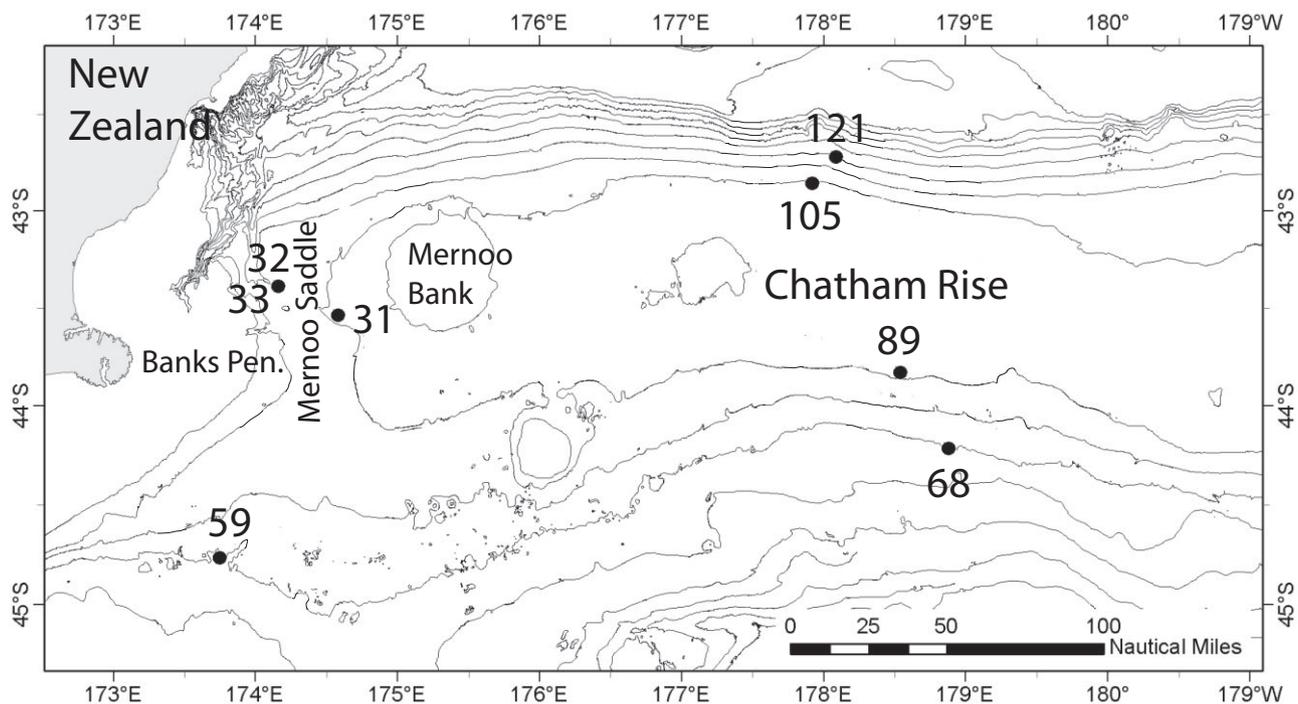


Figure 1. Location of samples examined and geographical names used in the text. Isobaths are at 250-m intervals from 250 m.

of MrBayes (Altekar *et al.*, 2004; Ronquist *et al.*, 2012). MrModeltest 2.3 (Nylander, 2004) was used to identify and select the appropriate models of sequence evolution. Each data set was run for 3 000 000 generations with a sample frequency of 1000 generations. The first 500 trees were discarded as burn-in, and 2500 trees were accepted from each run.

MORPHOLOGICAL CHARACTER ANALYSIS

All 31 genera, previously placed in the Epacteriscidae, Pseudocyclopidae, Ridgewayiidae, and Boholinidae, are represented in the in-group of an analysis of relationships among these genera to test the monophyly of these families (Table 1).

Out-group taxon: The out-group taxon was chosen in the context of the current phylogenetic hypotheses concerning the order Calanoida (Bradford-Grieve, Boxshall & Ahyong, 2010). From the superfamilies most closely related to the in-group, we chose the most plesiomorphic exemplar (*Disseta palumbii* Giesbrecht, 1889) from the most plesiomorphic family Heterorhabdidae in the superfamily Augaptiloidea (cf. Nishida & Ohtsuka, 1996). The Augaptiloidea differ from the currently studied genera chiefly in that the male antennule is typically geniculate on the left. In other respects, the swimming leg setation and the form of the male leg 5 can be very similar to the currently studied genera.

The character set: The character set was chosen based on defensible hypotheses of primary homology. These data were mainly taken from the literature, but some character states of the in-group taxa deposited at the Natural History Museum, London, were checked. Checked taxa included the genera *Azygonectes*, *Bomburiella*, *Caiconectes*, *Cryptonectes*, *Edaxiella*, *Enantronoides*, *Epacteriscus*, *Erebonectoides*, *Exumellina*, *Iboyella*, *Minnonectes*, *Oinella*, *Placocalanus*, *Pseudocyclops*, and *Robpalmeria*. For several of these species corrections are made for various characters. These are detailed below.

Hypotheses of primary homology relating to segmentation and setation are based upon our current understanding of copepod development (Bradford-Grieve *et al.*, 2010). Where we failed to arrive at a hypothesis of primary homology for particular mouthpart characters/states, this failure is evaluated in the sections below.

Characters and their states are listed in Appendix S3 and the data matrix is given in Appendix S4. On the figures, selected specific characters and states are indicated in the form '◀ 1:2'.

Cephalothorax (Fig. 2): Several characters of the cephalothorax were initially included. The arthrodivial mem-

branes between the head (cephalosome) and pedigerous somite 1, and between pedigerous somites 4 and 5, may be fully formed and functional or may fail to develop (characters 1, 2). The frontal margin of the head may be extended to form a rostrum of various shapes (a single plate, a bifurcate plate, or hardly extended; character 3). Rostral filaments may be present or absent (character 4). Rostral windows (paired areas of integumental perforations) are found in *Epacteriscus* and *Edaxiella* (Fosshagen *et al.*, 2001), but have yet to be reported for other genera (character 5).

Urosome (Fig. 2): The female gonopores on the genital double somite, closed off by a gonoporal plate (Cuoco *et al.*, 1997), may open directly to the outside environment or be covered by a genital operculum (see Bradford-Grieve *et al.*, 2010; character 6). In the former derived state the gonopores may be widely separated on the surface of the double somite, as in *Pseudocyclops* and *Boholina*.

Caudal ramus: The setation of the caudal rami is analysed according to the interpretation of Huys & Boxshall (1991), in which there are primitively seven setae. Seta I is either vestigial (new observation in *Epacteriscus dentipes* Fosshagen, Boxshall & Iliffe, 2001 and *Cryptonectes*) or absent (character 7). Seta II may be setiform, spiniform, or apparently absent (*Miheptneria*?) (character 8). The caudal rami show varying degrees of asymmetry. The female caudal rami may be of equal lengths, or longer on the left or right (character 9). The female caudal seta V may be of equal lengths on both rami, or longer on the left or right (character 10). The female left caudal seta VII may be similar to seta VII on the right side, or may be bordered by long medioproximal setules on one margin of the seta (character 11). The male caudal rami may be symmetrical or asymmetrical (character 12).

Antennule (Figs 3, 7, 8): Female and male antennule segmentation and the distribution of aesthetascs is analysed according to the interpretation of Huys & Boxshall (1991) and Boxshall & Huys (1998). Many of the species studied here exhibit the plesiomorphic calanoid state, with each segment separated by an arthrodivial membrane apart from segments XXVII–XXVIII. The presence or not of an arthrodivial membrane in the female between segments I and II, II and III, III and IV, X and XI, and XXVI and XXVII is recorded (characters 13–17). The distribution of aesthetascs in both sexes of some species was checked against the original descriptions and the results tabulated (Appendix S5a, b). A rather uniform distribution of aesthetascs among segments was revealed, with a tendency in a few taxa for aesthetascs to be absent on segments IV, VI, and VIII, or more segments in the case of *Oinella*, *Hondurella*,

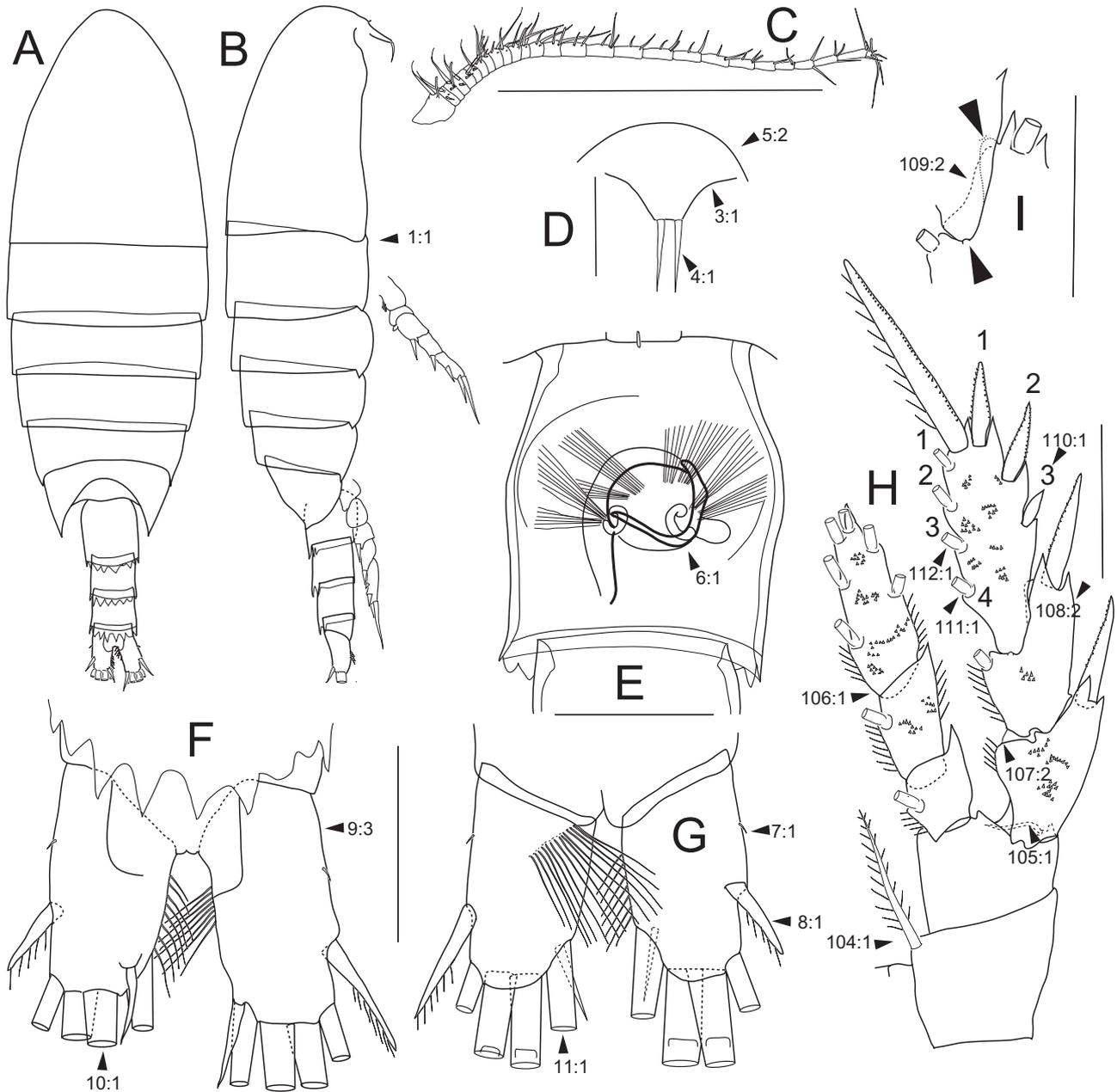


Figure 2. *Pinkertonius ambiguus* gen. et sp. nov. Female: A, dorsal view; B, lateral view; C, antennule at same scale as A and B; D, rostrum; E, genital double-somite ventral view; F, caudal rami dorsal view; G, caudal rami, ventral view; H, leg 5 anterior view; I, detail of joint between exopod segments 2 and 3 of leg 5, showing (large, unlabelled arrowheads) location of two pivot points. Selected characters and their states are indicated (e.g. ◀5:2). Scale bars: A–C, 1.0 mm; D–I, 0.1 mm.

Placocalanus, *Pseudocyclops*, and *Stygoridgewayia*. This absence of aesthetascs may be the result of the retention of character states of one or more developmental stages (Boxshall & Huys, 1998). The assignment of aesthetascs to segments of the compound proximal segments of *Pseudocyclops* was made on a comparative basis, using evidence from the timing of their ap-

pearance during development. In *Pseudocyclops umbraticus* (Costanzo, Crescenti & Zagami, 2004) three aesthetascs are present on the compound proximal segment at copepodid I. In species such as *Ridgewayia klausruetzleri* Ferrari, 1995, assumed to have the ancestral setation pattern (Boxshall & Huys, 1998), aesthetascs are found on segments III, V, and VII at

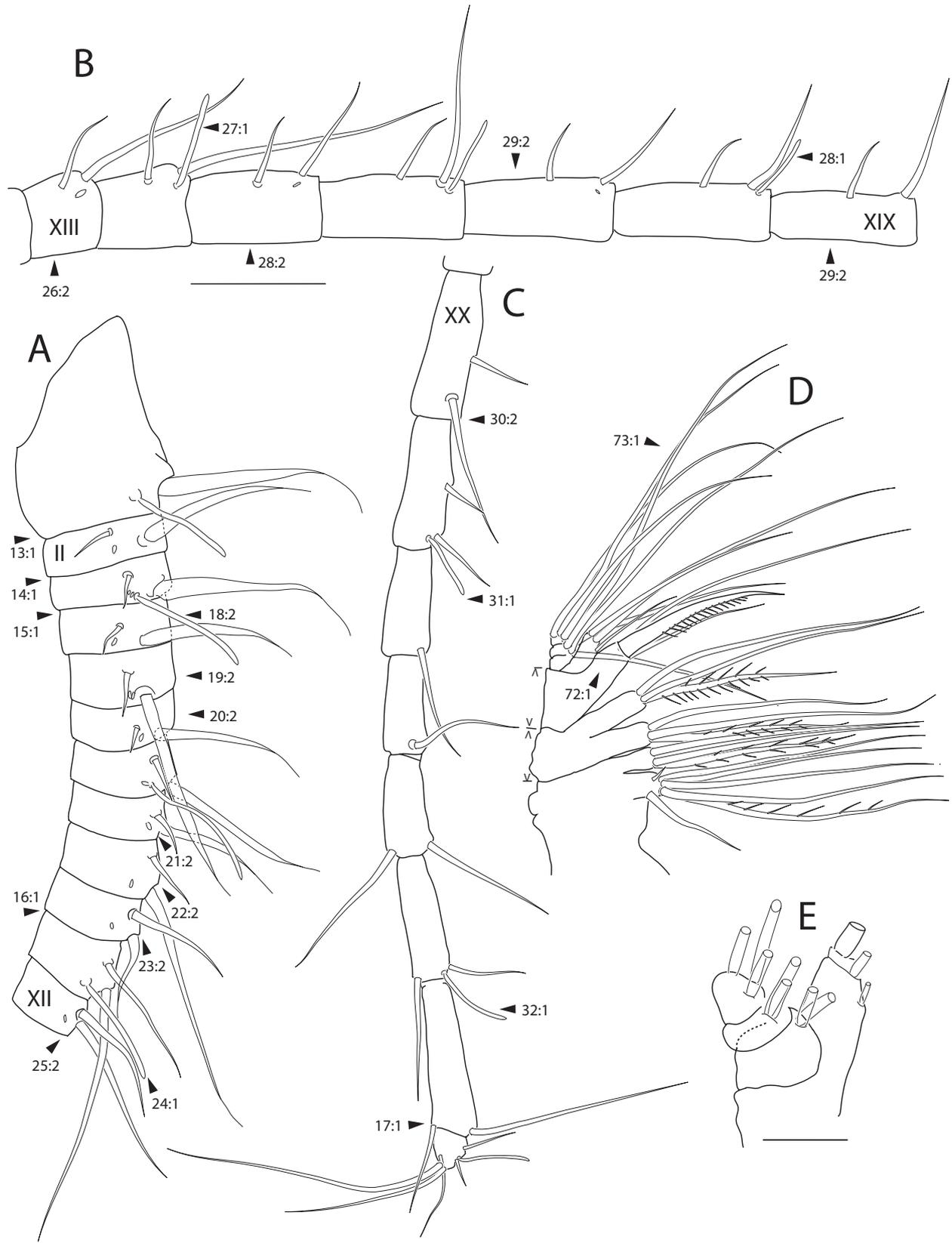


Figure 3. *Pinkertonius ambiguus* gen. et sp. nov. female: A, antennule ancestral segments I–XII; B, antennule ancestral segments XIII–XIX; C, antennules ancestral segment XX–XXVIII; D, maxilla; E, maxilla endopod. Scale bars: A–D, 0.1 mm; E, 0.02 mm.

copepodid III. Thus, the three aesthetascs on the proximal segment in *Pseudocyclops* are assumed to be derived from ancestral segments III, V, and VII. The presence or absence of aesthetascs on segments IV–VI, VIII–XVI, XVIII–XXI, and XXV in the female (characters 18–33) is recorded. On the right geniculate antennule of the male, the presence or absence of an arthrodistal membrane between segments I and II, III and IV, XXI and XXII, XXIV and XXV, and XXVI and XXVII (characters 34–39) is recorded. The presence or absence of aesthetascs on male segments IV, VI, VIII–XIX, XXI, and XXV is recorded (characters 40–55). Special fused setal elements may be found on segments XIX–XXIII (character 56), and a distoanterior process is present on segment XXV (character 57) of the male right geniculate antennule in some taxa (see Appendix S5).

Antenna (Fig. 4): The antenna is analysed according to the interpretation of Huys & Boxshall (1991). All these species exhibit the plesiomorphic state of the exopod with three terminal setae and one seta each for the remaining eight more-proximally located segments, some of which may or may not be separated by an arthrodistal membrane. The presence or absence of an arthrodistal membrane between exopod segments II and III, III and IV, and IV and V is scored (characters 58–60). The presence or absence of a seta on each of exopod segments I–IV is recorded (characters 61–64).

Mandible (Fig. 4): Mandibles are very variable, and it is difficult to establish robust homologies among the character states. The only characters used here reflect the state of development of the mandibular endopod: whether or not the ramus is two-segmented, one-segmented, or absent (character 66). In *Exumellina* and *Stargatia* endopod segment 2 is elongate and paddle-like (character 65).

Maxillule (Fig. 4): The presence or absence of a seta representing the basal exite is noted (character 67). The coxal epipodite develops consistently by the addition of setae proximally (see Bradford-Grieve *et al.*, 2010). Therefore, we consider setae to be homologous, counting from the distal part of the epipodite: the presence or absence of setae 6–9 (with seta 9 being the most proximal seta) is scored (characters 68–70, 74). In *Exumellina* and *Stargatia* the endopod segments are elongate, giving this ramus a paddle-like appearance (character 71).

Maxilla (Fig. 3): There is a group of genera that have an elongate basis and an endite that hardly projects from its segment. Here, we classify the basal endite as either an elongate lobe or lacking a distinct lobe (character 72). There is considerable variability in the

numbers of setae on endite 1 (between three and seven) and on the endopod (between five and 11). As there is no strong evidence upon which to base decisions about which setae are homologous, these characters are not used in this analysis. We do however include the form of the endopod setae, which may be fine and flexible or spine-like (character 73).

Maxilliped (Fig. 4): The presence or absence of the seta representing syncoxal endite 1 is noted (character 75). A group of genera have the length of the endopod (segments 2–6) reduced relative to the coxa (not including the praecoxa; Fig. 4D), being either greater than the coxa or less than the coxa (character 76). The maximum number of setae on the endopod segments is 2, 4, 4, 3, 3 + 1, 4. The endopod segments acquire setae during development in an order that is revealed by their length, i.e. the shorter setae are the most recently added (see Bradford-Grieve *et al.*, 2010). It is assumed that setae fail to develop in the reverse order from which they are added. The setae are numbered as shown in Figure 4. The presence or absence of individual setae is noted on endopod segments 2–5 (characters 77–81, 83). The only genus that does not appear to have an outer seta on endopod segment 5 is *Edaxiella*: both *Bomburiella* (new observation) and *Erebionectes* (Huys & Boxshall, 1991) have an outer seta, as do all other taxa in the in-group. In some genera endopod segment 6 is variously reduced in size or even fused to segment 5 (characters 84, 85). The state of endopod segment 6 in *Azygonectes plumosus* Fosshagen & Iliffe, 2007 was checked, and was found to be very small and separated from segment 5. The majority of endopod setae are fine and flexible, or spine-like (character 82).

Swimming legs 1–4 (Fig. 5): Leg 1 basis may or may not have an outer border seta (character 86), and may sometimes have the mediobasal seta absent (character 87). The posterior surface of the basis of leg 1 may also bear a specialized digitiform process: it is present in *Badijella*, *Boholina*, *Brattstromia*, *Placocalanus*, *Robpalmeria*, *Stargatia*, and *Stygoridgewayia*, as well as in the new species described here (character 88). Exopod segment 1 may sometimes have the inner seta absent (character 89), exopod segment 2 outer distal corner may be produced into a large spiny lobe, as in *Boholina* and *Stygoridgewayia* (character 90), and exopod segment 3 inner seta 4 may be absent (character 91). Endopod segment 2 inner seta 2 may be absent (character 92), endopod segment 3 may have the outer border seta absent (character 93), and inner seta 3 can also be lacking (character 94).

Leg 2 basis sometimes has an outer border seta present (character 95); if present, it is usually setiform, although in *Caiconectes* it is spiniform. Leg 3 basis may have the outer border seta present or absent, setiform

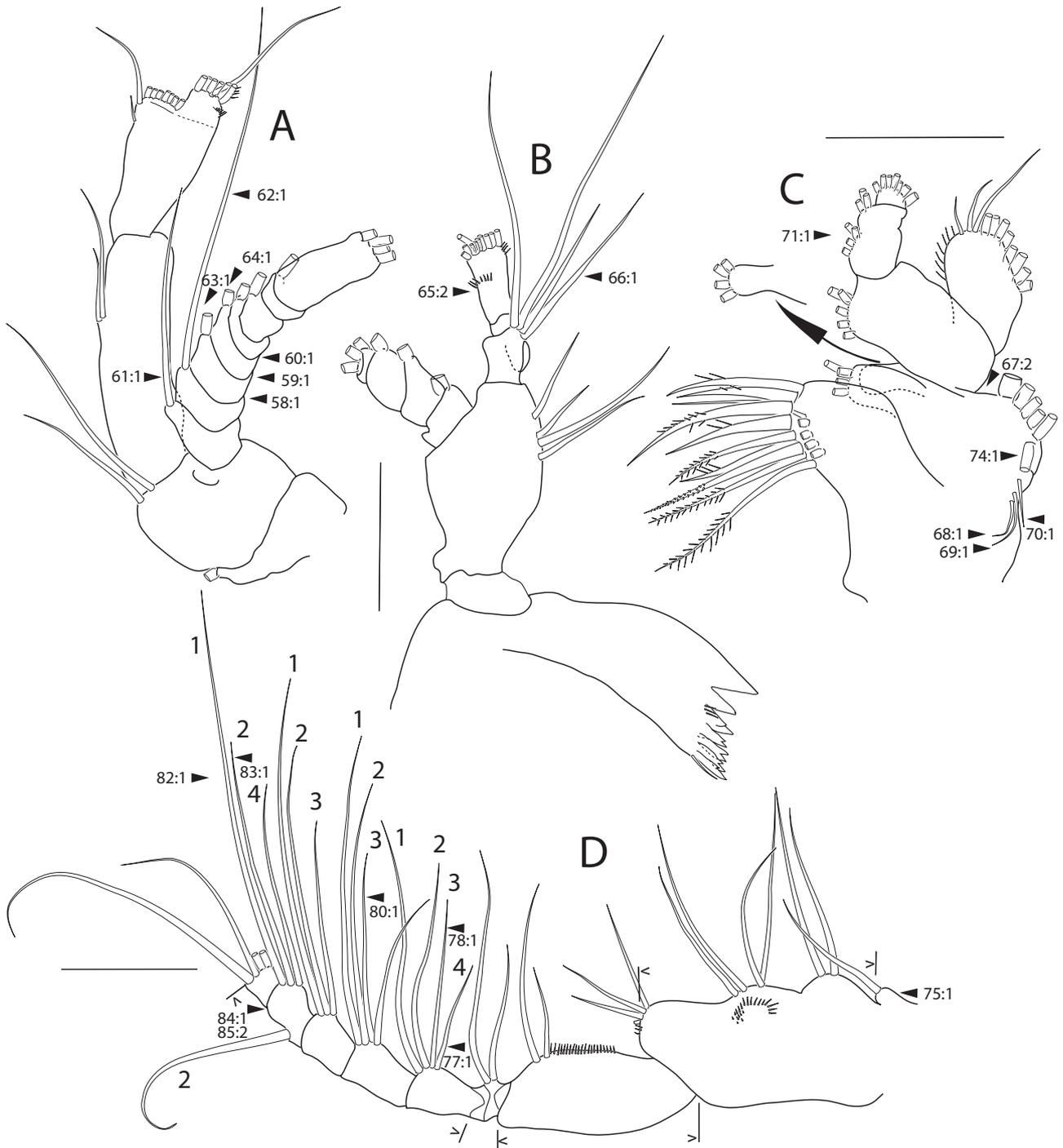


Figure 4. *Pinkertonius ambiguus* gen. et sp. nov. female: A, antenna; B, mandible; C, maxillule; D, maxilliped. Scale bars: 0.1 mm.

or spiniform (characters 96, 97). Leg 3 exopod segment 3 outer edge spine 3 may be absent (character 98), and if it is present, it is usually setiform, although in *Caiconectes* it is spiniform. The outer distal corners of endopod segments 1 and 2 may have one point, may be bifid, or trifold (characters 99, 100).

Leg 4 basis outer border seta may be present or absent (character 101), and exopod segment 2 outer spine 3 may also be absent (character 102). The outer proximal border of exopod segments 2 and 3 of legs 2–4 may bear a knob-like protrusion (character 103).

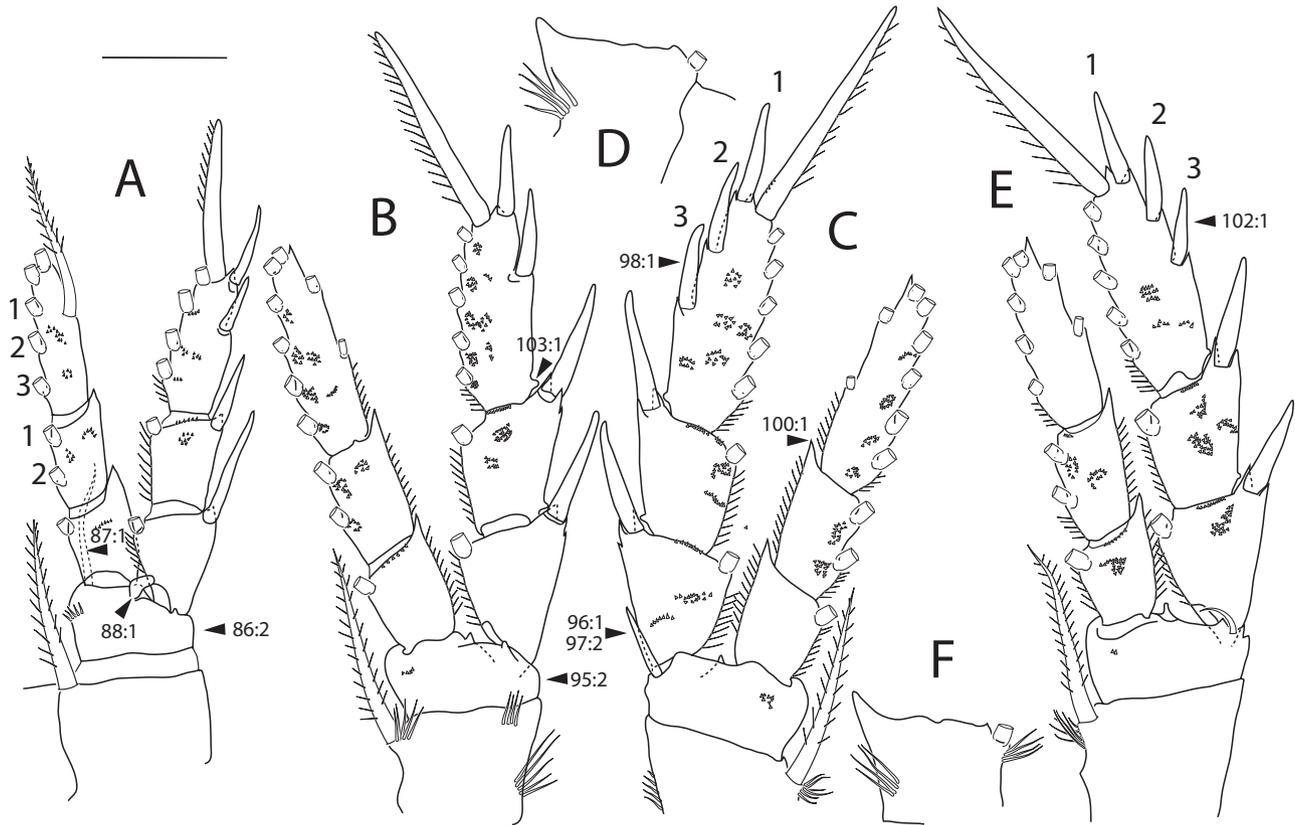


Figure 5. *Pinkertonius ambiguus* gen. et sp. nov. female: A, leg 1, posterior view; B, leg 2, posterior view; C, leg 3, posterior view; D, leg 3, coxa, anterior view; E, leg 4, posterior view; F, leg 4 coxa, anterior surface. Scale bars: 0.1 mm for all figures.

Female leg 5 (Fig. 2): The genus *Caiconectes* appears to have many characteristics of the female leg 5 that we envisage could have been derived unchanged from the ancestral calanoid. The basis has an outer edge seta, exopod segment 1 has an inner seta, exopod segment 3 has three outer border spines and four inner border setae, and the outer distal corners of endopod segments 1 and 2 are trifid and bifid, respectively. The coxa may have an inner seta (character 104; *Badijella*, *Boholina*, and the new genus), which is a relatively rare plesiomorphy in calanoids. The basis outer border seta may be absent (character 105; *Hondurella*, *Oinella*), endopod segments 2 and 3 may be fused (character 106; *Boholina*, *Hondurella*, *Ridgwayia*, *Stygoridgwayia*), and exopod segment I inner seta may be absent (character 107; *Azygonectes*, *Boholina*, *Epacteriscus*, *Exumellina*, *Gloinella*, *Hondurella*, *Iboyella*, *Normancavia*, the new genus, *Ridgwayia*, *Stygoridgwayia*). A number of genera have a striking modification of the articulation between exopod segments 2 and 3. This articulation is analysed here as being made up of two characters. The first character is the extent of the outer distal border of exopod segment 2 relative to the point of insertion of the

medioproximal seta – this may extend well beyond the proximal seta, as in *Ridgwayia*, or fall short of it, as in *Bunderia* (character 108). The second character is the width of the proximal articulating part of exopod segments 2 and 3 – this may be very narrow, as in *Ridgwayia* and a number of other taxa (i.e. less than half the width of exopod segment 3 at the level of the proximoinner seta), or wider (more than half the width of exopod segment 3 at the level of the proximoinner seta), as in the new genus and species described here (character 109). On exopod segment 3 there may be a reduction in the number of outer border spines (character 110) and inner border setae (characters 111, 112) (*Boholina*, *Badijella*, *Placocalanus*, *Stygoridgwayia*, *Robpalmeria*, *Normancavia*, *Brattstromia*, *Exumella*, *Stargatia*, *Exumellina*). It is noted that along with the reduction in inner setae there is a tendency for the terminal spine to migrate partly onto the distoinner border of exopod segment 3, a character not used here.

Male leg 5 (Fig. 6): The genus *Caiconectes* appears to have many characteristics of the male leg 5 that we envisage could have been unchanged from the

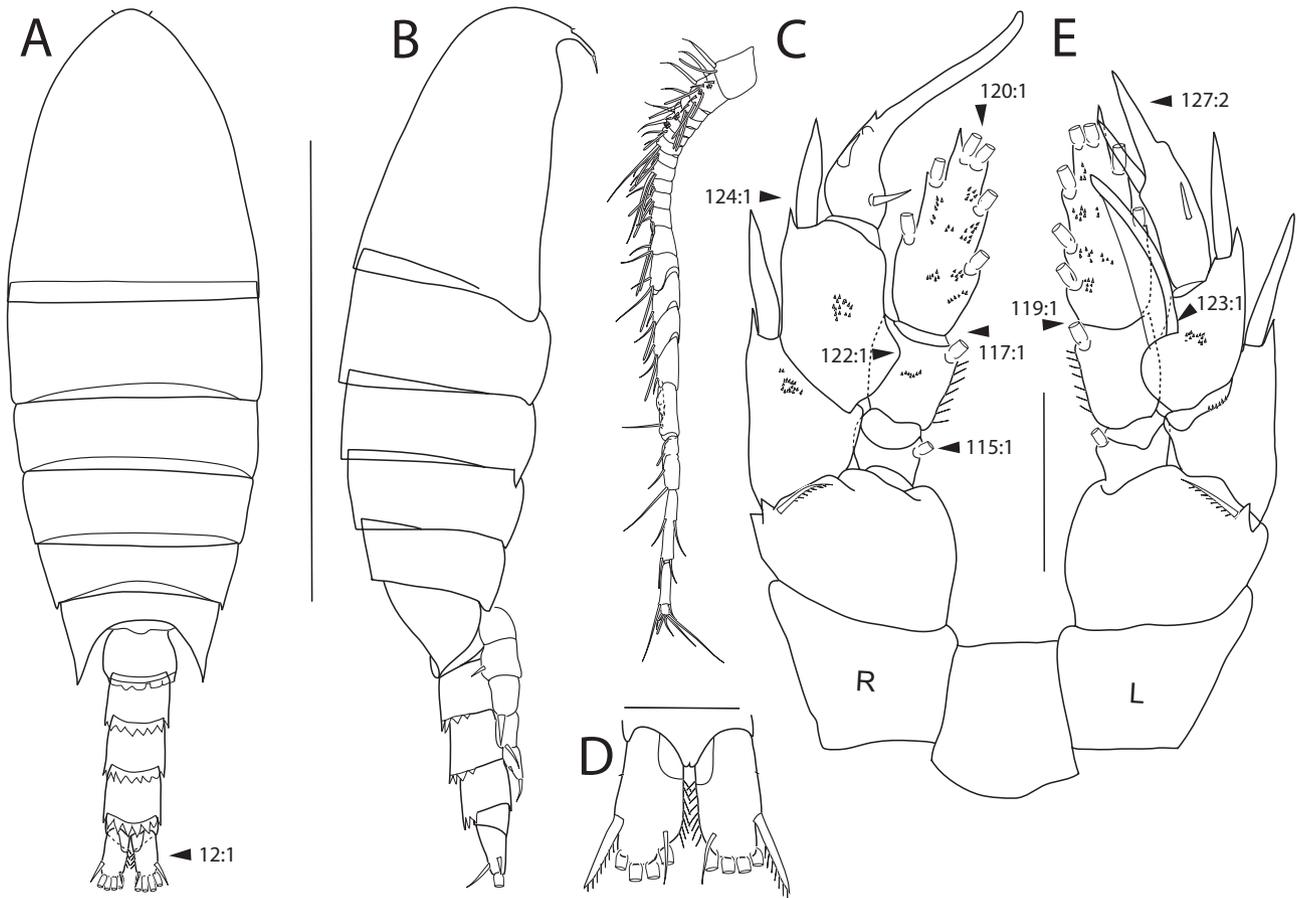


Figure 6. *Pinkertonius ambiguus* gen. et sp. nov. male: A, dorsal view; B, lateral view; C, right antennule at same scale as A and B; D, caudal rami, dorsal view; leg 5 posterior view (note the deformed left endopod segment 3 that has an extra inner seta – this seta is absent in another specimen). Scale bars: A–C, 1.0 mm; D, E, 0.1 mm.

ancestral calanoid. The fifth leg is only slightly asymmetrical, with the left exopod slightly longer than the right. The basis has an outer edge seta, and the inner border on both sides lacks any projection. Endopod segment 1 has an inner seta, and endopod segment 3 has 2, 2, 2 setae. Exopod segment 3 on both legs is simply built with a wide proximal part and a long slender terminal part fused to its segment, and with a pair of articulated spines at the base of the slender part. Numerous deviations from this pattern are found that include shortening and straightening of the terminal fused spine, strong modifications of the left endopod and exopod, or on both sides, and variously fused and reduced endopod segments. We have defined the following characters that we consider encapsulate the extent of the modifications of the basic limb.

The basis may have an inner projection on one or both sides or lacking any projection (characters 113, 114). Endopod segment I may or may not have an inner seta (character 115), and the endopod segments may be fused or separate on the right, or the left, or on

both sides (characters 116–119). Endopod segment 3 may have a full complement of 2, 2, 2 setae, or a reduced number on both sides (character 120). Where the number of setae is reduced, endopod segment 3 may have 2, 1, or 0 setae (character 121). The right and left exopod segment 2 may have an inner projection (characters 122, 123). The right and left exopod segments 2 and 3 may be fused or separate (characters 124, 125). The terminal spine on the right and left exopod segment 3 may be articulated or fused to its segment (characters 126, 127).

MORPHOLOGY-BASED ANALYTICAL METHODS

A database of 32 taxa including the out-group (Table 1) and 127 morphological characters (Appendix S3) was initially created using DELTA (Dallwitz, Paine & Zurcher, 1993), with output as a nexus file (Appendices S6, S7). The majority of characters are binary, although 12 characters have three states. Inapplicable characters were coded '?'. Characters were unordered

and equally weighted. As characters are unordered, the scores given for each state (0, 1, 2, 3, etc.) imply nothing about polarity.

Phylogenetic analysis under maximum parsimony was conducted in PAUP 4.0b10 (Swofford, 2002; Appendix S4). Analyses were conducted using the heuristic search (1000 replicates with random input order; branch swapping with tree bisection and reconnection). Strict consensus and majority-rule consensus trees were computed. Jackknife support using unweighted data was determined in PAUP (with 30% character deletion; 500 pseudoreplicates). The data set was finally analysed under a single round of successive weighting using the rescaled consistency index (Farris, 1969). Character state distribution was studied in MacClade 4.0 (Maddison & Maddison, 2000).

RESULTS

SYSTEMATIC DESCRIPTION

ORDER COPEPODA

CLASS CALANOIDA

FAMILY PSEUDOCYCLOPIDAE

PINKERTONIUS AMBIGUUS GEN. ET SP. NOV.

Material examined (Table 2): Type specimens deposited in the collection of the National Institute of Water and Atmospheric Research, New Zealand. Holotype: female, Stn 59, top net, NIWA 86594 (three slides). Paratypes: one male, Stn 59, top net, NIWA 86595 (three slides). Paratype lots in 4% formalin: one female, Stn 59, top net, NIWA 86596 (one vial, one slide); one female, Stn 59, bottom net, NIWA 86597 (one vial, one slide); two females, Stn 121, top net, NIWA 86600 (one vial); one female, one male, Stn 121, bottom net, NIWA 86599 (one vial); one female, one male, Stn 59, bottom net,

NIWA 86598 in 95% ethanol (one vial); one female, Stn 59, top net, NIWA 85993, NHMUK 2013.25.

Etymology: The generic name *Pinkertonius* (gender masculine) is derived from the name of Dr Matt Pinkerton who is project leader for the NIWA research programme 'Marine Food Web Dynamics' on the Chatham Rise, where these specimens were found. The specific name *ambiguus* derives from the Latin, referring to the ambiguous nature of the morphology of this species in not exactly fitting into any of the previously described families.

Genetic description

The genetic description, from one specimen, is based on the nuclear large (28S) and small (18S) subunits ribosomal RNA and the mitochondrial genes for the proteins cytochrome *c* oxidase subunit I (*COI*) and cytochrome *b* (*cyt b*). These sequences were deposited in GenBank accession nos: KF753813–KF753816, respectively, which correspond to the catalogued specimen, Co.449.1.1, held in the Marine Science Department, University of Connecticut.

Morphological description

Female: Total length 1.8–2.0 mm, urosome 28% of total length (Fig. 2A, B). Prosome ovoid with head (cephalosome) and pedigerous somite 1 separate; pedigerous somites 4 and 5 separate, posterolateral corners of pedigerous somites 1–4 extend into small triangular projections, pedigerous somite 5 produced into pointed lappets extending more than halfway along genital double somite. Rostrum in form of ventrally directed rounded plate with pair of distal filaments (Fig. 2D). Urosome of four free somites, first three bordered posteriorly by unevenly serrated hyaline fringe,

Table 2. Material examined

Station no.	Latitude	Longitude	Depth	Bottom net	Top net
31	43°32.11'S	174°34.91'E	494 m	–	Nil
32	43°23.65'S	174°10.44'E	568 m	–	Nil
33	43°23.36'S	174°10.50'E	570 m	–	Nil
59	44°41.95'S	173°41.94'E	904 m	1 female 1.8 mm	1 female 2.0 mm 1 male 1.88 mm 1 female 2.0 mm 1 male 1 female (NHM)
68	44°13.26'S	178°52.92'E	1007 m	Nil	Nil
89	43°49.55'S	178°33.14'E	463 m	Nil	Nil
105	42°53.84'S	177°50.09'E	420 m	–	Nil
121	42°43.50'S	178°05.09'E	976 m	1 female 1.9 mm 1 male 1.8 mm	2 females 1.9, 2.0 mm

–, samples not examined.

Table 3. Length of antennule segments (μm)

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
67 160	21	20	25	25	25	27	27	29	29	29	29	36	58
XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII– XXVIII	
72	82	84	86	86	85	78	63	53	57	73	86	23	

Measurements taken along posterior border of each segment but two (posterior (shortest) and anterior) measurements taken of ancestral segment I.

largely intact ventrally. Genital double somite with slight anterior swelling in dorsal view; in lateral view swollen ventroanteriorly; in ventral view genital field asymmetrical, gonopores slightly unequally developed, being larger on left, genital operculum skewed to left with hinge aligned at about 45° to anterior–posterior axis of somite, so that right gonopore not completely covered, right side of genital field bordered by ridge aligned anterior–posteriorly, no such flange on left, copulatory pore and seminal receptacle not obvious, although sac on left apparently linked to left gonopore (Fig. 2E). Caudal rami slightly asymmetrical, longer on right, with seven setae each (Fig. 2F, G) more or less symmetrically arranged on each side. Seta I vestigial, seta II spiniform, seta V longest, about 1 mm long, seta IV next longest followed by setae VI and III, seta VII small and spiniform and inserted on dorsoinner distal corner, left caudal ramus inner border lined with fine setules; on right ramus row of long setules arranged obliquely on anterior part of ventral surface.

Antennule (Table 3): Twenty-seven segmented, extending to posterior border of pedigerous somite 5 (Fig. 2C; Fig. 3A, B, C).

One aesthetasc present on each of segments I, III, VII, XI, XIV, XVI, XVIII, XXI, XXV, and XXVIII; small cuticular thickenings found on segments II–X, XII, XIII, XV, and XVII; one seta on eight proximal segments, wider than other setae, and attenuated distally into curved narrow tip. Setal formula: I, 1s, 1a; II, 2s; III, 2s, 1a; IV, 2s; V, 2s; VI, 2s; VII, 2s, 1a; VIII–X, 2s; XI, 2s, 1a; XII to XIII, 2s; XIV, 2s, 1a; XV, 2s; XVI, 2s, 1a; XVII, 2s; XVIII, 2s, 1a; XIX to XX, 2s; XXI, 2s, 1a; XXII to XXIII, 1s; XXIV, 1 + 1s; XXV, 1 + 1s, 1a; XXVI, 1 + 1s; XXVII–XXVIII, 5s, 1a.

Antenna: Coxa and basis separate, coxa with one seta, basis with two setae (Fig. 4A). Endopod two-segmented, with traces of fusion between segments 2 and 3, and between segments 3 and 4; segment 1 with two inner setae, segment 2 with nine plus seven terminal setae

and outer transverse row of spinules marking boundary between putative endopod segments 3 and 4. Exopod shorter than endopod, eight-segmented, ancestral segments VIII and IX fused, segments I–VII each with one well-developed seta, compound distal segment VIII–IX with three terminal setae and one inner subterminal seta, outermost seta on terminal endopod segment lined proximally with small spinules.

Mandible: Gnathobase with seven marginal teeth, ventralmost largest, five dorsal teeth bicuspid, small spinulated seta inserted dorsally (Fig. 4B). Basis with four apparently naked setae; endopod two-segmented, segment 1 with inner lobe and four setae, segment 2 with ten terminal setae, distoinner border with short row of spinules, transverse row of spinules at about midlength. Exopod five-segmented, with 1, 1, 1, 1, 2 setae.

Maxillule: Praecoxal arthrite with 14 spines and setae, including four on posterior surface and one on dorsal surface (Fig. 4C); coxal endite with four setae; basal endites 1 and 2 with four and five setae, respectively; endopod segments 1 and 2 fused, segments 2 and 3 separate, with four, three, and seven setae, respectively; exopod with 11 setae, of which three terminal setae short and bordered by fine setules along inner border; basal exite without seta; coxal epipodite with nine setae, of which three proximal setae short.

Maxilla: Praecoxa, coxa, and basis clearly separated, endites 1–4 with seven (one very short), three, three, and three setae, respectively (Fig. 3D, E); basal endite with three setae, one of them stout and spiniform; inner setae on endites 2–5 lined with long spinules; endopod segment 1 endite with three setae, segments 2–4 with two, two, and three setae, respectively.

Maxilliped: First syncoxal endite with one seta (Fig. 4D); endites 2 and 3 with two and four spinulose setae, respectively, crescent-shaped row of fine spinules at base of endite 3 on inner surface; endite 4 with three setae

Table 4. Setal formulae for swimming legs: Roman numerals indicate spines; Arabic numerals indicate setae; outer border setation listed first in each segment, with group separated by ;

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	0-1	I-1; I-1; II, I, 4	0-1; 0-2; 1, 2, 3
Leg 2	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 3	0-1	I-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 4
Leg 4	0-1	I-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 3
Leg 5 female	0-0	I-0	I-0; I-1; III, I, 4	0-1; 0-1; 2, 2, 2
Leg 5 male left	0-0	I-0	I-0; I-1; I, 0, I	0-1; 0-1; 2, 2, 2*
Right	0-0	I-0	I-0; I-0; I, 0, I	0-0; 0-1; 2, 2, 2

*Illustrated specimen with extra seta, absent in another specimen.

and small peg-like structure, and a few small spinules. Basis with two setulose setae and proximal border lined by spinules. Endopod well-developed, longer than basis, endopod segment 1 apparently separate from basis, endopod segments 1–6 with two, four, four, three, three plus one, and four spinulose setae, respectively, outer seta of segment 6 wider and longer than adjacent seta, and terminally inserted.

Swimming legs (Table 4): Legs 1–5 biramous, all rami three-segmented (Fig. 5).

Leg 1 basis with distal lobe on posterior surface situated between exopod and endopod (this lobe is visible in lateral view in the whole animal, as it projects posteriorly); ornamented with transverse row of inner setules; inner distal seta slightly curved. Coxae of legs 2–5 decorated with patches of long spinules on posterior surface; basis of legs 2–4 with blunt tooth on anterior surface between endopod and exopod; outer seta on basis of leg 3 spiniform; outer proximal border of exopod segments 2 and 3 of legs 2–4 each with small knob-like projection; exopods of legs 2–5 with heavily built outer spines bordered by very small blunt denticles, terminal exopod spines also heavily built, and with outer edge lined by tiny small blunt teeth. Surfaces of both rami of legs 1–5 ornamented with patches of very small spinules, most dense on posterior surfaces.

Leg 5 articulation of exopod segment 3 with segment 2, oblique and reminiscent of species of *Ridgewayia* (Fig. 1I, H); proximal end of segment 3 narrowing, distance between two pivot points (Fig. 2H) ensures region of articulation is as wide as exopod segment 3 at level of proximal inner seta of exopod segment 3. Outer distal extension of exopod segment 2 not reaching origin of proximal outer spine of exopod segment 3.

Male: Total length 1.80–1.88 mm, urosome 28% of total length (Fig. 6A, B). Prosome ovoid with head and pedigerous somite 1 separate; pedigerous somites 4 and 5 separate, posterolateral corners pedigerous somites 1–4 extended into small triangular projections, pedigerous

somite 5 extending into pointed lappets reaching beyond posterior border of genital somite. Rostrum in form of ventrally-directed rounded plate, with pair of distal filaments. Urosome of five free somites, anterior four somites bordered posteriorly by unevenly serrated hyaline fringe. Caudal rami symmetrical, with seven setae each: seta I vestigial, seta II spiniform, seta V longest, seta IV next longest followed by setae VI and III, seta VII small and spiniform and inserted on dorsoinner distal corner, inner borders of caudal rami lined with fine setules (Fig. 6D).

Antennule: Twenty-six segmented on left, 25-segmented on right, extending just beyond posterior border of fourth pedigerous somite; right antennule geniculate between ancestral segments XX and XXI, segments XV–XIX enlarged (Fig. 7). One seta on proximal segment on each side wider than other setae and attenuated distally into curved narrow tip. Left antennule with following setation: I, 1 s, 1a; II–III, 3s, 5a (two on segment II, three on segment III); IV, 2s, 1a; V, 2s, 2a; VI, 2s, 1a; VII, 2s, 2a; VIII to XVIII, 2s, 1a; XIX to XX, 2s; XXI, 2s, 1a; XXII to XXIII, 1s; XXIV, 1 + 1s; XXV, 1 + 1s, 1a; XXVI, 1 + 1s; XXVII–XXVIII, 5s, 1a. Right antennule (Fig. 8) with following setation: I, 1s, 1a; II–IV, 6s, 5a (one on segment II, three on segment III, one on segment IV); V, 2s, 2a; VI, 2s, 1a; VII, 2s, 2a; VIII to XVIII, 2s, 1a; XIX to XX, 2s (on segment XIX distal seta fused spatulate distal extension, segment XX with anterior proximal ridge, proximal seta modified as fused spine-like element and one seta); XXI–XXIII, two fused spine-like elements, 1a, 2s; XXIV–XXV, 2 + 2s (without fused distal process; see Appendix S5), 1a; XXVI, 1 + 1s; XXVII–XXVIII, 5s, 1a.

Antenna, mandible, maxillule, maxilla, maxilliped, and swimming legs 1–4 identical to those of female.

Leg 5: Leg 5 biramous on both sides, with both rami three-segmented, asymmetrical (Fig. 6E); exopod slightly longer on right. Right exopod segment 2 with triangular inner attenuation, segment 3 in form of claw

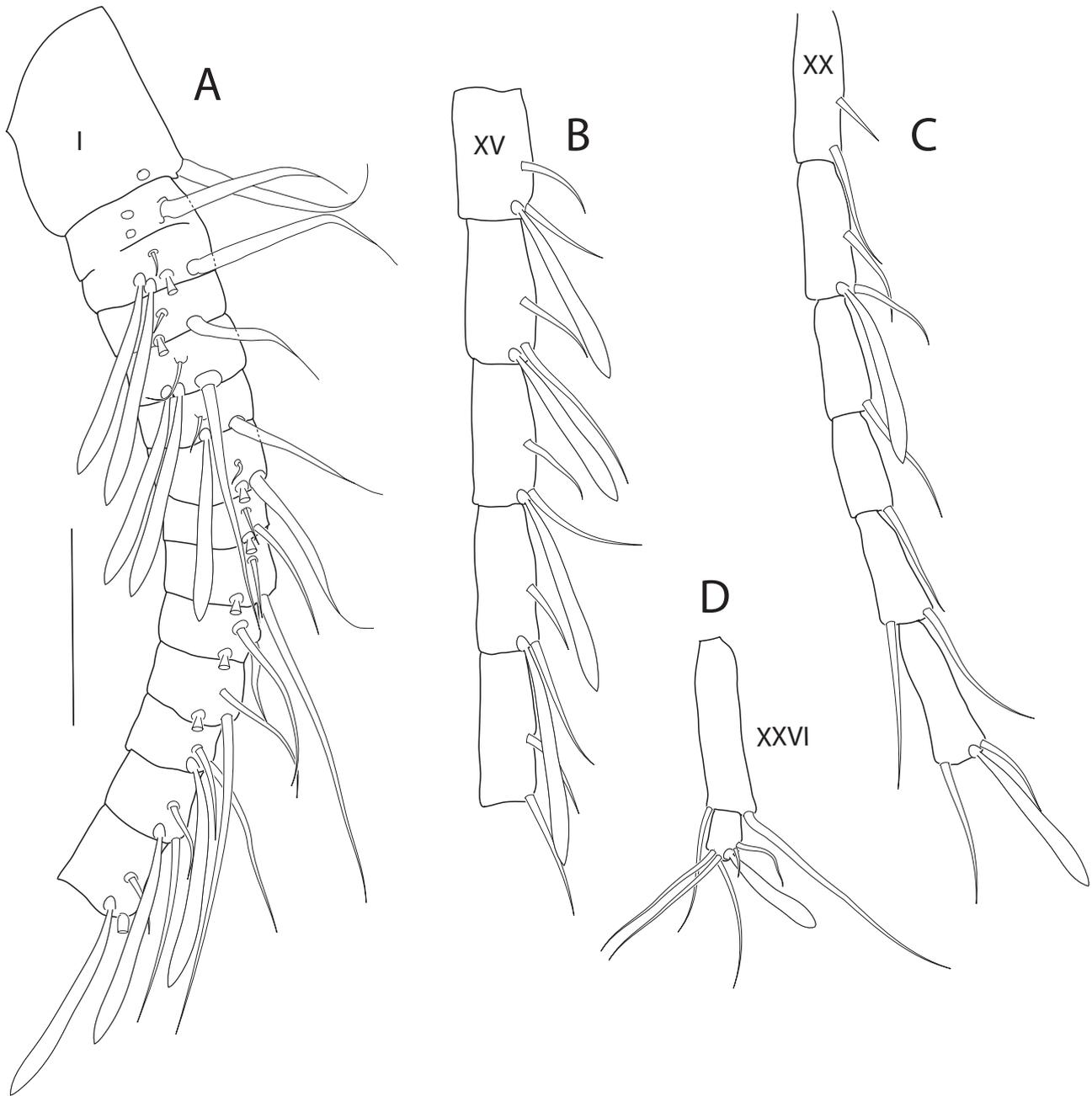


Figure 7. *Pinkertonius ambiguus* gen. et sp. nov. male, left antennule ancestral segments: A, segments I–XIV; B, segments XV–XIX; D, segments XX–XXV; E, segments XXVI–XXVIII. Scale bar: 0.1 mm.

with terminal spine, rounded distally, fused to segment, with two articulated spines (one medioproximal, other on outer border more distally inserted), with additional outer fused spinule and pore opening. Left leg exopod segment 2 with inner border swollen, bearing seta modified into scalpel-shaped element thickened along its outer border; exopod segment 3 simple, about twice as long as wide, short terminal spine fused to segment at level adjacent to lateral pore opening, one articu-

lated inner spine and outer articulated spine more proximally inserted on posterior surface. Posterior surfaces of exopods and endopods ornamented with scattered patches of very small spinules.

Ecological notes: The samples that contained *P. ambiguus* sp. nov. came from the hyperbenthic zone above sediments with high pelagic calcium carbonate content on the flanks of the Chatham Rise,

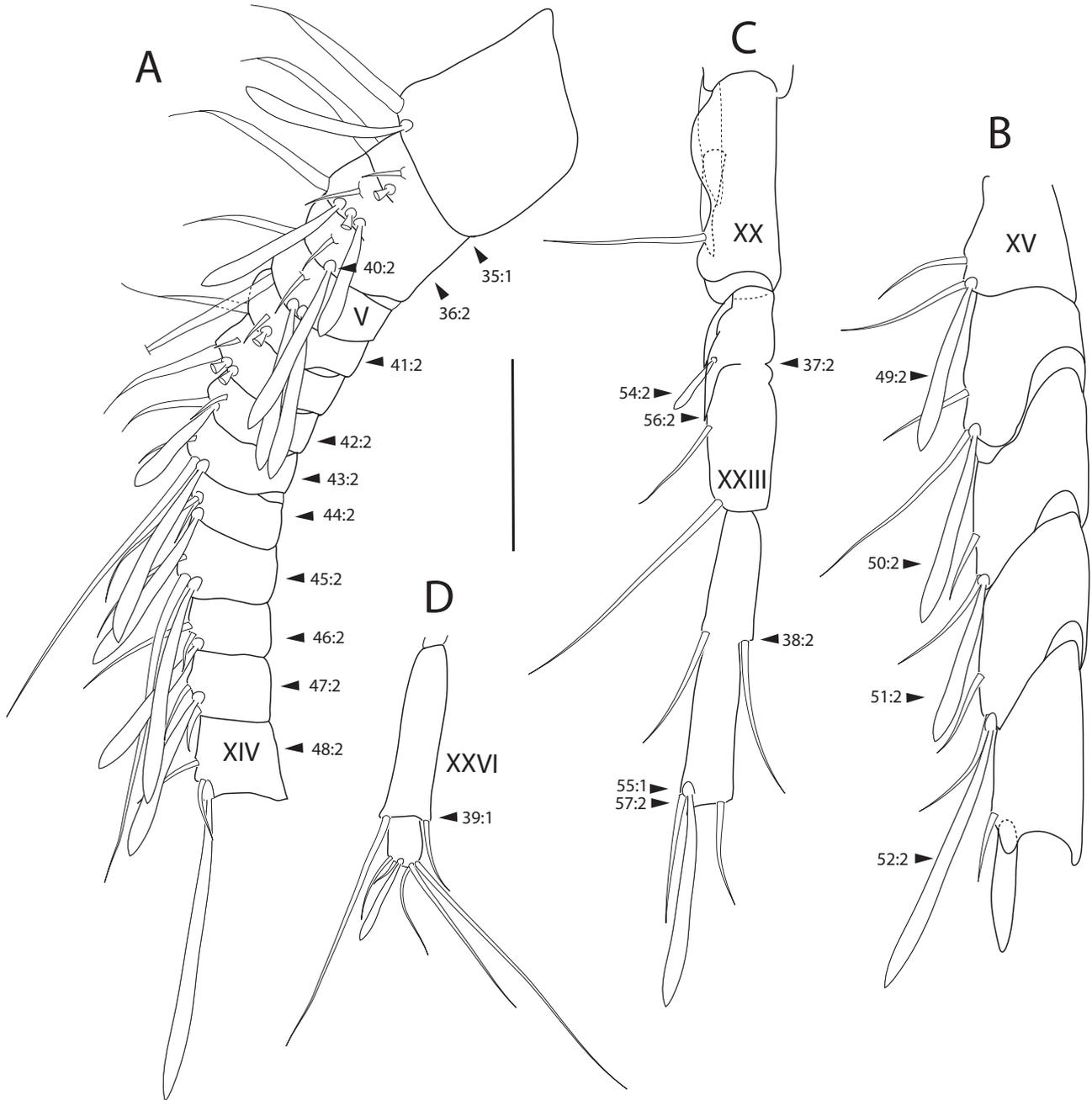


Figure 8. *Pinkertonius ambiguus* gen. et sp. nov. male, right antennule ancestral segments: A, segments I–XIV; B, segments XV–XIX; C, segments XX–XXV; D, segments XXVI–XXVIII. Scale bar: 0.1 mm.

but were absent from shallower regions on the Mernoo Saddle or on the scoured slopes of the rise (Carter, Neil & McCave, 2000). The Chatham Rise traps the Subtropical Front east of New Zealand, and is the location of year-round elevated primary productivity (Bradford-Grieve *et al.*, 1997).

Remarks: *Pinkertonius ambiguus* gen. et sp. nov. retains a number of plesiomorphic features: the female genital

double somite has a genital operculum; the caudal rami have a vestigial seta I; the female antennule has all of ancestral segments I–XXVII separated; all swimming legs have both rami on each side three-segmented, male leg 5 of relatively simple construction; the proximal seven segments of the antenna exopod are separate; the endopod of the mandibular palp is two-segmented, well-developed, and bears ten terminal setae on segment 2; the maxillule has nine setae on the coxal epipodite,

four setae on the posterior surface of the praecoxal arthrite, and 11 exopod setae; on the maxilla, endopod segment 1 and its endite are separated from the basis; the maxilliped endopod segment 1 is separate from the basis, and endopod segment 5 has an outer seta.

Superficially, *P. ambiguus* sp. nov. resembles *Miheptneria abyssalis*, especially in general body shape, but differs from this species and all other genera currently in the Epacteriscidae, Pseudocyclopidae, Ridgewayiidae, and Boholinidae in having several autapomorphies: in the female the longer caudal ramus is on the right and there is an oblique row of long setules on the ventral surface, which we consider to be homologous with the inner row of setules on the left ramus; the segmental distribution of aesthetascs differs greatly between the sexes (female ancestral segments II, IV–VI, VIII–X, XII–XIII, XV, and XVII are without aesthetascs, whereas the male has aesthetascs on all of segments I to XVIII). *Pinkertonius ambiguus* gen. et sp. nov. is unique among the above group of families in having the apomorphic condition of multiple aesthetascs on some segments in the male (three on ancestral segment III and two each on segments V and VII on both sides) and one aesthetasc on right segment II and two aesthetascs on left segment II. The proximal outer borders of exopod segments 2 and 3 of legs 2–4 each have a knob-like projection.

The following analyses allow us to place *P. ambiguus* sp. nov. in a systematic hierarchy.

MORPHOLOGY-BASED CLADISTIC ANALYSIS

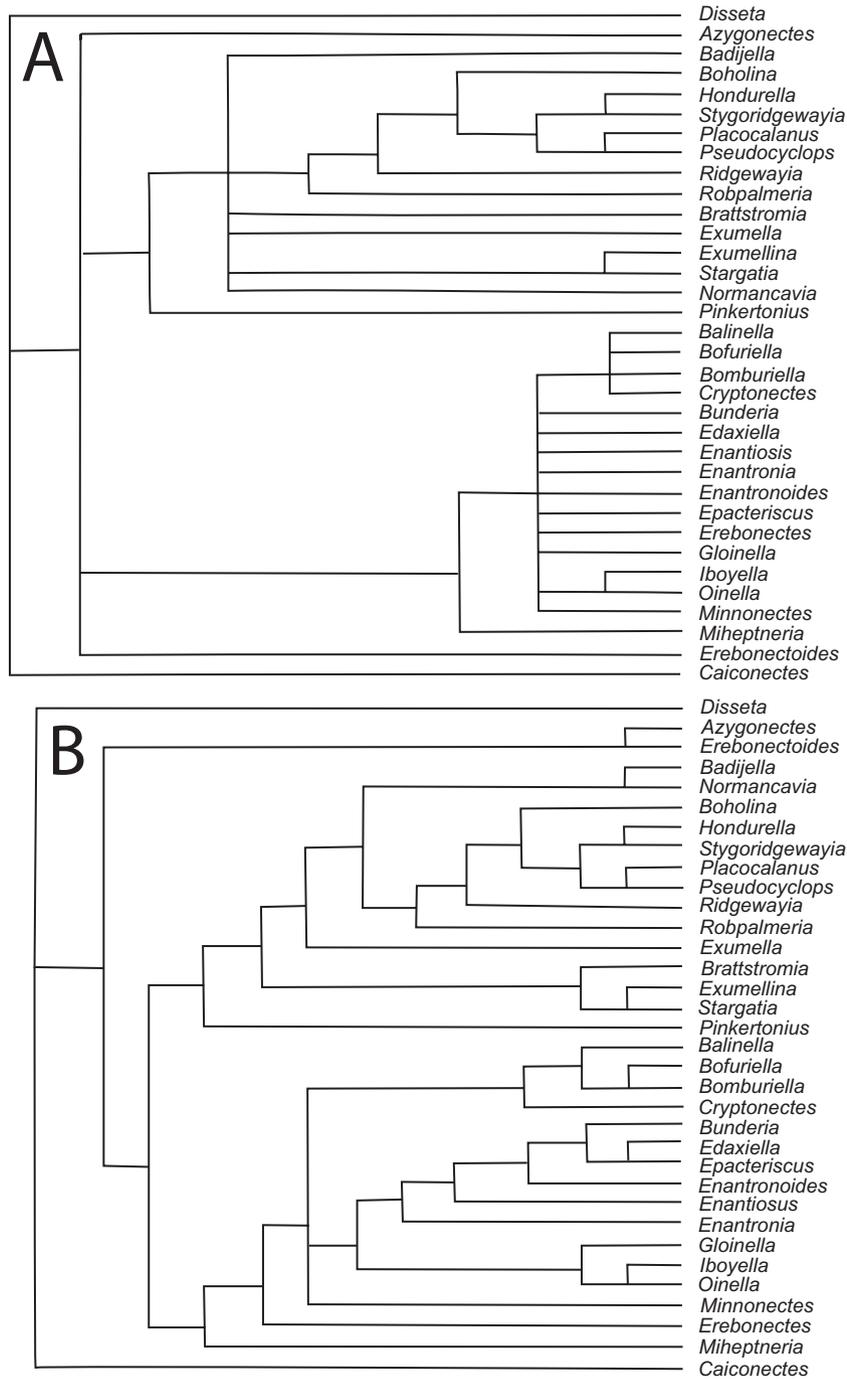
The heuristic search retrieved 39 most parsimonious trees of length 475, consistency index 0.29, and retention index 0.56. The strict and 50% majority-rule consensus trees differ (Fig. 9). Although both consensus trees have two major groups of genera, the strict consensus tree contains many unresolved relationships, whereas in the 50% majority rule tree most relationships are resolved. In the 50% majority rule tree there are three main clades: one containing *Azygonectes* and *Erebnectoides*, one containing mainly ridgewayiids, and another containing mainly epacteriscids, with *Caiconectes* being sister to all other clades. Only one monophyletic clade is retrieved with moderate jackknife support (81%), and is composed of Ridgewayiidae, *Boholina*, and *Pseudocyclops* (Fig. 10). Within this clade there is one highly supported subclade comprising *Boholina*, *Hondurella*, *Stygoridgewayia*, *Placocalanus*, and *Pseudocyclops* (99%). The sister clade to the large ridgewayiid clade, although monophyletic, is poorly supported (< 50%) in the jackknife analysis, although a subclade comprising most epacteriscids apart from *Miheptneria* is moderately supported (76%). Further subclades, one comprising *Iboyella* and *Oinella*, are highly supported (97%), and another more weakly sup-

ported clade comprises *Bofuriella* and *Bomburiella* (71%). The small clade containing *Azygonectes* and *Erebnectoides* is poorly supported (< 50%), but forms a sister clade to the two major clades (Fig. 10). Finally, *Caiconectes* is recovered as the sister to all three clades.

As indicated by the rescaled consistency index (*RC*) for each character (Appendix S3), 79 out of the 127 characters made a small contribution (*RC* < 0.20) to the resulting topologies. Very few characters with *RC* > 0.20 have been recognized in the family classification: characters 5, 8, 9, 18, 22, 24, 26, 27–30, 33, 39, 40, 42–49, 51, 63, 65, 68–72, 99, 106, and 117–121 (Appendix S3). Of particular significance are: the state of seta II on the caudal ramus (spiniform, setiform, or absent); the presence or absence of an aesthetasc on female ancestral segments IV, IX, XI, XIII, XIV–XVIII, and XXI; absence of an expressed arthrodistal membrane between male right antennule ancestral segments XXVI and XXVII; presence and/or number of aesthetascs on male right antennule ancestral segments IV, VIII, IX–XV, XVII; the presence or absence of a seta on antenna exopod segment III; the form of mandibular endopod segment 2 (short or elongate and paddle-shaped); the presence or absence of setae 9–7 on the maxillule coxal epipodite; the form of endopod segments (short or elongate and paddle-shaped); the form of endite 5 on the maxilla (elongate lobe or distinct lobe absent); the nature of the outer corner of leg 3 endopod segment 1 (with between one and three points); absence of an arthrodistal membrane between endopod segments of leg 5 in both sexes and their numbers of setae. In contrast, characters relating to the setation of the swimming legs (characters 86, 87, 89, 91–94, 96–98, 100–106, and 107–112) have low *RC*, as do some specialized characters (e.g. characters 88, 90) relating to leg 1, which are homoplasious in this analysis.

One round of successive weighting yielded one most parsimonious tree (Fig. 11). The strict and 50% majority-rule consensus trees are identical. The main topological differences, after one round of successive weighting, from the 50% majority-rule tree using unweighted data, are: *Badijella* becomes sister to all genera in clade 11, *Brattstromia* transfers to clade 9, *Cryptonectes* becomes sister to *Bomburiella* in clade 20, and *Gloinella* transfers to clade 18, and is no longer sister to *Iboyella* and *Oinella*, which are in clade 17 (c.f. Figs 9 and 10).

In the weighted analysis, which reduces the influence of the numerous homoplasious characters, *Caiconectes* is sister to all other taxa in the analysis (Fig. 11). Clade 1 (with little jackknife support) is united by three characters that are homoplasious within the clade: female antennule ancestral segment XX aesthetasc (character 32: absent); male geniculate antennule ancestral segment XIX aesthetasc (character 53: present); leg 2 basis outer seta (character 95: absent). Clade 2 has little jackknife support and is united by two



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Figure 9. Strict consensus (A) and 50% majority-rule consensus (B) of 39 trees, length 475, consistency index (CI) = 0.29, retention index (RI) = 0.56. The out-group is *Disseta palumbii* Giesbrecht, 1889. For genus exemplars see Table 1.

characters that are homoplasious outside the clade: female ancestral segments X and XI (character 16: fused); leg 3 exopod segment 3 outer edge spine 3 (character 98: absent). Clade 3 is united by two characters that are homoplasious within the tree: female caudal rami (character 9: of equal length); male caudal rami (character 12: symmetrical).

Clade 3 divides into two sister taxa, clade 4 that has moderately strong jackknife support (81%) and clade 5 that has little jackknife support. Clade 4 is united by two characters that are homoplasious outside the clade: caudal rami seta II (character 8: spiniform); the aesthetasc on female antennular ancestral segment IV (character 18: absent). Clade 4 is also united by three

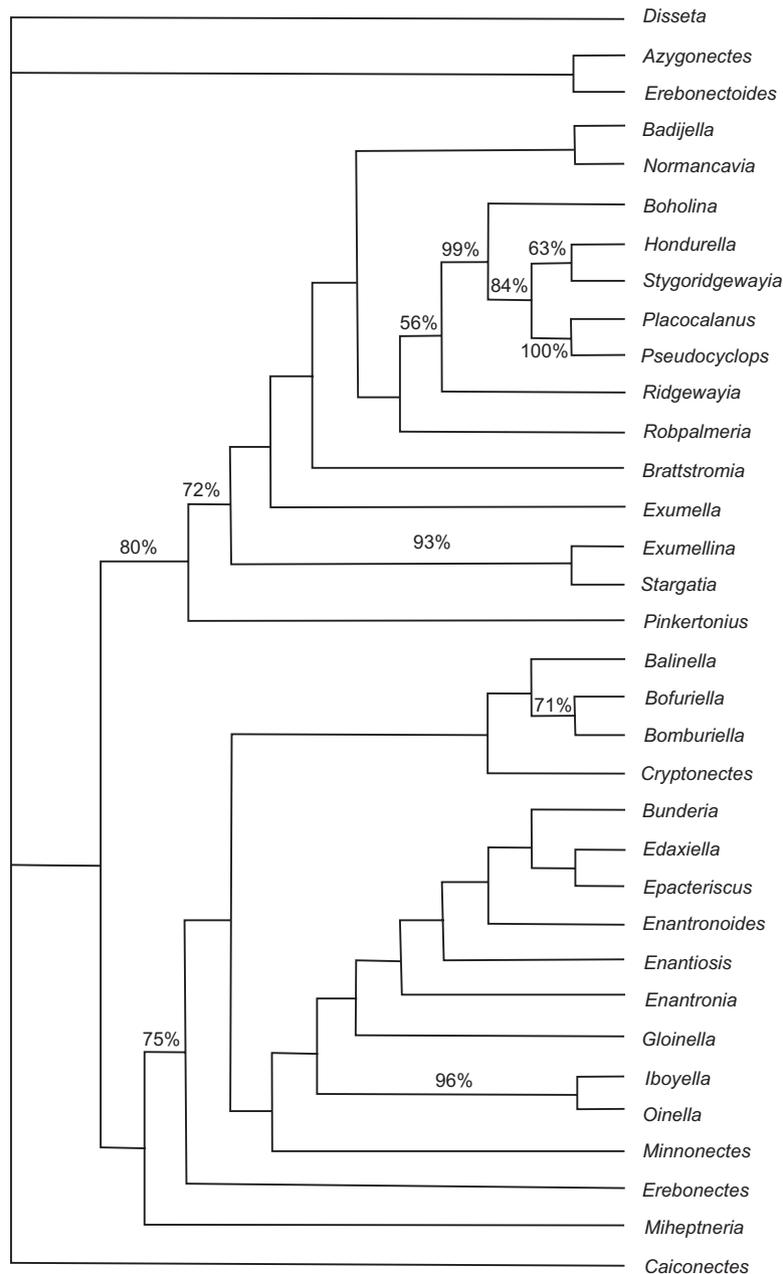


Figure 10. Jackknife 50% majority-rule consensus tree showing jackknife support > 50%. The out-group is *Disseta palumbii* Giesbrecht, 1889. For genus exemplars see Table 1.

characters that are homoplasious outside and within the clade: maxilliped endopod segments 2–6 (character 76: longer than length of coxa); male left leg 5 basis inner projection (character 113: absent); and male right leg 5 exopod segment 2 inner process (character 122: present). Clade 5 is united by one character that is homoplasious outside the clade – seta 9 of the maxillule coxal epipodite (character 67: absent) – and by one character that is homoplasious inside and outside the clade – male right and left leg 5 basis inner projection (character 114: present).

In clade 4 the new genus and species (*Pinkertonius ambiguus* gen. et sp. nov.) is sister to all other taxa in the clade. Clade 6 is united by two characters that are homoplasious outside the clade: the female antennular ancestral segments II and III (character 14: fused), the male geniculate antennule ancestral segment IV aesthetasc (character 40: 0); one character that is homoplasious above in the tree – female leg 5 exopod segment 3 inner seta 4 (character 111: absent); and two characters that are homoplasious inside and outside the clade – male left leg 5 exopod segments 2 and 3

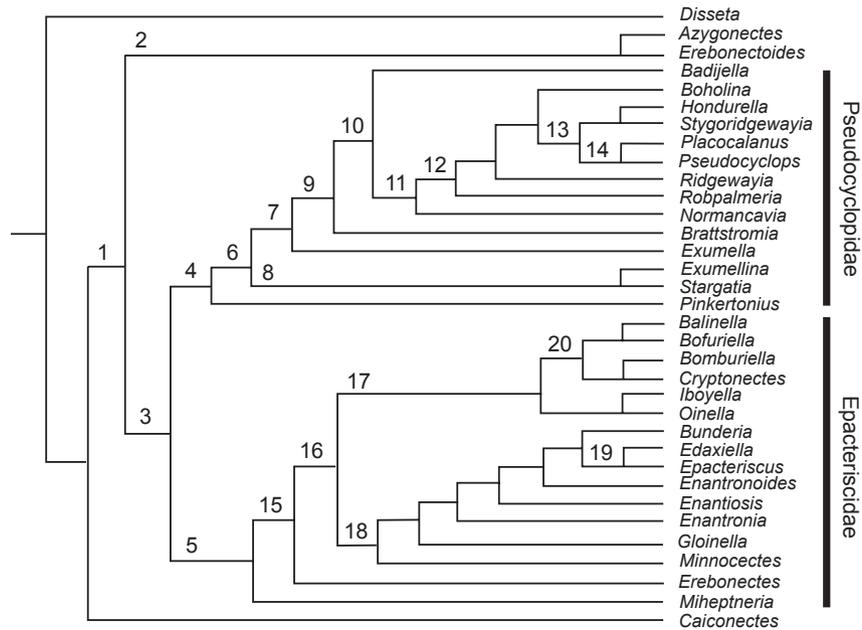


Figure 11. Strict consensus of one tree after one round of successive weighting. Clade numbers above the line. The outgroup is *Disseta palumbii* Giesbrecht, 1889. For genus exemplars see Table 1.

(characters 124 and 125: fused). Clade 8 is unambiguously supported by two unique characters that are uniform above in the clade: mandibular endopod segment 2 (character 65: elongate and paddle-like) and maxillule endopod segments (character 71: elongate and paddle-like). This clade is also united by seven other characters that are homoplasious outside the clade (Table 5). Additional clades above this in the tree are linked by unique character changes (Table 5), and clade 14 is highly supported in the jackknife analysis (100%; Figs. 10, 11).

Within clade 5, *Miheptneria* is sister to the remaining epacteriscids. This clade contains several smaller clades that are united by unique character changes not changing above in the clade: clades 15, 16, and 19 (Figs. 10, 11). Clade 15, which has moderate jackknife support (75%), has unique characters uniting it: maxillule coxal epipodite seta 8 (character 86: absent) and maxilla basal endite 5 (character 72: without a distinct lobe). A unique character change also unites clade 16 – maxillule coxal epipodite seta 7 (character 70: absent) – and clade 19 – rostral windows (character 5: present).

Remarks: The outcome of the phylogenetic analysis presented here is made less certain because of the large number of homoplasious character states. Homoplasious character states result in low jackknife support and unstable and unresolved relationships at the base of the tree. Nevertheless, two major groupings (clades 4 and 5) represent monophyletic taxa that can be as-

signed existing family names. Clade 4 contains the oldest described family, the Pseudocyclopoidea Giesbrecht, 1893, but also includes genera from the Boholinidae and Ridgewayiidae, which are therefore considered to be junior synonyms of Pseudocyclopoidea. Clade 5 contains most of the taxa previously assigned to the Epacteriscidae Fosshagen, 1973, apart from *Azygonectes*, *Erebnectoides*, and *Caiconectes*. Outside the two major monophyletic families sit *Caiconectes* and clade 2 (comprising *Azygonectes* and *Erebnectoides*), which are the most plesiomorphic genera. There are no grounds on which we can assign more than one superfamily name to the group of genera studied, and we recognize a single basal superfamily, the Pseudocyclopoidea, as already noted by Andronov (2007). Therefore, the name Epacteriscoidea becomes a junior synonym of Pseudocyclopoidea Giesbrecht, 1893.

The superfamily Pseudocyclopoidea and families Pseudocyclopoidea, Epacteriscidae, and the genus *Pinkertonius* gen. nov. are diagnosed below.

GENE-BASED PHYLOGENY OF CALANOIDA

Amplification of the mitochondrial genes (*COI* and *cyt b*) was successful for *P. ambiguus* sp. nov., but was not successful for any of the *Pseudocyclops* species; however, the *18S* and *28S* amplification and sequencing was successful in all cases. The topology of the resulting tree (Fig. 12) was similar to that obtained previously (Blanco-Bercial *et al.*, 2011), although with generally lower support values. We note that the relative

Table 5. Unambiguous character state changes for the one most parsimonious tree after one round of successive weighting (the strict consensus and 50% majority rule consensus trees have the same topology; Fig. 11)

Clade 1	32, 1 → 2; 53, 2 → 1; 95, 1 → 2
Clade 2	16, 1 → 2; 98, 1 → 2
Clade 3	9, 2 → 1; 12, 2 → 1
Clade 4	8, 2 → 1; 18, 1 → 2; 76, 2 → 1; 113, 1 → 2; 122, 2 → 1
Clade 5	68, 1 → 2; 114, 2 → 1
Clade 6	14, 1 → 2; 40, 2 → 1; 111, 1 → 2; 124, 1 → 2; 125, 1 → 2
Clade 7	108, 2/3 → 1
Clade 8	4, 1 → 2; 41, 2 → 1; 42, 2 → 1; 65, 1 → 2; 81, 1 → 2; 98, 1 → 2; 102, 1 → 2; 114, 2 → 1; 117, 1 → 2
Clade 9	113, 2 → 1; 118, 1 → 2; 120, 1 → 2
Clade 10	116, 1 → 2; 126, 2 → 1
Clade 11	59, 1 → 2; 119, 1 → 2
Clade 12	113, 1 → 2; 117, 1 → 2; 121, 1 → 3
Clade 13	22, 1 → 2; 23, 1 → 2; 26, 1 → 2; 28, 1 → 2; 43, 2 → 1; 47, 2 → 1; 49, 2 → 1
Clade 14	13, 2 → 1; 24, 1 → 2; 27, 1 → 2; 30, 1 → 2; 33, 1 → 2; 35, 1 → 2; 45, 2 → 1; 48, 2 → 1; 52, 2 → 1; 54, 2 → 1; 57, 2 → 1; 76, 1 → 2; 106, 2 → 1; 107, 2 → 1
Clade 15	3, 1 → 2; 69, 1 → 2; 72, 1 → 2; 73, 1 → 2; 82, 1 → 2
Clade 16	66, 1 → 2; 70, 1 → 2
Clade 17	3, 2 → 3; 8, 2 → 1; 39, 1 → 2
Clade 18	58, 1 → 2
Clade 19	5, 2 → 1; 62, 1 → 2; 63, 1 → 2

Unique character changes at nodes not changing above in the tree are set in **bold**.

positions of the Augaptiloidea and the Centropagoidea are preserved (although with very poor posterior probability in the BI analysis).

A basal Pseudocyclopoidea clade is sister to all other families in the Calanoida (Fig. 12). Within this basal clade, *P. ambiguus* sp. nov. is sister to a clade that contains *Exumella* and *Pseudocyclops* spp. Although members of the Epacteriscidae are not available to be included in this analysis, the present phylogeny is in accord with our new conclusions, based on morphology, on the rearrangement of these basal taxa into families within an enlarged Pseudocyclopoidea.

The added data contributed to an improvement in the resolution of some relationships in the previous phylogeny. We note the presence of the weakly supported clade containing the Eucalanoidea and a subclade comprising a monophyletic Megacalanoidea (barely supported in the ML analysis and not supported by the BI approach) and Bathypontioidea. Another change was the sister relationship of the now added families

Pseudodiaptomidae and Diaptomidae to the rest of the Centropagoidea (Fig. 12).

SYSTEMATICS

SUPERFAMILY PSEUDOCYCLOPOIDEA

GIESBRECHT, 1893

Epacteriscoidea Fosshagen, 1973

Diagnosis: Plesiomorphic calanoid copepods with underlying pattern of full development of arthrodial membranes between body somites and limb segments, with some exceptions: antennule of female up to 27-segmented, ancestral segments XIX, XX, and XXIII usually without aesthetascs; male antennule always geniculate on right and tendency for ancestral segment XXV to have distoanterior process. Male and female mouthparts identical. Antenna exopod nine-segmented, segments I–VIII with one seta each. Maxilliped endopod segment V nearly always with outer border seta (except *Edaxiella*); swimming legs 1–5 with both rami usually three-segmented; legs 1 and 2 exopod segment 3 with two or three outer border spines, leg 3 exopod segment 3 with two or three outer border spines, legs 4 and 5 (female) with two or three outer border spines.

FAMILY PSEUDOCYCLOPIDAE GIESBRECHT, 1893

Ridgewayiidae M.S. Wilson, 1958

Boholinidae Fosshagen & Iliffe, 1989

Diagnosis: Female caudal rami of equal lengths (except *Pinkertonius* gen. nov., longer on right); male caudal rami usually symmetrical (except *Stargatia*); seta II spiniform. Female antennule ancestral segment IV without aesthetasc. Tendency for aesthetascs to be absent from additional segments. Mandible endopod well-developed, two-segmented, ranging from greater than half length of exopod to extending well beyond exopod (except *Exumella*), segment 1 usually with four setae (exceptions *Robpalmeria*, *Normancavia*, *Exumella*, and *Stargatia*), segment 2 usually with between eight and 11 setae, except for *Normancavia*, *Exumella*, and *Exumellina*, which have six, six, and seven setae, respectively. Maxillule coxal epipodite always with nine setae. Maxilla basis usually less than twice estimated length of coxa (except *Exumella*), basal endite usually elongate (except in *Placocalanus*), and endopod setae normal (exception in *Boholina*, which has spine-like setae). Maxilliped endopod segments 2–6 usually longer than length of coxa (not including praecoxa) (exception *Placocalanus* and *Pseudocyclops*) and endopod setae normal. Swimming legs 1–5 with both rami three-segmented. Leg 1 basis posterior surface with tendency to have posterior surface process; exopod segment 2 with tendency to have outer distal corner produced into spinous lobe inner to articulated spine; exopod segment 3

Table 6. Spine and seta formulae of swimming legs: Roman numerals indicate robust setae; Arabic numerals indicate setae; outer border setation listed first in each segmental group, and separated by ;

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	0/1-0/1	I-0/1; I-1; II, I, 2/3/4/5	0-1; 0-2; 1, 2, 2/3 0-0; 0, 0, 3 0, 0, 3/4
Leg 2	0-1	0/I-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 3	0-1	0/I/1-0	I-0/1; I-1; II/III, I, 5	0-1; 0-2; 2, 2, 4
Leg 4	0-1	0/1-0	I-0/1; I-1; III, I, 5	0-1; 0-2; 2, 2, 3
Leg 5 female	0-0/1	0/1-0	I-0/1; I-1; III, I, 2/3/4	0-0/1; 0-1; 1/2, 2, 2/3 0-1; 2, 2, 3

Table 7. Swimming legs 1–5 biramous, with both rami three-segmented, spine and seta formula as follows: Roman numerals indicate robust setae; Arabic numerals indicate setae; outer border setation listed first in each group, separated by ;

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	0-1	I-0/1; I-1; II, I, 4	0-1; 0-2; 1, 2, 3
Leg 2	0-1	0/I-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 3	0-1	0/I/1-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 4
Leg 4	0-1	0/1-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 3
Leg 5 female	0-0	0/1-0	I-0/1; I-1; III, I, 4	0-1; 0-1; 2, 2, 2

that of female, except for exopods: I-0; I-1; I, 0, I (left) and I-0; I-0; I, 0, I (right); left and right exopod segment 2 with inner processes.

Type species: *Pinkertonius ambiguus* gen. et sp. nov. by original designation.

Remarks: The most distinctive shared characteristics that link this genus to the family Pseudocyclopidae are: the absence of an aesthetasc on ancestral antennular segment IV; the presence of a well-developed, elongate two-segmented mandibular endopod with ten terminal setae; the presence of nine setae on the coxal epipodite of the maxillule; the presence of a posterior surface process on the basis of leg 1; exopod segment 2 of female leg 5 is distally extended, and the articulation between segments 2 and 3 is at an oblique angle to the main axis of the limb; and the right exopod segment 2 of male leg 5 has a triangular inner process and left exopod segment 2 has a scalpel-like inner projection that is directed distally.

FAMILY EPACKTERISCIDAE FOSSHAGEN, 1973

Diagnosis: Female caudal rami usually of equal lengths (exceptions: *Balinella* longer on right; *Gloinella* longer on right); male caudal rami symmetrical or asymmet-

rical, seta II spiniform, setiform, or apparently absent in *Miheptneria*, female seta VI tending to be asymmetrical, with one seta having one side bearing short setules, the other with long setules (exceptions *Balinella*, *Cryptonectes*, *Oinella*, *Bunderia*, *Edaxiella*, and *Enantronoides*). Female antennule ancestral segment IV with aesthetasc (except for *Oinella*). Mandible with exopod and basis forming major axis of palp; endopod poorly developed, two- or one-segmented, or absent, being at most half length of exopod segment 1, which when present, with at most two setae, segment 2 with four or fewer setae, except for *Erebionectes* and *Miheptneria*, which have seven and nine setae, respectively. Maxillule coxal epipodite with seta 9 absent (exception *Miheptneria*), and tendency for further setae to be lost up to seta 6. Maxilla basis usually more than twice estimated length of coxa, basal endite with low profile, and endopod setae spine-like (exception *Miheptneria*, which has elongate basal endite and normal setae). Maxilliped endopod segments 2–6 shorter than length of coxa (not including praecoxa) and endopod setae spine-like (exceptions *Miheptneria*, *Bomburiella*, and *Edaxiella*, which appear to have normal setae on endopod). Leg 3 endopod segment 1 with outer distal corner bifid or trifold in clade 20. Male left leg 5 without inner process present on exopod segment 2 (only exception *Balinella*) (Table 7).

Type genus: Epacteriscus Fosshagen, 1973.

Remarks: The genera included in this family are: *Balinella*, *Bofuriella*, *Bomburiella*, *Bunderia*, *Cryptonectes*, *Edaxiella*, *Enantiosis*, *Enantronia*, *Enantronoides*, *Epacteriscus*, *Erebonectes*, *Gloinella*, *Iboyella*, *Miheptneria*, *Minnonectes*, and *Oinella*.

DISCUSSION

POSITION OF PSEUDOCYCLOPOIDEA IN CALANOIDA

Genetic data and a revised phylogeny confirm the basal position of a newly defined superfamily Pseudocyclopoidea (which now includes families previously assigned to the synonymized Epacteriscoidea) within the Calanoida, as well as improving the organization and resolution of the relationships among superfamilies.

The revised molecular phylogeny (Fig. 12) reinforces the results obtained in the previous gene-based study (Blanco-Bercial *et al.*, 2011), although the lack of members of the basal families, the epibenthic Ridgewayiidae, Boholinidae, Pseudocyclopidae, and Epacteriscidae in the previous analysis was problematic. With the inclusion of sequences from the superfamily Pseudocyclopoidea, the reconstructed phylogeny agrees partially with the topology of early morphology-based phylogenies (Andronov, 1974; Park, 1986). Here superfamilies Pseudocyclopoidea, Augaptiloidea, and Centropagoidea sequentially split off from a main stem. The remaining superfamilies form a single clade, the topology of which is similar to the topology described in Bradford-Grieve *et al.* (2010) based on morphological data.

The lower support than that found by Blanco-Bercial *et al.* (2011), evident in many parts of the reconstructed phylogeny, could result from incomplete gene coverage of the new taxa added (Appendix S1). It is likely that the addition of *COI* and *cyt b* to the clades where they are missing would add stronger support to the analyses. For example, the inclusion of mitochondrial genes can improve resolution at deeper nodes (Fisher-Reid & Wiens, 2011; Cornils & Blanco-Bercial, 2013). We found that without the addition of mitochondrial gene sequences from *P. ambiguus* sp. nov., not only was the phylogeny not recovered, as it is here, but the resulting superfamilies were also polyphyletic or paraphyletic in some cases (data not shown).

All superfamilies were recovered as monophyletic, even in cases where very divergent groups were included, supporting the conclusions drawn from morphological characters (Ho, 1990; Huys & Boxshall, 1991). This fact is very significant in Centropagoidea, where the two divergent families Diaptomidae (the only entirely freshwater family) and Pseudodiaptomidae clustered with the other Centropagoidea in a single clade.

The resolution of monophyletic clades, representing the superfamilies Eucalanoidea, Megacalanoidea, and Bathypontoidea, sister to the Clausocalanoidea and Spinocalanoidea, does not agree with morphological studies (Andronov, 1974; Park, 1986; Bradford-Grieve *et al.*, 2010). Although interesting, this result should be considered with caution, because the family identified as intermediate between these two clades (Ryocalanidae; superfamily Ryocalanoidea) is missing from the molecular analysis, and its addition might result in changes to the present topology of the phylogeny. Thus, the presented revised molecular phylogeny provides testable hypotheses for future work.

PHYLOGENY OF PSEUDOCYCLOPOIDEA

Among the pseudocyclopoidean genera, the morphology-based phylogenetic signal was possibly obscured by the high degree of homoplasy and may have interfered with accurate tree inference. Some of the homoplasy in our data may be the result of the possibility that we are not always dealing with homologous characters or that the character states are not accurately recorded in the literature. It is also possible that some characters reflect lifestyle rather than preserving a phylogenetic signal. In this analysis, it was not possible to determine homologies in some characters relating to the mandible upon which feeding niche strongly impacts (Itoh, 1970). We note, however, that certain types of modification of the mandible, maxilla, and maxilliped are strongly linked to the two major clades (Pseudocyclopidae and Epacteriscidae), and so may contain a robust phylogenetic signal.

In the Pseudocyclopidae, the mandible generally has small teeth (although *Exumellina* and *Stargatia* have two elongate ventral teeth) and the endopod is well developed, often with four setae on segment 1 and more than nine setae on segment 2. The maxilla has a normally developed basis with an elongate endite and normal endopod setae. The maxilliped usually has an elongate endopod that is furnished with normal setae. We note that this family contains genera that live in open water habitats and the only freshwater ground-water genus, as well as marine cave-dwellers. It is deduced from the form of the mouthparts that many of these genera are fine-particle feeders. Those that deviate in having reduced mandibular endopod setation appear to have other modes of feeding. For example, *Exumella* seems to be a benthic scavenger (Jaume & Boxshall, 1995), and *Exumellina* and *Stargatia*, which also have paddle-like modification to the mandible and maxillule endopods, may be raptorial feeders feeding on delicate prey (Fosshagen & Iliffe, 1998). A large subset of these genera also have female leg 5 modified so that the mode of articulation of exopod segment 3 directs this segment into the midline, or

ensures that it has an even greater arc of movement (*Badijella*, *Pseudocyclops*, *Ridgewayia*, *Robpalmeria*, *Normancavia*, *Brattstromia*, *Exumella*, *Exuminella*, *Pinkertonius* gen. nov., and possibly *Hondurella*, *Placocalanus*, and *Stargatia*). It is tempting to hypothesize that these genera are adapted to digging in sediment.

In the Epacteriscidae, the mandible generally has the ventral tooth enlarged and separated by a much larger gap than exists between the remaining teeth; an even more specialized gnathobase is found in *Epacteriscus*, where the cutting blade has a prominent extension bearing sharp teeth that extends well out from the body (Fosshagen, 1973). Most species have a reduced endopod that is either one-segmented with only one seta or is absent (except for *Balinella*, *Bofuriella*, *Erebnectes*, and *Miheptneria*, which have two-segmented endopods). The endopods of the maxillae and maxillipeds are very condensed and nearly always have spine-like setae, with a few exceptions (*Miheptneria*, *Bomburiella*, and *Edaxiella*), and the maxilla basis is enlarged and has a very low-profile endite. Taken together, these character states indicate that most genera are carnivores.

The basal taxa in each family (*Pinkertonius* gen. nov. and *Miheptneria*) appear to be adapted to fine-particle feeding. Their mandibles have short teeth, and the endopods of the maxilla and maxilliped bear normal setae. The remaining genera in each clade have rather uniform modifications to the mandible, maxilla, and maxilliped. A similar situation exists in the family Heterorhabdidae, within which distinct transformations occur from small-particle feeders such as *Disseta*, to highly specialized carnivores, such as *Heterorhabdus* and *Neorhabdus*. This evolutionary shift in feeding type is accompanied by a strong reduction or loss of dorsal teeth on the mandible and an increase in size of the ventralmost tooth (Nishida & Ohtsuka, 1996). The phylogenetic analysis of the Heterorhabdidae by Ohtsuka, Soh & Nishida (1997) recovered the small particle-feeding genera as basal branches and the specialized carnivorous genera as terminal branches. We infer that carnivory has evolved independently within all of these families.

Our phylogenetic analysis and conclusions concerning the taxonomic hierarchy at the base of the calanoid phylogeny are similar to the suggestions of Andronov (2007). Nevertheless, we have raised the rank of his family and subfamily names. Our analysis places *Miheptneria* in the same taxon as that of Andronov (2007), but separates out *Azygonectes*, the affinities of which he was not sure about, along with *Erebnectoides* and *Caiconectes*. We consider these genera currently to be *incertae sedis* (of uncertain placement). There is reasonably strong evidence for a differential diagnosis of a new family based on *Caiconectes*. It has a

uniquely primitive setation pattern on the endopod of leg 1. It is the only calanoid with seven setae on the third endopodal segment. It also is the only calanoid we are aware of with five setae on the basis of the maxilla (most others have a maximum of four setae). In addition to these unique plesiomorphies, there is a cluster of other shared maximum plesiomorphic states relating to the distribution of aesthetascs on the antennules in both sexes, especially on segments XIX and XX (Appendix S5a, b). In addition, there are some apomorphies (e.g. reduced setation on the mandibular endopod and maxillule, and the coarse outgrowths of the long feeding setae on the maxilliped). At least on the basis of morphology, we would expect *Caiconectes* to be robustly recovered as the basal offshoot of the Calanoida in future analyses. On the other hand, *Azygonectes* and *Erebnectoides* is a potentially unstable group. These two genera share many key characters with other Epacteriscidae: presence of an aesthetasc on segment IV in female; the form of the mandible palp, with a reduced endopod; the asymmetry of caudal seta VI in the female, and of the caudal rami in the male; and endopod segment 1, of at least leg 3, with its distal outer corner bifid or trifold. But these character states alone are obviously not enough (on balance) to cluster them with the Epacteriscidae in this tree.

The Pseudocyclopoidea are characterized by numerous homoplasious character states that ensure that the phylogenetic signal in the data is weak; therefore, none of the higher level taxa definitions is based on unique synapomorphies. The wide range of combinations of character states among the taxa in this superfamily hints at these taxa being a sparse sampling of a once much more diverse, ancient taxon, from which the ancestors of the Augaptiloidea, Centropagoidea, and a clade containing the remaining superfamilies evolved (Fig. 12). Part of their evolutionary capacity may have included the possession of character states that were easily reversed or independently reacquired, hence the currently observed diversity of character state combinations. Although the addition of new taxa or the revision of some character states may change relationships at the base of the tree, in our judgement, the pseudocyclopid and epacteriscid clades are likely to remain intact.

The old concepts of the monogeneric Pseudocyclopidae and Boholinidae were based, at least in part, on the obvious external separation of the paired gonopores of the adult female (e.g. Huys & Boxshall, 1991: fig. 2.2.16). A similar arrangement was noted in at least some members of the Arietellidae, but the variation in structure of the female genital system within the family prompted Ohtsuka, Boxshall & Roe (1994) to recognize five major trends involving fusion of copulatory pores to form a single common pore, various migra-

tions of gonopores and copulatory pores, and the asymmetrical enlargement of copulatory pores. Despite this variability in structure, Bradford-Grieve *et al.* (2010) were only able to use three characters based on female genital systems in their phylogenetic analysis of the Calanoida, and two of those were based on seminal receptacles and ducts. Variability in female genital structures can be found even in the more derived calanoid taxa, such as the clausocalanoidean family Stephidae. Unlike all of its congeners, the adult female of *Stephos vivesi* Jaume, Boxshall, Gràcia, 2008 possesses a pair of separate gonopores. Jaume *et al.* (2008) interpreted this condition as secondary, possibly derived by the loss of the genital operculum concealing the paired gonopores and their subsequent migration and separation. This serves to highlight the scale of intrafamilial variability in certain calanoid families, and the variability within the revised and enlarged concept of the family Pseudocyclopidae should be interpreted from this perspective.

It is interesting to note that the two taxa that are basal to the Epacteriscidae and Pseudocyclopidae live in the open ocean at depth, and that the terminal taxa are cave-dwelling in the Epacteriscidae and cave-dwelling, shallow-water, or even groundwater-dwelling in the Pseudocyclopidae. Thus, it appears that any hypothesis about the sequence of events surrounding the colonization of anchialine cave environments may be exactly the opposite from that proposed by Boxshall & Jaume (2000) for the Misophrioida. The misophrioid family Speleophriidae currently comprises eight genera and 19 species, almost all of which occur only in coastal anchialine habitats. The exception is *Archimosphria* Boxshall, 1983, which contains two species, both found in the deep hyperbenthic community of the tropical Atlantic. This genus is not basal within the family, and is recovered as the sister taxon of the cave-dwelling genus *Expansophria* Boxshall & Iliffe, 1987 (Boxshall & Jaume, 2000). In the case of the speleophriids, Boxshall & Jaume (2000) inferred that the presence of species in the deep sea was secondary, and that they were probably descended from shallow-water ancestors.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Taxon and gene sampling of Calanoid superfamilies analysed for this study.

Appendix S2. Gene alignment nexus file.

Appendix S3. Morphological characters/states used for morphology-based phylogenetic analysis, followed by the consistency index and rescaled consistency index.

Appendix S4. Data matrix of character state scores.

Appendix S5. Distribution of female antennule aesthetascs and segmental fusions.

Appendix S6. Morphological data matrix as a DELTA file.

Appendix S7. Nexus file of morphological data.