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Phylogenetic relationships within *Amphiglena* Claparède, 1864 (Polychaeta: Sabellidae), description of five new species from Australia, a new species from Japan, and comments on previously described species

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Abstract

In reviewing material in Australian museum collections from the Australian and Japanese coasts it became clear that many specimens of *Amphiglena* did not match the diagnosis of any current species. To allow for variability observed among the specimens, and hence within nominal species, three specimens from each locality were included in a cladistic analysis, along with all currently accepted *Amphiglena* species, new characters, and a variety of apomorphic sabellins. This allowed a reassessment of the monophyly of *Amphiglena*, its sister group, and relationships with close taxa. Phylogenetic relationships among the species of *Amphiglena* are presented and six new species are described.

Keywords: *Amphiglena*, Australia, Japan, new species, Polychaeta, Sabellidae, systematics

Introduction

Amphiglena Claparède, 1864 is a group of small sabellids found worldwide in shallow waters associated generally with coralline algae, calcareous substrates, or coarse sand. The monophyly of the genus has been assessed (Rouse and Gambi 1997, 1998a; Fitzhugh and Rouse 1999), but its phylogenetic position and relationships with other sabellid taxa are uncertain. Recent studies suggest that either *Laonome* Malmgren, 1866 (Rouse and Fitzhugh 1994; Rouse and Gambi 1997, 1998a), *Terebrasabella* Fitzhugh and Rouse, 1999 (Fitzhugh and Rouse 1999), or a clade comprised of *Caobangia* Giard, 1893 and *Terebrasabella* (Fitzhugh 2003; Nogueira et al. 2004; Murray and Rouse forthcoming) is the sister group to *Amphiglena*.

The six *Amphiglena* species described to date have been the subject of a range of anatomical, morphological, reproductive mode, and phylogenetic studies (Rouse 1993; Rouse and Gambi 1997, 1998a, 1998b; Fitzhugh and Rouse 1999). The various species were described based on unique combinations of characters, dealing with features such as ventral basal flanges, dorsal lip radiolar appendages, shape of chaetae, gamete arrangement, and sperm morphology. In reviewing a range of various Australian museum collections it

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became apparent that material from Australia and Japan could not be assigned to any of the six currently accepted *Amphiglena* species.

This study can be broken into three aspects: (1) a reassessment of the relationships of *Amphiglena* and other apomorphic Sabellinae with additional morphological characters; (2) a new phylogenetic hypothesis within the genus that results in (3) the description of several new *Amphiglena* species.

Materials and methods

Sampling and study of specimens

Specimens collected from the Australian and Japanese coasts and housed in the Australian Museum (AM), South Australia Museum (SAM), and Museum of Victoria (MV) collections were studied. The collecting was undertaken in several surveys by L. Albertson, M. Capa, P. Hutchings, A. Murray, T. O'Hara, G. Rouse, R. Springthorpe, and G. D. F. Wilson. All the specimens were fixed in formalin and transferred to 80% ethanol. Some additional specimens were collected by the authors for live study and preserved in 95% ethanol for future molecular analyses.

Some parapodia (from the first and the second last thoracic chaetigers and others from the mid-abdominal region) were removed and mounted in permanent slides with polyvinyl-lactophenol for examination of the chaetae. The internal structure of the radioles and lips were studied by mounting thin transverse sections of these structures in permanent slides, also with polyvinyl-lactophenol. Line drawings were made to scale with a drawing tube attached to the stereo and light microscopes, and digitized. Micrographs were taken with a Nikon Coolpix 995 camera attached to a compound microscope. Some specimens were dehydrated in ethanol, critical-point dried and covered with 20 nm of gold and examined under a Leo 435VP scanning electron microscope, using Robinson backscattered and ET secondary electron detectors. Some abbreviations have been used for labelling figures: vbf, ventral basal flange; p, pinnule; dra, dorsal radiolar appendage; pe, peristomial eye; apr, anterior peristomial ring; ppr, posterior peristomial ring; py, pygidial eyes; eg, eggs.

Ingroup and outgroup

For the cladistic analysis, 21 terminals were included as the ingroup (Appendix 1). This includes all six currently accepted species of *Amphiglena* (character states were scored from the holotype, except for *A. pacifica*, where the original description refers only to the range of variation within the species) plus three *Amphiglena* specimens from each locality studied here that differed from previously described species. Also, one species from each of the proposed most closely related genera, *Kirkia* Nogueira et al., 2004, *Terebrasabella*, *Laonome*, and *Perkinsiana* Knight-Jones, 1983, were included as the ingroup. Note that *Kirkia* would appear to be a junior synonym of *Kirkia* Pollonera, 1909 (<http://uiio.mbl.edu/NomenclatorZoologicus/>) and a replacement name may be needed. *Caobangia* has been shown to be sister group to *Terebrasabella* by Fitzhugh (2003), but this has not as yet been fully documented in an explicit matrix. For this reason this genus has not been included in the study. The outgroup selected was *Potamilla* Malmgren, 1866 based on it being a closely related taxon but arguably not the sister to any member of the ingroup (Fitzhugh 1989; Rouse and Gambi 1997, 1998a; Fitzhugh and Rouse 1999). The matrix was scored obtaining data from live and preserved specimens and description from literature (Appendix 2).

Characters

Thirty-seven characters were used in the cladistic analysis. Some were those included in previous analyses (Rouse and Gambi 1997, 1998a; Fitzhugh and Rouse 1999) and others are developed here for the first time (Appendix 3). Unfortunately, some of the characters related to the reproductive modes or reproductive features could not be observed in the specimens studied.

(1–12) *Crown*. The number of radioles (1) is a character that has been used traditionally to distinguish among species of *Amphiglenea*. It can vary slightly within one species as it does in *A. lindae* with four to five pairs (Rouse and Gambi 1997), *A. nathae* with five to six pairs (Rouse 1994), and *A. terebro* with four to five pairs (Rouse 1993), but in other species the number of radioles seem to be constant, as in *A. jimenezi* with four pairs of radioles (Capa and López 2004) and *A. mediterranea* with five pairs (Rouse and Gambi 1997). Other taxa of the ingroup are polymorphic: *Laonome* species have numerous radioles (Fitzhugh 1989) while *Kirkia* (Rouse 1996; Nogueira et al. 2004) and *Terebrasabella* (Fitzhugh and Rouse 1999) have fewer pairs, especially *Terebrasabella* with only two pairs of radioles (Fitzhugh and Rouse 1999; Murray and Rouse forthcoming). The radiolar skeleton (2) of many Sabellinae is made up of four or more rows of cells across the base of any given radiole, a condition that is different from that seen in *Kirkia*, *Laonome*, *Terebrasabella*, and *Amphiglenea*, where there are only two cells in cross-section (Fitzhugh 1989; Fitzhugh and Rouse 1999). Note that this latter condition is also seen in plesiomorphic sabellins (Fitzhugh and Rouse 1999), such as *Amphicorina* Quatrefages, 1850 and *Desdemonia* Banse, 1957. *Amphiglenea* has a small number of pinnules (3) in their radioles (Figures 2B, C, 6A, 7A, 8A, 9A, 11A) compared to most other apomorphic Sabellinae most of which tend to have 15–20 pairs, though *Terebrasabella* has four to eight pairs. Note that the number can vary among *Amphiglenea* species and specimens. *Amphiglenea gracilis* sp. nov. has a maximum of six pairs, the lowest number of pinnules among *Amphiglenea*, and the rest of the species have between 6 and 10 pairs, except *A. jimenezi*, *A. nathae*, and *A. lenae* sp. nov. that have always between 15 and 20 pairs of pinnules on each radiole. The relative length of the pinnules along the radioles (4) varies among the species of *Amphiglenea*, being practically uniform from the proximal part to the distal end of radioles in some taxa (e.g. *A. terebro*, Figure 11A), whereas in other species there is a clear enlargement (Figure 9A) or reduction in their length along the radioles (Figure 2A). The basal membrane (5), also called palmate membrane, is an “extension of the lateral sides of two adjacent radioles and is covered on both sides by columnar epithelium, between which is a layer of skeletal sheath material” (Perkins 1984; Fitzhugh 1989). Some sabellids have been described as lacking this membrane, such as *Amphiglenea* (Fitzhugh 1989; Rouse 1994; Rouse and Gambi 1997), *Kirkia* (Nogueira et al. 2004), *Terebrasabella* (Fitzhugh and Rouse 1999), and *Laonome* (Fitzhugh 1989), but it should be noted that a slight membrane is present in some *Laonome* and *Amphiglenea* (Hsieh 1995, see Figures 6C, 7C). We code here the low or slight membrane as the same state as absent pending further investigation.

Fitzhugh (1989) showed as an apomorphy (though homoplastic) for *Amphiglenea* the presence of “long ventral basal flanges extended posteriorly to the posterior peristomial ring”. He defined basal ventral flanges (6) as “a pair of thin, erect, membranous flanges, which extend anteriorly from the ventral side of peristomium to the outer ventral margins of the branchial lobes”. Rouse and Gambi (1997) stated that ventral basal flanges were absent in *A. terebro* and described these structures in the rest of the species of *Amphiglenea* as “extending from the base of radioles and posteriorly across the anterior peristomial ring”.

We interpret ventral basal flanges as present in all species of *Amphiglena*, but in some species, including *A. terebro* (Figure 11B), the flanges do not extend beyond the margin of the crown and the peristomium and are limited to the base of the branchial crown, while in others they continue posteriorly across the peristomium (7). Ventral basal flanges have been described for *Amphiglena*, *Terebrasabella* Fitzhugh and Rouse, 1999 (Murray and Rouse forthcoming), *Anamobaea* Kröyer, 1856, *Notaulax* Tauber, 1879, and *Pseudopotamilla* (Perkins 1984; Fitzhugh 1989; personal observation) but it is still uncertain if they are homologous structures across all genera (Fitzhugh 1989). In *Anamobaea*, *Notaulax*, and *Pseudopotamilla* the ventral basal flanges seem to be a prolongation of the outer margin of the radioles, similar to the basal membrane or a radiolar flange on the ventralmost radiole (Perkins 1984; Fitzhugh 1989; personal observation). However, in *Amphiglena* (Figures 2A, 5B, 8A, 9B, 11B) and *Terebrasabella* (Murray and Rouse forthcoming) these flanges seem to be extensions from the first pinnule of the ventralmost radiole to the base of the crown. On the current cladistic hypothesis for Sabellinae (Fitzhugh 2003) the two forms of ventral basal flanges do appear independently.

Amphiglena species lack ventral lips (8) and parallel lamellae (9) as does *Terebrasabella*, while the other taxa included in the analysis have these features (Rouse 1996; personal observation). Note that though all the species of *Laonome* have ventral lips (Perkins 1984; Fitzhugh 1989; Hsieh 1995), only some, such as *L. albicigillum* Hsieh, 1995, *L. indica* Southern, 1921 (Hsieh, 1995), and *L. triangularis* (personal observation) have parallel lamellae. *Amphiglena* as well as *Terebrasabella* and some species of *Laonome* (Fitzhugh 2003) have dorsal lips with dorsal radiolar appendages (10), while *L. albicigillum*, *K. riwo*, and the outgroup lack them (Hsieh 1995; Rouse 1996; Fitzhugh 2003; Nogueira et al. 2004). However, in *Amphiglena* there is no supporting skeleton, whereas in some *Laonome* species such as *L. triangularis*, there is a branchial skeleton extension along the dorsal radiolar appendages (Fitzhugh 2003; personal observation). The relative length of dorsal radiolar appendage (e.g. Figures 5C, 8C, 11B) compared to the branchial crown (11) has been formulated here as a character rather than its length compared to the dorsal lips (as in Rouse and Gambi 1997) owing to the small size of these structures and the difficulty of recognizing them. Dorsal pinnular appendages (12) are only found in the outgroup.

(13–17) *Peristomium and thorax*. Sabellids have a peristomium divided in two “rings”, a feature most easily seen in small sabellids or when they lack a collar (Fitzhugh 1989). Most terminals used here have an anterior peristomial ring of even height all around, with no anterior projection. But within *Amphiglena* some variation can be noticed in these features. The anterior peristomial ring is divided by a mid-ventral incision in most cases (Figures 2D, 5B, 6B, 8B). In some *Amphiglena* it is thinner than in other sabellins and may be fused to the posterior ring, so it is not visible all the way around (13). The posterior peristomial ring is generally longer than the anterior ring. The anterior margin of the posterior peristomial ring (14) can be horizontal (Figures 8B, 9B, 11C) or prolonged ventrally on both sides of the mid-ventral incision (Figures 2D, 5B, 6B, 7B, 10A). A posterior peristomial ring collar (15) is absent in *Amphiglena* but present in the rest of the ingroup and outgroup. *Amphiglena*, as well as some other small sabellids such as *Kirkia* (Rouse 1996; Nogueira et al. 2004), has peristomial eyes (16) (e.g. Figure 3B), but these are not found in *Terebrasabella* (Simon, personal communication). This character has not previously been included in any cladistic analysis.

Most sabellids have a fixed number of thoracic chaetigers, usually eight, although there are a few species where the number is variable. This character (17) can be variable within *Amphiglena* species as shown here.

(18–26) *Chaetae*. All *Amphiglena* species have broadly hooded superior thoracic chaetae (18) on chaetigers 2–8 (Rouse and Gambi 1997), including the new species described here (e.g. Figures 2F, 5D, 6D, 7F, 9E), while the outgroup has narrowly hooded superior chaetae. The inferior thoracic chaetae (19) are paleate in the outgroup and most of the ingroup, with the exception of *Terebrasabella*, where broadly hooded capillaries are present (Murray and Rouse forthcoming). Uncini have been regarded as diagnostic characters and used in previous cladistic analysis at different levels within Sabellidae (see Fitzhugh 1989; Rouse and Gambi 1997). Features used have been the development of the uncini breast (20, 25) or the length of the handle (22, 26) in both thoracic and abdominal chaetigers. States of characters vary within *Amphiglena* as there is a range of length of uncini handle from short to long, as well as either narrow or well-developed uncinal breasts. Another character referring to the relative length of the breast with reference to the space between the breast to main fang (21) is used here as well (Figure 4). The presence of companion chaetae is shared in all the terminals included in the present analysis, but its shape (23) is different in *Amphiglena* in having a symmetrical membrane with a long tip and a denticulated mucro, while the others have asymmetrical companion chaetae (Figures 2I, 5E, 6E, 7E, 8E, 9F, 10C). Unlike other apomorphic Sabellidae, *Amphiglena* have only a single row of neurochaetae in the abdominal chaetigers (24), while the other taxa have two rows of chaetae (Figures 2G, 5F, 6F, 7F, 8F, G, 9G, 10D).

(27) *Pygidium*. Most sabellids lack pygidial eyes, and this is a character that has been used in previous cladistic analysis (Fitzhugh 1989). All species of *Amphiglena* (Figure 3C) and *Kirkia* have pygidial eyes, while *Potamilla*, *Terebrasabella*, or *Laonome* lack them.

(28–37) *Gamete arrangement and reproduction*. Eggs are easily recognizable in mature individuals, while other characters related to the reproductive mode require fresh material or histology. The character states used for the present analysis are: eggs begin in the anterior part of the abdomen (from the first to third abdominal chaetiger) or posterior to that (Figure 3D). No terminal taxa had eggs in thoracic segments. Characters related to the reproductive mode, presence of spermathecae, sperm morphology, and spermiogenesis were all obtained from Rouse and Gambi (1997) and Fitzhugh and Rouse (1999). Scores from Rouse and Gambi (1997, 1998a) for *A. pacifica* were based on the specimens here referred to *A. nishii* sp. nov.

Cladistic analyses

Characters were of equal weights and in the case of the multistate characters, unordered. Unknown characters were coded with “?”, inapplicable with “–”, and polymorphic characters with the possible states (/). The matrix was developed in NEXUS Data Editor (Page 1998) for PC and the cladistic analysis was performed using NONA 2.0 (Goloboff 1993). Tree searches were completed using the heuristic search command, with the multiple tree bisection and reconnection (TBR + TBR) strategy, selecting 10,000 replicates and holding 10,000 trees at each step and starting three trees per replicate. Analyses were also run using PAUP* version 4.0b10 (Swofford 2002) with 100 random addition heuristic searches. Cladogram topologies and character-state optimization were examined using Winclada (Nixon 2002), showing only unambiguous changes.

Key to Amphiglena species

A matrix was constructed in DELTA 1.04 (Dallwitz et al. 2000) including as terminals all species of *Amphiglena* from Australia and Japan. The characters included were only those

from the cladistic analysis that vary among the *Amphiglena* species and are easy to observe with a dissecting or compound microscope. Those visible in live specimens only, or by electron microscopy (e.g. gamete arrangement and reproductive mode, length of the anterior peristomial ring) were not included. Variation within the species was scored as polymorphisms. The matrix was translated into a key format and then tested with some specimens of each species.

Results of the cladistic analysis

Parsimony analysis using PAUP* resulted in five most parsimonious trees that were 94 steps long (Consistency Index (CI)=0.62, Retention Index (RI)=0.73). This result differed from that obtained using WINONA, where three trees (branches not supported under all possible parsimonious optimizations are collapsed, resulting in only three possible topologies) with 91 steps (WINONA counts transformations regarding polymorphisms differently from PAUP*) were found. One of these is shown in Figure 1A with transformations indicated and Figure 1B shows the strict consensus of the five trees. The monophyly of *Amphiglena* is supported by the absence of a posterior peristomial ring collar (15) and the presence of companion chaetae with a symmetrical membrane (23), an elongate sperm head (nucleus) (31), the presence of a sperm nuclear fossa (34), and elongate and spiral sperm mitochondria (35). *Amphiglena* and *Terebrasabella* share the presence of ventral basal flanges (6), the absence of ventral lips (8) and parallel lamellae (9), dorsal lips with dorsal radiolar appendages without a skeleton (10), superior thoracic chaetae broadly hooded (18), and neurochaetae on abdominal chaetigers arranged in single rows (24) and cytophores with less than 100 spermatids (30).

In all five trees, *A. pacifica* is the sister group to the remaining *Amphiglena*; within this clade *A. terebro* is sister to the remaining *Amphiglena*, and the clade *A. lenae* sp. nov., *A. bondi* sp. nov., and *A. magna* is the most derived *Amphiglena*. The strict consensus tree (Figure 1B) shows two polytomies inside *Amphiglena*, one is *A. nishii* sp. nov. and the non-monophyletic species *A. maiteae* nov. sp. and the other is *A. jimenezi*, *A. nathae*, and the clade comprised of *A. lenae* sp. nov., *A. bondi* sp. nov., and *A. magna* sp. nov. Based on the three specimens included for each six species described here, five are monophyletic. The monophyly of *A. gracilis* sp. nov. is supported by the small number of pinnules (3) (homoplastic, occurs also in *Terebrasabella*), and the unique occurrence of long dorsal radiolar appendages (11); *A. bondi* sp. nov. by the occurrence of eggs from the third abdominal chaetiger (28) (homoplastic, seen also in *A. jimenezi*, *A. lindae*, and some *A. maiteae* sp. nov.); *A. nishii* sp. nov. by the short thoracic uncini handles (22) (homoplastic, seen also in *A. lenae* sp. nov.); *A. lenae* sp. nov. by the short thoracic uncinal breast (21) (homoplastic, seen also in *A. terebro*) and short thoracic uncini handles (22) (homoplastic, seen also in *A. nishii* sp. nov.), and *A. magna* by (7) the occurrence of ventral basal flanges stopping at the base of the crown (homoplastic, also in *Terebrasabella*, *A. gracilis* sp. nov., *A. mediterranea*, and *A. terebro*). The sixth species named here, *A. maiteae* sp. nov. (from Australia), appears as a clade in three of the five most parsimonious trees but is otherwise paraphyletic with respect to *A. nishii* sp. nov. (from Japan). When monophyletic this is supported by the even height of the anterior peristomial ring (13) (homoplastic, also in *A. gracilis* sp. nov., *A. jimenezi*, *A. pacifica*, and *A. terebro*). In the two other shortest trees either one specimen of *A. maiteae* sp. nov. or the remaining two form a clade with *A. nishii* sp. nov. This is because of the polymorphism with respect to location of the eggs in the abdomen (28) for *A. maiteae* sp. nov. and the unknown state for this character for *A. nishii*



Figure 1. (A) One of the most parsimonious cladograms (L 95, CI 0.62, RI 0.73) from the matrix in Appendix 3. Black circles indicate synapomorphies and white circles homoplasies showing unambiguous changes only. (B) Strict consensus of five most parsimonious trees.

sp. nov. Also the mode of larval development is unknown for *A. maiteae* sp. nov. The possible most parsimonious transformations for these two characters results in *A. maiteae* sp. nov. specimens “b” and “c” grouping with *A. nishii* sp. nov. on one shortest tree and *A. maiteae* sp. nov. specimen “a” (the holotype) grouping with it on the other.

Discussion of the cladistic analysis

There is no requirement for species to be monophyletic in order to be recognized as taxa. If this were so then species would merely be clades and so would be no different from other more inclusive clades in phylogenetic hypotheses (see Pleijel 1999; Pleijel and Rouse 2000a, 2000b). The approach adopted allowed for variation within species with respect to the characters coded, though ultimately only one name-bearing type is allocated as is required by the International Code of Zoological Nomenclature (ICZN). For ease of use we here name only clades formed by the *Amphiglana* specimens from given localities as species with the exception of *A. maiteae* sp. nov., which as pointed out in the results was not monophyletic due to unknown character states in two characters.

Three phylogenetic analyses with regard to relationships within *Amphiglana* have been conducted to date (Rouse and Gambi 1997, 1998a; Fitzhugh and Rouse 1999). In two of these analyses (Rouse and Gambi 1997; Fitzhugh and Rouse 1999), *A. terebro* and *A. pacifica* appear as apomorphic *Amphiglana* taxa sharing the features of a narrow uncinal breast in both the thorax and abdomen. In both of these studies *A. mediterranea* and *A. nathae* formed a clade that was sister group to the remaining *Amphiglana*, which formed a clade based on the presence of long-handled thoracic uncini. In the other study, Rouse and Gambi (1998a) incorporated new characters based on reproductive features and larval development and the results showed *A. terebro* as the sister group to the rest of the *Amphiglana* and that, for instance, the narrow uncinal breast had appeared independently in both *A. terebro* and *A. pacifica*. Fitzhugh and Rouse (1999) used the same extra characters but had additional sabellid species including the newly discovered *Terebrasabella* and this resulted in the switch in topology. In the present analysis the topology differs yet again with respect to the plesiomorphic *Amphiglana* species and *A. terebro* and *A. pacifica* form a basal clade. We do not expect that the current results will be stable in the future; further characters and perhaps DNA sequence data are probably required.

Taxonomic account

SABELLIDAE Johnston, 1846
Amphiglana Claparède, 1864, emended

Type species. *Amphiglana mediterranea* Leydig, 1851 designated by Bush (1905).

Diagnosis

Four to eight pairs of radioles. Radiolar skeleton with two rows of cells. Palmate membrane and radiolar flanges absent. Dorsal lips and dorsal radiolar appendages present, without skeleton but with a longitudinal blood vessel and sheath tissue; dorsal pinnular appendages absent. Ventral lips, ventral sacs, or parallel lamellae absent. Anterior margin of anterior peristomial ring narrow, divided ventrally by the midventral incision; posterior peristomial

ring of even height all around, collar absent. Peristomial eyes present. Ventral basal flanges from the proximal pinnule of the ventralmost radiole extending posteriorly to peristomium. Chaetae of first chaetiger in two groups, superior chaetae narrowly hooded, inferior broadly hooded; rest of thoracic segments with broadly hooded superior thoracic chaetae, paleate inferior thoracic chaetae arranged in a single row. Avicular uncini with rows of small teeth over the main fang, hood absent; companion chaetae with a long and thin distal mucro, an inflated and subdistally dentate end, and long and straight handle. Abdominal chaetae broadly hooded, arranged in a single row; avicular uncini with a main fang surmounted by several rows of small teeth. Pygidial eyespots present.

Remarks

The value of studying the external and internal structure of the dorsal lips and dorsal radiolar appendages in the taxonomy and the systematics of Sabellidae was highlighted by Fitzhugh (2003). He stated that in *A. pacifica* dorsal lips have pointed dorsal radiolar appendage with no skeleton cells but only sheath tissue and a longitudinal blood vessel, as well as a dorsal lamella. This observation has been verified in the present study in other species of the genus such as *A. magna* sp. nov. and *A. nishii* sp. nov., though further study is needed for remaining *Amphiglana* species. The structure of the peristomium shows clear differences among the *Amphiglana* species that has not been previously considered. The anterior peristomial ring has been described in the past as low and of even height all around (Rouse 1994; Rouse and Gambi 1997), but it is actually divided ventrally by the midventral incision. The anterior peristomial ring is also not always low and even. It can be distinguished from the posterior ring in being separated from it by a groove (Figures 2B, C, 5B, 11B, D) or it can be largely hidden between the posterior ring and the base of the branchial crown (Figures 7B, 8C, 9B). The posterior peristomial ring is always broader than the anterior ring. In most *Amphiglana* species the anterior margin of the posterior peristomial ring is extended on each side of the mid-ventral incision (e.g. Figure 2D), but these extensions are not the same as the ventral lappets of other sabellids since *Amphiglana* lacks a posterior peristomial ring collar. All *Amphiglana* species possess peristomial eyes (Figure 3B) as seen in other closely related sabellids (e.g. *Kirkia*). The ventral basal flanges, as described by Rouse and Gambi (1997), are not present in all the species of *Amphiglana* since in some they do not continue beyond the margin of the crown and the peristomium (*A. gracilis* sp. nov., *A. magna* sp. nov., *A. mediterranea*, and *A. terebro*) and so this has been removed from the diagnosis of the genus. Some *Amphiglana*, such as *A. lindae*, are described as having the narrowly hooded notochaetae on the first chaetiger (Rouse and Gambi 1997). Examination of type material shows that the chaetae of the first chaetiger are arranged in two fascicles, with the superior ones as narrowly hooded chaetae but the inferior ones are broadly hooded (following Fitzhugh's (1989) definition of sabellid chaetal types).

Amphiglana gracilis sp. nov. (Figures 2, 3, 4A, B)

Material studied

Australia, New South Wales. Holotype: AM W30002, North-west of Julian Rocks, Byron Bay, 28°36'48" S, 153°37'48" E, shell and gravel, 15 m depth, 3 March 1992. Paratypes:

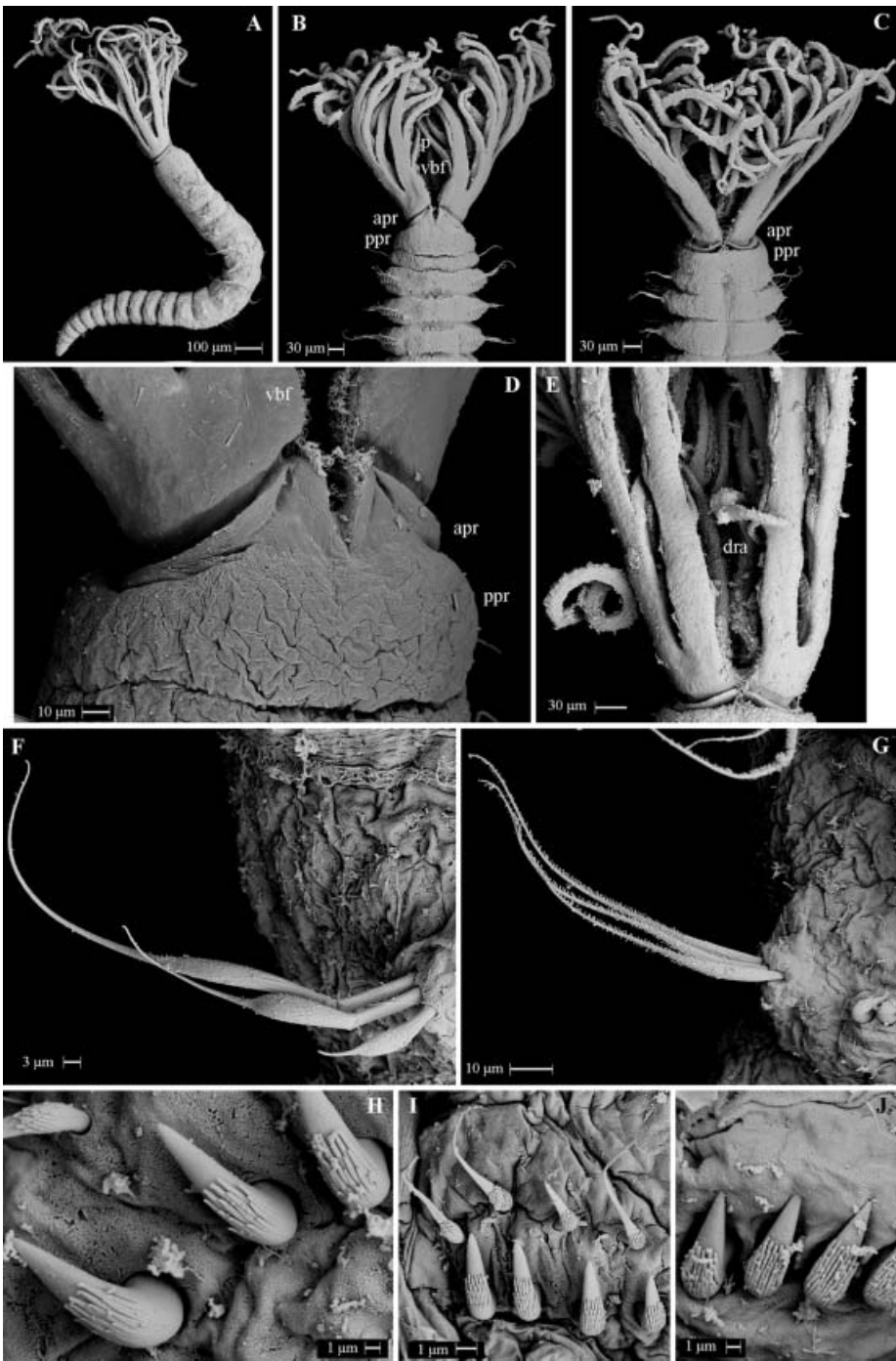


Figure 2. *Amphiglena gracilis* sp. nov. SEM preparation, paratypes, AM W30402–AM W30404, AM W30483, AM W30484. (A) Whole specimen, lateral view; (B) anterior end, ventral view; (C) anterior end, dorsal view; (D) detail of base of crown and peristomium, ventral view; (E) base of crown and dorsal radiolar appendages; (F) notochaetae of fourth thoracic chaetiger; (G) mid-abdominal neurochaetae; (H) uncini, second thoracic chaetiger; (I) uncini and companion chaetae seventh thoracic chaetiger; (J) mid-abdominal uncini.

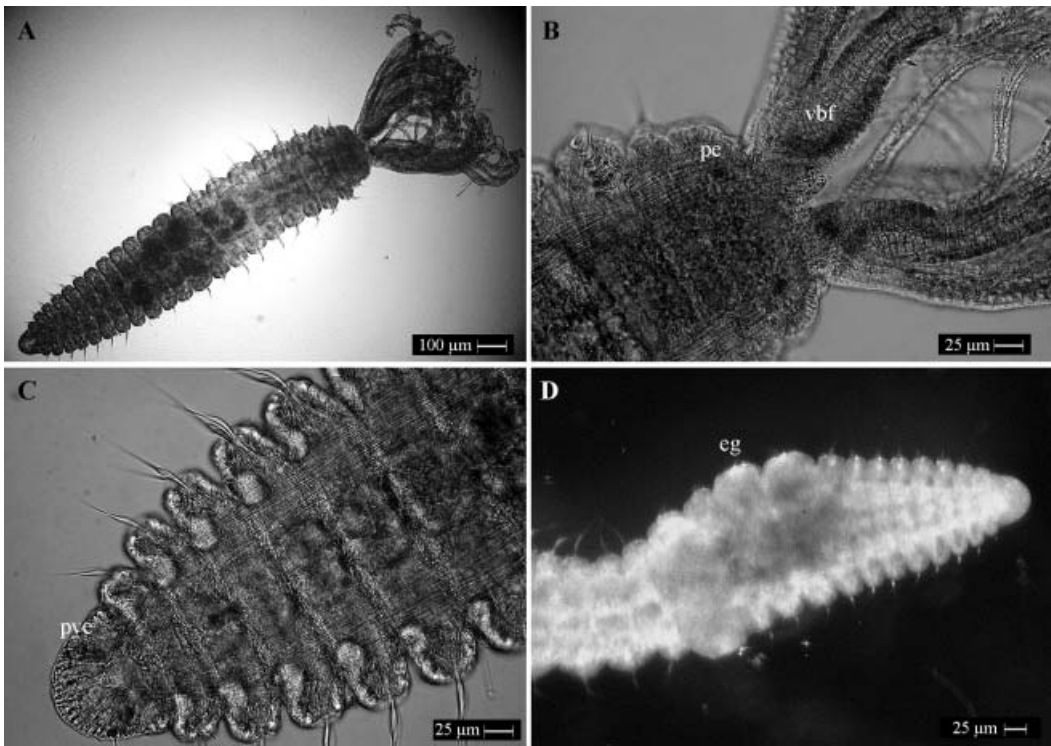


Figure 3. *Amphiglena gracilis* sp. nov. Micrographs. (A) Whole specimen ventral view; (B) detail of anterior segments, ventral view; (C) pygidium and posterior segment, ventral view; (D) last thoracic and abdominal segments, ventral view.

AM W26004, same sample (76 specimens); AM W30402, AM W30403, AM W30404, AM W30483, AM W30484, same sample (SEM). Additional material: AM W26005, north-west of Julian Rocks, Byron Bay, 28°36'48" S, 153°37'48" E, gravel, 15 m depth, 3 March 1992 (four specimens). AM W26006, north-west of Julian Rocks, Byron Bay, 28°36'48" S, 153°37'48" E, shelly sand, 15 m depth, 4 March 1992 (six specimens).

Description

Holotype 2 mm long, crown one-quarter of its length, with eight thoracic and 15 abdominal chaetigers. Crown with four pairs of radioles with a few long pinnules arranged in two rows (Figures 2A–C, 3A); tip of radioles without pinnules, longer than the pinnules (Figure 2C). Dorsal lips about one-third of crown length, reaching about pinnule 3 of each radiole (Figure 2E). Anterior peristomial ring short all around (Figure 2A–E). Posterior peristomial ring collar absent. Ventral basal flanges present as two extensions from the first pinnule of the ventralmost radiole to junction of peristomium and crown (Figure 2B, D), not fused to the peristomial ring; appearing glandular (Figure 3B). Peristomial eyes brownish and ellipsoid (Figure 3B). Thorax longer than wide with quadrangular ventral shields similar in size. Superior notochaetae of first thoracic chaetiger narrowly hooded, inferior chaetae broadly hooded, two per fascicle (Figure 2F). Remaining thoracic chaetigers with one broadly hooded superior thoracic notochaeta and two paleate inferior thoracic notochaetae per fascicle. Second to eighth thoracic chaetiger with four to six thoracic uncini per fascicle,

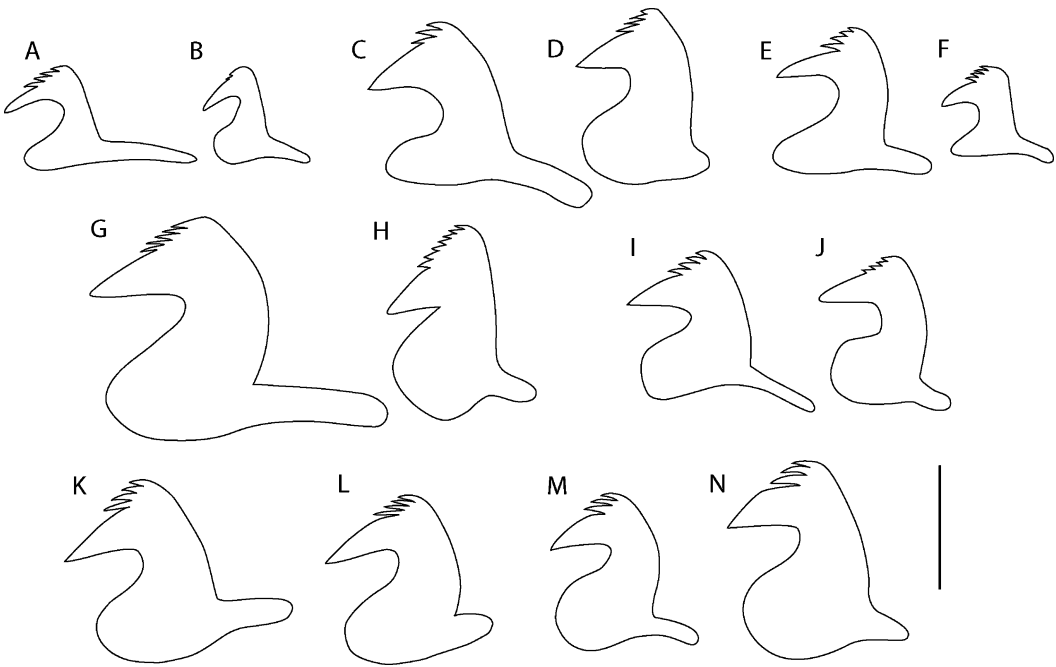


Figure 4. Line drawings of uncini. (A, B) *A. gracilis* sp. nov.: (A) thoracic uncini, seventh chaetiger; (B) mid-abdominal chaetiger. (C, D) *A. bondi* sp. nov.: (C) thoracic uncini, seventh chaetiger; (D) mid-abdominal chaetiger. (E, F) *A. lenae* sp. nov.: (E) thoracic uncini, seventh chaetiger; (F) mid-abdominal chaetiger. (G, H) *A. magna* sp. nov.: (G) thoracic uncini, seventh chaetiger; (H) mid-abdominal chaetiger. (I, J) *A. maiteae* sp. nov.: (I) seventh chaetiger; (J) mid-abdominal chaetiger. (K–N) *A. nishii* sp. nov.: (K, L) seventh chaetiger; (M, N) mid-abdominal chaetiger. Scale bar: 10 μ m.

progressively increasing in size ventrodorsally on each torus, with about six rows of small and long teeth similar size above main fang, occupying half of its length (Figure 2E–H); breast well developed, handles medium to long (Figure 4A). Companion chaetae geniculated, with straight shaft and very elongate mucro with several basal teeth of similar size (Figure 2H). Three thin broadly hooded abdominal neurochaetae in each fascicle (Figure 2G). About four abdominal uncini per torus, with several rows of small teeth of similar size above the main fang; breast well developed, short handle, about half of the uncini width (Figure 4B). Pygidial eyes a pair of orange-brown spots on lateral margins of pygidium (Figure 3C). Statocysts not seen. Tube unknown. Eggs in the second and third abdominal chaetigers of holotype (Figure 3D).

Variation

The paratypes and rest of the additional material vary in the body length from 1.2 to 2.2 mm with the branchial crown from 0.5 to 0.8 mm. All have eight thoracic and between 12 and 16 abdominal chaetigers. The crown is one-third to half the length of the body, and in all the specimens observed there are four radioles with long pinnules; the tip of radioles is always longer than any of the pinnules. Some specimens have eggs from the first to the fourth abdominal chaetiger.

Remarks

Amphiglena gracilis sp. nov. is distinguished from other species in the genus by having a few very long pinnules from the proximal part of the radioles and long dorsal radiolar appendages (about one-third of the length of the crown). It is also characterized by the combination of anterior peristomial ring narrow all around, glandular flanges not fused to peristomium, and medium to long thoracic uncini handles. The abdominal neurochaetae are narrower than in other species of the genus. *Amphiglena gracilis* sp. nov. is similar to *A. terebro* in having ventral basal flanges not extending to the posterior peristomial ring, but the uncini of *A. terebro* show a narrow breast while in *A. gracilis* sp. nov. they are well developed.

Etymology

This species name refers to the Latin term for slender.

***Amphiglena bondi* sp. nov.**
(Figures 4C, D, 5)

Material studied

Australia, New South Wales. Holotype: SAM E3636, scrapings of intertidal algal mat, largely made up of red calcareous *Amphiroa* sp. near Mermaid Rock, North Bondi Rocks, Sydney, 33°54' S, 151°17' E, intertidal, June 2001. Paratypes: SAM E3637, same locality, October 2001 (three specimens); AM W30480, same locality (one specimen, SEM).

Description

Holotype 2 mm long, branchial crown 0.8 mm long. Eight thoracic and 22 abdominal chaetigers. Six radioles on each side of the branchial crown. Palmate membrane and radiolar flanges absent (Figure 5B, C). Radioles with two longitudinal rows of 10–15 pinnules similar in length along the radiole. Tip of radioles longer than distal pinnules (Figure 5A). Radiolar skeleton with two rows of cells. Ventral basal flanges from proximal pinnule of ventralmost radiole to posterior peristomial ring (Figure 5B). Dorsal lips with elongate, ciliated, dorsal radiolar appendages of about one-sixth the length of the branchial crown, not longer than length of proximal pinnule in ventralmost radiole (Figure 5C); with no skeleton. Dorsal pinnular appendages absent. Ventral lips absent. Anterior peristomial ring narrow, only visible ventrally (Figure 5B, C). Posterior peristomial ring with mid-ventral incision, where ventral basal flanges attach; collar absent (Figure 5B). Peristomial eyes present, two brown and circular spots. Thorax longer than wide with quadrangular ventral shields similar in size. Four chaetae on first chaetiger, superior chaetae narrowly hooded, inferior broadly hooded (Figure 5B, D). Rest of chaetigers with three superior thoracic broadly hooded notochaetae, broader than collar chaetae, and up to four paleate inferior thoracic notochaetae, in single row (Figure 5D). Second to eighth thoracic chaetiger with up to eight thoracic uncini per fascicle, increasing in size from ventral to lateral margin of torus (Figure 5D, E), with about four to five rows of small, similarly sized teeth, above main fang, arranged in upper half of main fang; breast well developed, handles

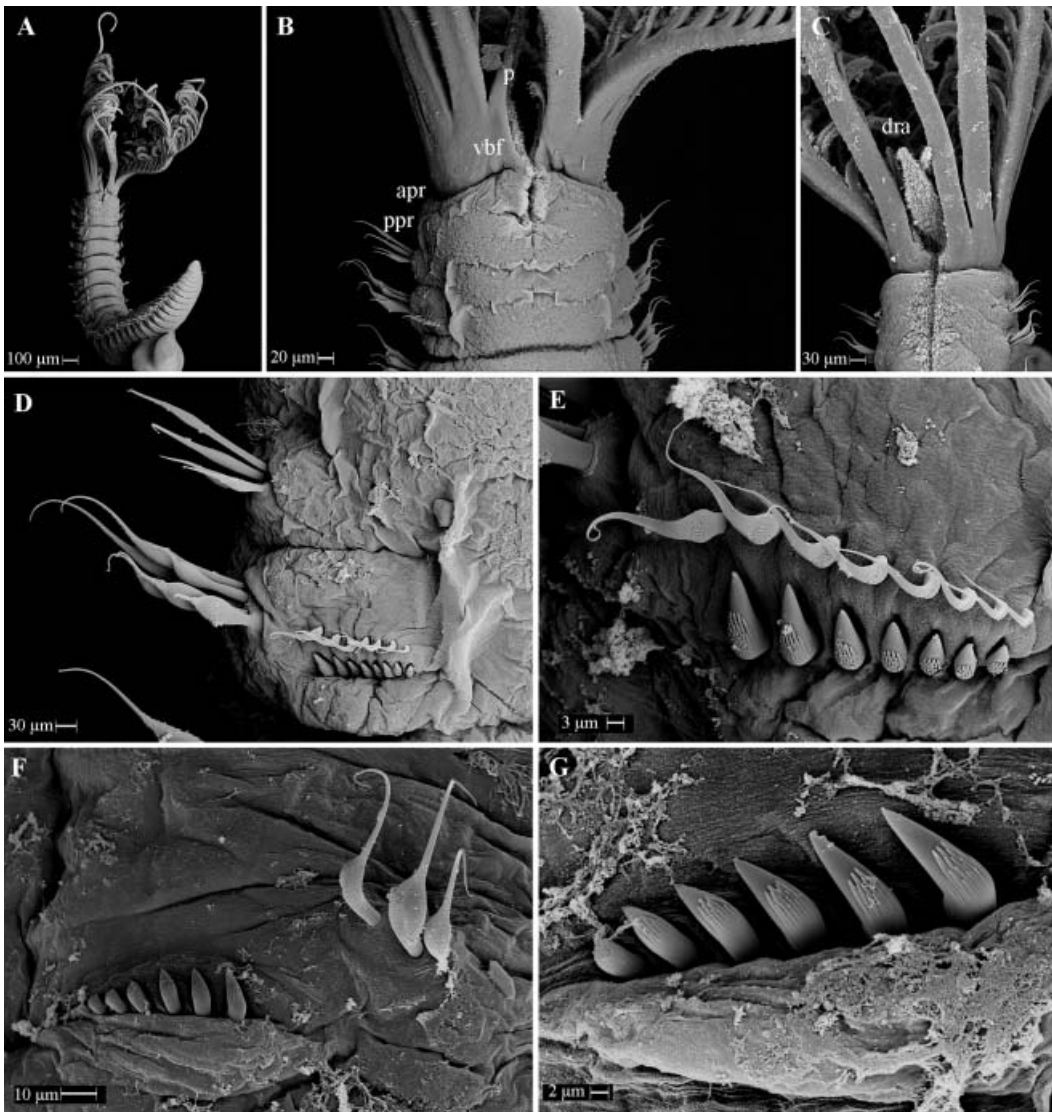


Figure 5. *Amphiglena bondi* sp. nov. SEM preparation, paratypes, AM W30480. (A) Whole specimen, ventral view; (B) anterior segments and base of crown, ventral view; (C) anterior segments, dorsal view; (D) first and second thoracic chaetigers, ventral view; (E) uncini and companion chaetae second thoracic chaetiger; (F) parapodia, first abdominal segment; (G) uncini, first abdominal segment.

medium in length (Figure 4A). Companion chaetae geniculated, with straight shaft and elongate mucro with several basal teeth of same size (Figure 5D, E). Abdominal chaetigers with up to three broadly hooded abdominal neurochaetae in each fascicle (Figure 5F) and up to six uncini per torus with several rows of small teeth of similar size above main fang, breast well developed, short handle (Figures 4B, 5F, G). Pygidial eyes present, pair of irregular brown spots on lateral margins of pygidium. Statocysts not seen. Tube clear mucous sheath with some sediment. Eggs observed from the third to the tenth abdominal chaetiger.

Variation

Some specimens can have seven thoracic and from nine to 19 abdominal chaetigers. The number of radioles does not vary among the specimens. The number of thoracic and abdominal uncini may differ from holotype. Eggs normally begin in the second abdominal chaetiger.

Remarks

Based on the results of the cladistic analysis, the presence of eggs from the second abdominal chaetiger is an apomorphy for *A. bondi* sp. nov., though this is also seen in *A. jimenezi*, *A. lindae*, and some *A. maiteae* sp. nov. and so may not remain an apomorphy if the *Amphiglena* tree topology changes. *Amphiglena bondi* sp. nov. is also characterized by a unique combination of characters: the presence of six pairs of radioles, small dorsal radiolar appendages, thoracic uncini with a well-developed breast, medium in width and with medium or short handles. The most similar species are *A. magna* sp. nov. and *A. lenae* sp. nov. *Amphiglena bondi* sp. nov. is distinguished from *A. magna* sp. nov. by the location of the eggs, in that they are from the first abdominal segment in *A. magna* sp. nov. Also the ventral basal flanges continue to the peristomium in *A. bondi* sp. nov. while in *A. magna* sp. nov. they are folded in the junction of crown and thorax. *Amphiglena lenae* sp. nov. is distinguished from *A. bondi* sp. nov. by the shape of the uncinial breast, which is well developed but short and pointed.

Etymology

This species is named after the type locality, one of Australia's most famous beaches.

***Amphiglena lenae* sp. nov.**
(Figures 4E, F, 6)

Material studied

Australia, Western Australia. Holotype: AM W30003, Red Bluff, Kalbarri, 27°42' S, 114°09' E, rocky shore, mixed coralline algae, 3–4 m, 10 January 1984. Paratypes: AM W30004, same sample (70 specimens); AM W30478, AM W30479, same sample, (SEM). Additional material: AM W30005, Red Bluff, Kalbarri, 27°42' S, 114°09' E, rocky shore, mixed brown algae, 3–4 m, 10 January 1984 (10 specimens).

Description

Holotype with 2.5 mm body length and 1 mm branchial crown length. Eight thoracic and 15 abdominal chaetigers. Five radioles on each side of branchial crown (Figure 6A). Palmate membrane and radiolar flanges absent (Figure 6B, C). Radioles with two longitudinal rows of pinnules alternating along the length, similar in size except for distally (Figure 6A); tip of radioles longer than distal pinnules. Radiolar skeleton with two rows of cells. Ventral basal flanges present as rounded lappets from the base of crown, continuing posteriorly and fused to posterior peristomial ring (Figure 6B, C). Dorsal lips with elongate, ciliated, dorsal radiolar appendages about one-quarter length of branchial crown, not longer than proximal pinnule in ventralmost radioles; with no skeleton. Dorsal pinnular

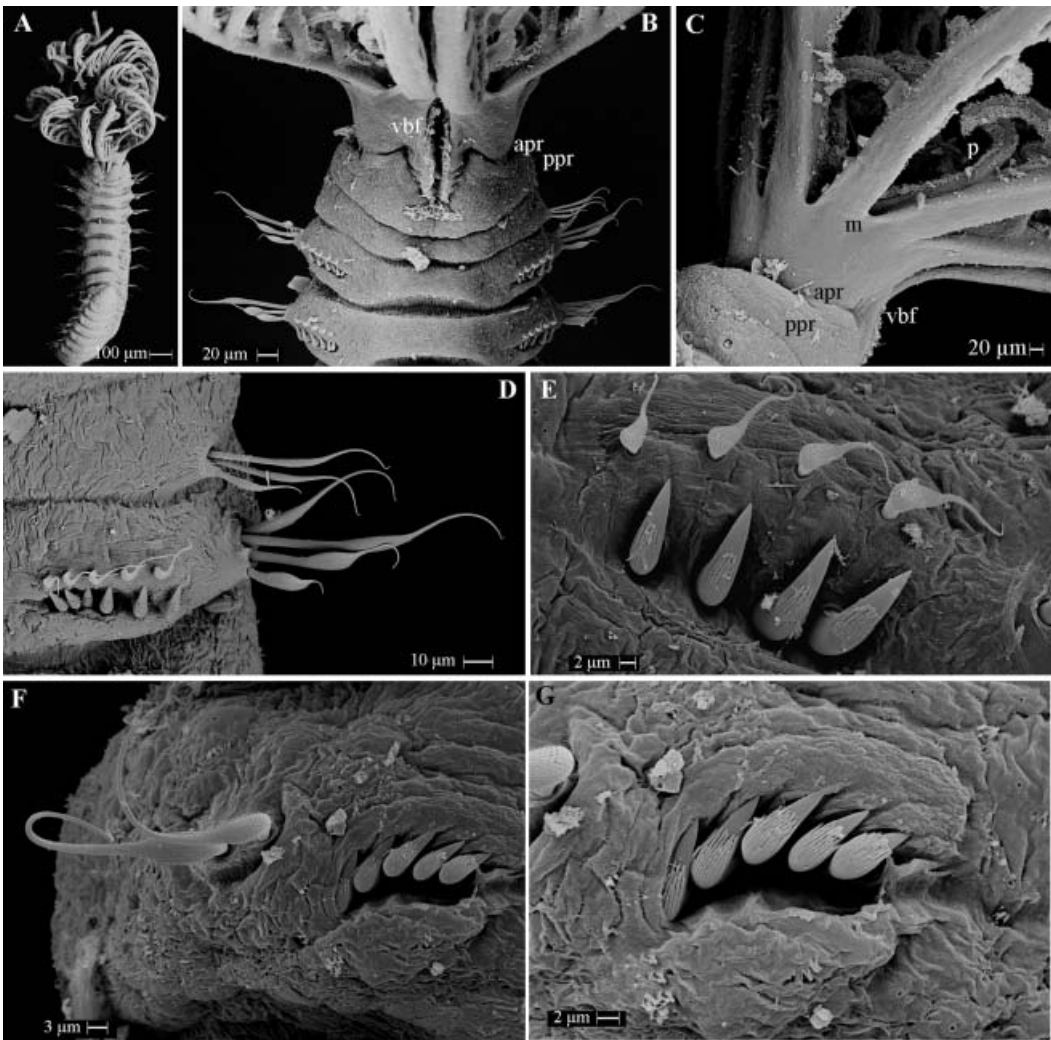


Figure 6. *Amphiglena lenae* sp. nov. SEM preparation, paratype, AM W30479. (A) Whole specimen, ventral view; (B) anterior segments and base of crown, ventral view; (C) base of crown and peristomium, lateral view; (D) first and second thoracic chaetigers, ventral view; (E) uncini and companion chaetae, seventh thoracic chaetiger; (F) neurochaetae and uncini, ventral view, mid-abdominal segment; (G) uncini, same segment.

appendages absent. Ventral lips absent. Anterior peristomial ring inconspicuous (Figure 6B, C). Posterior peristomial ring with a mid-ventral incision and margins prolonged anteriorly; collar absent. Peristomial eyes as two reddish and circular spots. Thorax longer than wide with quadrangular ventral shields similar in size. One narrowly hooded and two broadly hooded notochaetae of first thoracic chaetiger (Figure 6D). Other thoracic chaetigers with one superior broadly hooded notochaeta and two paleate inferior thoracic notochaetae (Figure 6D). Up to eight thoracic uncini per torus, increasing in size from the ventral side to lateral margin of torus, with numerous rows of small teeth of similar size above main fang, arranged in upper half of uncini (Figure 6D, E); breast well developed, reaching level to tip of main fang, handles medium to short (Figure 4C). Companion chaetae geniculated, with straight shaft and elongate mucro with several basal

teeth of similar size (Figure 6D, E). Abdominal chaetigers with two broadly hooded abdominal neurochaetae per fascicle (Figure 6F) and up to eight uncini per torus, with several rows of small teeth of similar size above main fang, breast well developed, short handled (Figures 4D, 6G). Pygidial eyes present, pair of irregular brown spots on lateral margins of pygidium. Statocysts not seen. Tube unknown. Eggs from chaetiger one to seven, though not all of these.

Variation

A few specimens have four radioles, but most have five radioles with long tips and pinnules similar in length all along. The number of chaetae and uncini vary slightly among specimens. Some specimens lack eggs, which if present, appear from the first abdominal chaetigers for up to seven segments.

Remarks

This species is one of the apomorphic *Amphiglena* species and shares with *A. bondi* sp. nov. and *A. magna* sp. nov. the presence of pinnules of similar length along the radioles. It is distinguishable from other species of *Amphiglena* by the shape of the thoracic uncini. It is the only species with a well-developed but short breast (the space from breast to main fang is larger than the breast). There is also a character combination unique to this species in the presence of five (sometimes four) pairs of radioles with between 10 and 15 pinnules, long ventral basal flanges extending to peristomium with posterior peristomial ring extending forward ventrally on each side of the median incision and small radiolar appendages (less than one-quarter of the length of radioles).

Etymology

It is our pleasure to name this species for Dr Elnae Kupriyanova (University of Adelaide) for her work on serpulid polychaetes and on assessing their relationship with sabellids.

***Amphiglena magna* sp. nov.** (Figures 4G, H, 7)

Material studied

Australia, Victoria. Holotype: MV F109494, Cheviot Beach, Point Nepean, 38°12' S, 144°40' E, 3.5–5 m, 31 March 1998. Paratypes: MV F91001, same locality (46 specimens), AM W30486, same locality (one specimen, SEM). Additional material: MV F90998, Off Eagles Nest, Bunurong, 38°40' S, 145°39' E, 1 April 1997 (13 specimens).

Description

Holotype 5 mm long (branchial crown 1.5 mm). Eight thoracic and 21 abdominal chaetigers. Seven radioles on each side of the branchial crown. Radioles with numerous pinnules in two rows, alternating along length, similar in size all along; tip of radioles as long as distal pinnules (Figure 7A). Palmate membrane inconspicuous and radiolar flanges absent (Figure 7B, C). Radiolar skeleton with two rows of cells. Short ventral basal flanges

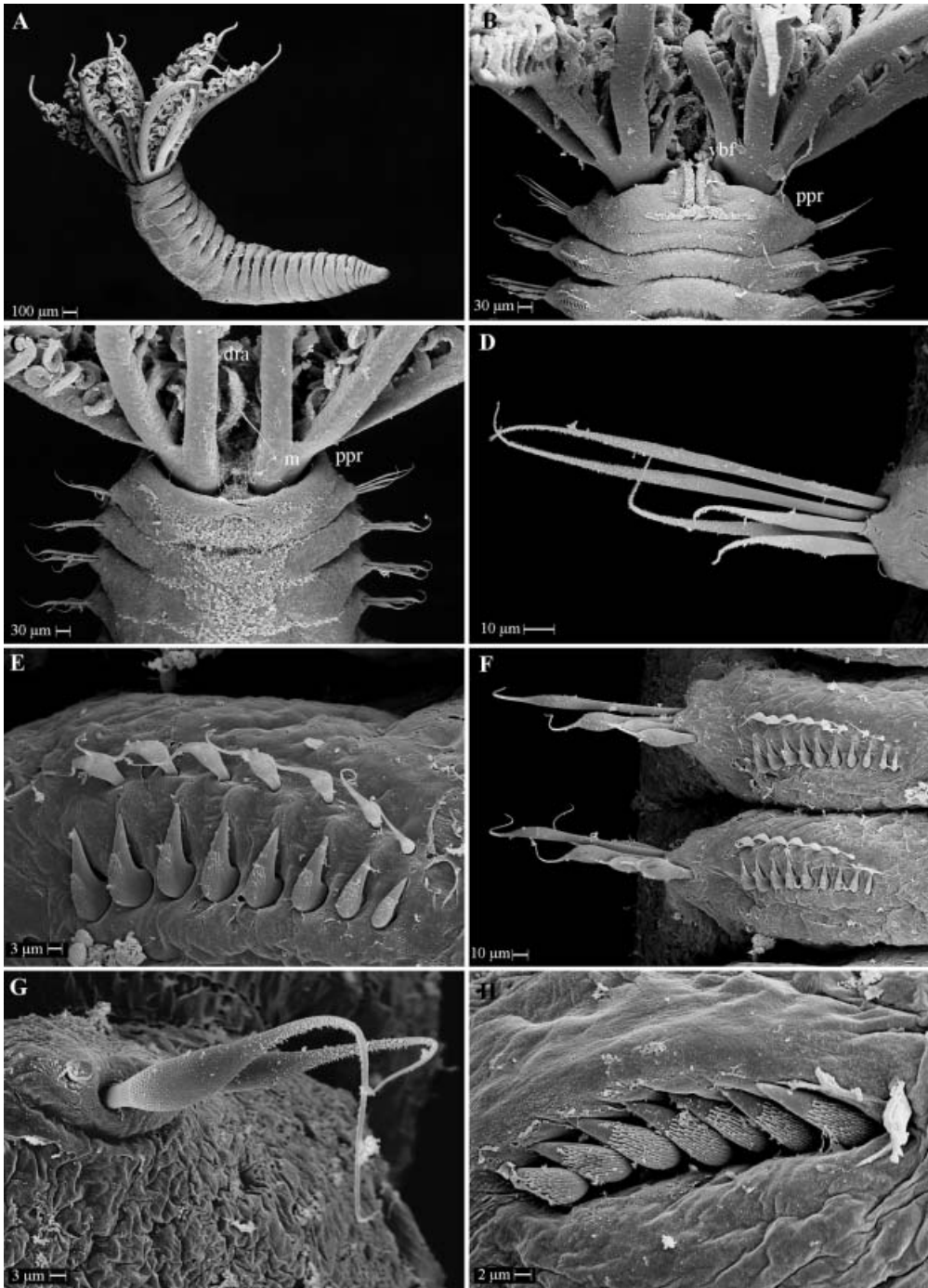


Figure 7. *Amphiglena magna* sp. nov. SEM preparation, paratype, AM W30486. (A) Whole specimens, dorsolateral view; (B) anterior segments and base of crown, ventral view; (C) same, dorsal view; (D) notochaetae, first thoracic chaetiger, ventral view; (E) uncini and companion chaetae second thoracic chaetiger; (F) six and seventh thoracic chaetigers, ventral view; (G) neurochaetae, anterior abdominal segment; (H) uncini, anterior abdominal chaetiger.

restricted to base of crown, not continuing posteriorly (Figure 7B). Dorsal lips with elongate, ciliated, dorsal radiolar appendages of about one-quarter the length of branchial crown. Dorsal pinnular appendages absent. Ventral lips absent. Anterior peristomial ring indistinguishable (Figure 7A–C). Posterior peristomial ring with a mid-ventral incision and ventral margins prolonged anteriorly on both sides of incision; collar absent. Peristomial eyes as two brownish spots. Thorax longer than wide with quadrangular ventral shields similar, not in contact with the thoracic tori. Two narrowly hooded superior and three broadly hooded notochaetae on first chaetiger (Figure 7D). Other thoracic chaetigers with one or two superior broadly hooded notochaetae and three paleate inferior thoracic notochaetae (Figure 7F). Up to 10 thoracic uncini per torus, increasing in size from the ventral side to the lateral margin of torus, with numerous rows of small teeth of similar size above the main fang, arranged in upper half of uncini (Figure 7E, F); breast well developed, reaching the level of tip of the main fang, handles medium in length (Figure 4G). Companion chaetae geniculated, with straight shaft and elongate mucro with several basal teeth of similar size (Figure 7E, F). Abdominal chaetigers with two broadly hooded abdominal neurochaetae per fascicle (Figure 7G) and up to eight uncini per torus, with several rows of small teeth of similar size above the main fang (Figure 7H), breast well developed, with a convex base, short handle (Figure 4H). Pygidial eyes as a brown spot on each side of pygidium. Statocysts not seen. Tube unknown.

Variation

Amphiglana magna sp. nov. is generally larger than other species of *Amphiglana*, except *A. pacifica* which can measure up to 20 mm (Rzhavsky 1995), but small specimens have also been found, measuring 2 mm long and with 15 abdominal chaetigers. Some specimens show slight variation in the number of radioles in that some have six pairs with up to 20 pinnules along radioles. Some specimens have eggs in the anterior abdominal chaetigers.

Remarks

The specimens of *A. magna* sp. nov. are large compared to other *Amphiglana*. Additionally, a distinguishing feature is the shape and length of the ventral basal flanges that terminate at the junction of crown and thorax, though this is seen in *A. gracilis* sp. nov., *A. terebro*, and *A. mediterranea*. However, these species are distinguishable from *A. magna* sp. nov. in having fewer radioles, the pinnules along radioles not being similar in length, and having larger dorsal radiolar appendages.

Etymology

The name of this species refers to its size, one of the largest in the genus.

***Amphiglana maiteae* sp. nov.** (Figures 4I, J, 8)

Material studied

Australia, New South Wales. Holotype: AM W30510, Sand Point, south of Lennox Head, New South Wales, Australia (28°51' S, 153°36'30" E), high energy rock platform, scrapings

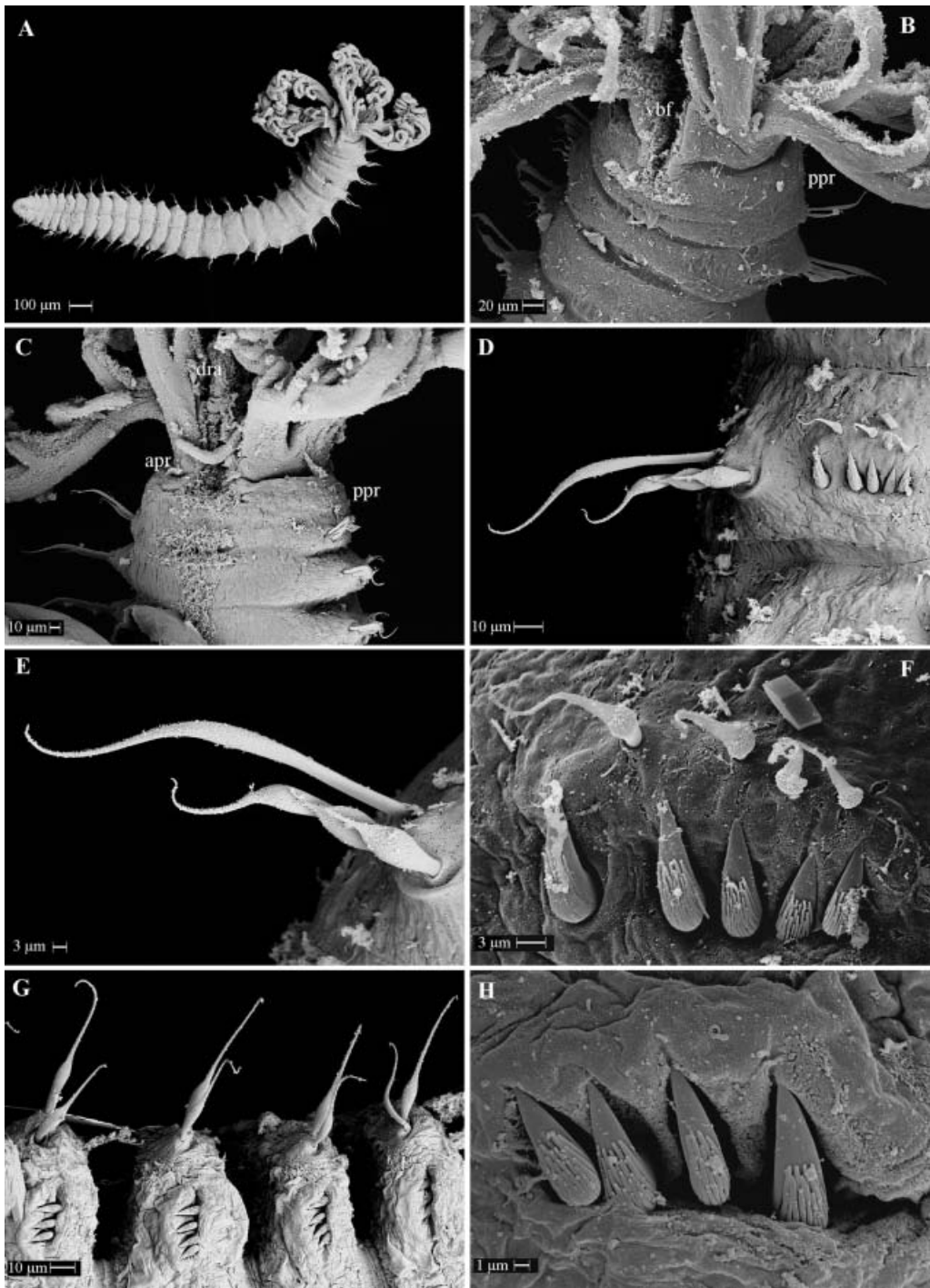


Figure 8. *Amphiglana maiteae* sp. nov. SEM preparation, paratypes, AM W30399, AM W30400. (A) Whole specimen, ventral view; (B) anterior segments and base of crown, ventral view; (C) same, dorsal view; (D) second thoracic chaetiger, ventral view; (E) notochaetae, second thoracic chaetiger; (F) uncini and companion chaetae, second thoracic segment; (G) mid-abdominal segment, dorsal view; (H) mid abdominal uncini.

from rocks and boulders, intertidal, 1 March 1992. Paratypes: AM W25995, same sample (29 specimens); AM W30400, same sample (one specimen, SEM).

Description

Holotype 2 mm long, branchial crown 0.5 mm long. Eight thoracic and 16 abdominal chaetigers. Four radioles on each side of the branchial crown. Palmate membrane and radiolar flanges absent (Figure 8B, C). Radioles with two longitudinal rows of pinnules alternating along the length, in the distal half of the radiole they are longer than in the proximal half (Figure 8A). Tip of radioles not longer than the distal pinnules. Radiolar skeleton with two rows of cells. Ventral basal flanges from the proximal pinnule of the ventralmost radiole, continuing and fused to the posterior peristomial ring, with a transversal incision (Figure 8B). Dorsal lips with elongate, ciliated, dorsal radiolar appendages of about one-quarter the length of the branchial crown, not longer than the length of the proximal pinnule in the ventralmost radiole; with no skeleton. Dorsal pinnular appendages absent. Ventral lips absent. Anterior peristomial ring narrow, only visible over the posterior peristomial ring on both margins of the faecal groove (Figure 8C). Posterior peristomial ring with a mid-ventral incision, where the ventral basal flanges are attached; collar absent (Figure 8B). Peristomial eyes as two brown circular spots. Thorax longer than wide with quadrangular ventral similarly sized shields. Three chaetae on the first chaetiger, superior chaetae narrowly hooded, inferior broadly hooded (Figure 8D). Rest of chaetigers with one superior thoracic notochaeta broadly hooded, broader than collar chaetae, and up to three inferior paleate thoracic notochaetae, in a single row (Figure 8E). Second to last thoracic chaetiger with up to six thoracic uncini per fascicle, increasing in size from the ventral to the lateral margin of torus, with numerous rows of small, similarly sized teeth, above the main fang, arranged in the upper half of the main fang (Figure 8F); breast well developed, handles medium to short (Figure 4I). Companion chaetae geniculated, with straight shaft and elongate mucro with several teeth of the same size in the basis (Figure 8F). Abdominal chaetigers with up to three broadly hooded abdominal neurochaetae in each fascicle (Figure 8G) and up to four uncini per torus with several rows of small teeth of similar size above the main fang (Figure 8H), breast well developed, short-sized handle (Figure 4J). Pygidial eyes as irregular brown spots in both lateral margins of pygidium. Statocysts not seen. Tube unknown. Eggs observed in the second and third abdominal chaetiger.

Variation

Some specimens have seven thoracic and from nine to 19 abdominal chaetigers. The number of radioles seems not to vary among the specimens. The number of thoracic and abdominal chaetae are as the holotype. Eggs begin normally in the second abdominal chaetiger but they can begin at up to the sixth abdominal chaetiger.

Remarks

Amphiglena maiteae sp. nov. is the only species we erect in this study that is not monophyletic in our cladistic analysis. However, as discussed above, monophyly is not a requisite for the erection of species taxa. *Amphiglena maiteae* sp. nov. (from Australia) is most similar to *A. nishii* sp. nov. (from Japan) but clearly differs from it in the length of the uncini handles and number of radioles. In trees in which *A. maiteae* sp. nov. is

monophyletic (in three of the five shortest trees) the species is supported by the even height of the anterior peristomial ring, though this is seen also in *A. gracilis* sp. nov., *A. jimenezi*, *A. pacifica*, and *A. terebro*.

Etymology

It is our pleasure to name this species for María Teresa Aguado, a good friend and in recognition of her valuable contributions to polychaete taxonomy.

***Amphiglana nishii* sp. nov.**

(Figures 4K–N, 9)

Amphiglana pacifica (Rouse and Gambi 1997, 1998a; Fitzhugh and Rouse 1999; not *Amphiglana pacifica* Annenkova, 1934).

Material examined

Japan, Misaki. Holotype: SAM E3638, intertidal algal mats, 139°36'8" E, 35°09' N, 20 May 1998. Paratypes: SAM E3639, same sample (five specimens), AM W30485, same sample (one specimen, SEM).

Description

Holotype 1.8 mm long, branchial crown 1 mm long, with eight thoracic and 15 abdominal chaetigers. Crown is about half body length, with four pairs of radioles with about nine pairs of alternating long pinnules; tip of radioles is longer than pinnules (Figure 9A). Dorsal lips about one-quarter of length of crown (Figure 9C). Anterior peristomial ring short, only visible ventrally (Figure 9B, C). Posterior peristomial ring of even height all around, slightly enlarged ventrally on margins of mid-ventral groove; collar absent (Figure 9B, C). Ventral basal flanges present from basal pinnules of ventralmost radioles to middle of posterior peristomial ring (Figure 9B). Peristomial eyes brown and circular. Thorax longer than wide with quadrangular similarly sized ventral shields. Two notochaetae in first thoracic chaetiger, one narrowly hooded and another broadly hooded (Figure 9D). Rest of chaetigers with one superior broadly hooded thoracic notochaeta, and two inferior thoracic paleate notochaetae, in a single row (Figure 9D). Second to 8th chaetiger with four to six thoracic uncini per fascicle, similar in size, with about four rows of small, similarly sized teeth, above the main fang, arranged in the upper half of uncini (Figure 9D, E); breast well developed, as long as the tip of main fang, handles short (Figure 4K, L). Companion chaetae geniculated, with straight shaft and elongate mucro with several basal teeth of similar size (Figure 9D, E). About four abdominal uncini per torus with several rows of small teeth of similar size above main fang (Figure 9F, G), breast well developed, handle short, about half of uncini width (Figure 4M, N). Three broadly hooded abdominal neurochaetae in each fascicle (Figure 9G). Pygidial eyes as four to six orange-brown spots on each side of lateral margins of peristomium. Statocysts not seen. Tube unknown.

Variation

Length of the body 0.8–3 mm, branchial crown 0.2–1 mm. Four specimens with eight thoracic chaetigers and one with six, the number of abdominal chaetigers is between 10 and

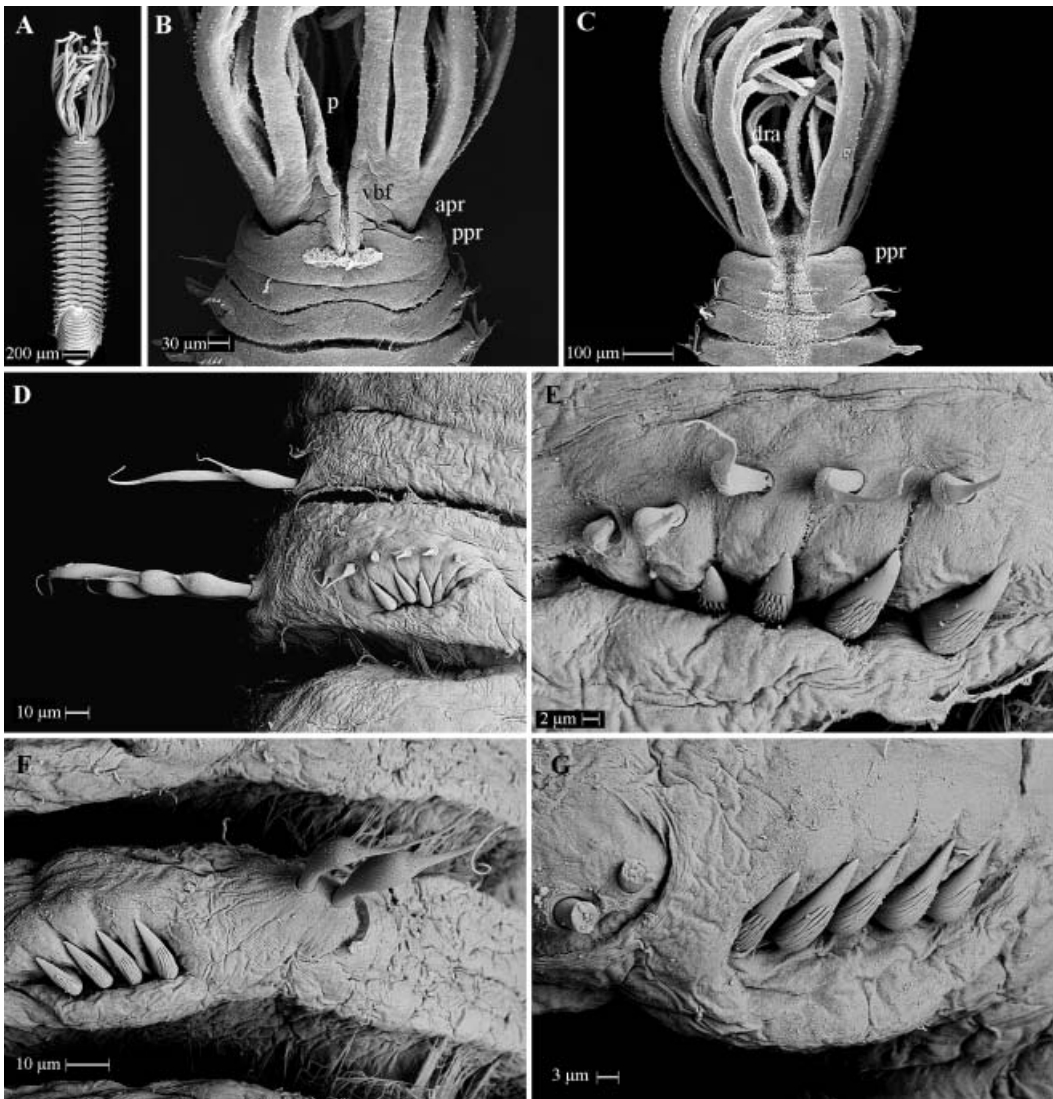


Figure 9. *Amphiglena nishii* sp. nov. SEM preparation, paratype, AM W30485. (A) Whole specimen, ventral view; (B) anterior segments and base of crown, ventral view; (C) crown and anterior segments, dorsal view; (D) first and second thoracic chaetigers, ventral view; (E) uncini and companion chaetae second thoracic chaetiger; (F) parapodia, mid-abdominal segment; (G) uncini, mid-abdominal segment.

36. Two juvenile specimens with five and eight thoracic and one and three abdominal chaetigers, respectively. The crown is one-third to half the length of the body, with four or five radioles with scarce alternating and very long pinnules arranged in the proximal part of radioles. None of the specimens have gametes.

Remarks

This species is characterized by short thoracic uncini in thoracic and abdominal chaetigers with a broad and long breast. It also has a short posterior peristomial ring of even height,

only slightly projecting forward on both sides of the mid-ventral incision. The most similar species is *A. maiteae* sp. nov. but they differ in the length of the uncinal handles. *Amphiglana nishii* sp. nov. differs from the other species of the genus recorded in Japan, *A. pacifica*, in the length of the thoracic and abdominal uncini handles, which are both much shorter in the new species. The uncinal breast is also shorter and narrower in both thoracic and abdominal uncini of *A. pacifica* and the abdominal uncini have a long neck (Annenkova 1934; Rzhavsky 1995). The data on reproductive mode and sperm morphology given by Rouse and Gambi (1997, 1998b) and Fitzhugh and Rouse (1999) for *A. pacifica* refer to *A. nishii* sp. nov. and not to *A. pacifica* Annenkova.

Etymology

It is our pleasure to name this species for Dr Eijiroh Nishii, Yokohama National University, Japan in recognition of his valuable contributions to polychaete taxonomy.

Amphiglana jimenezzi Capa and López, 2004

(Figure 10)

Amphiglana jimenezzi Capa and López 2004, p 67–69, Figures 3, 4.

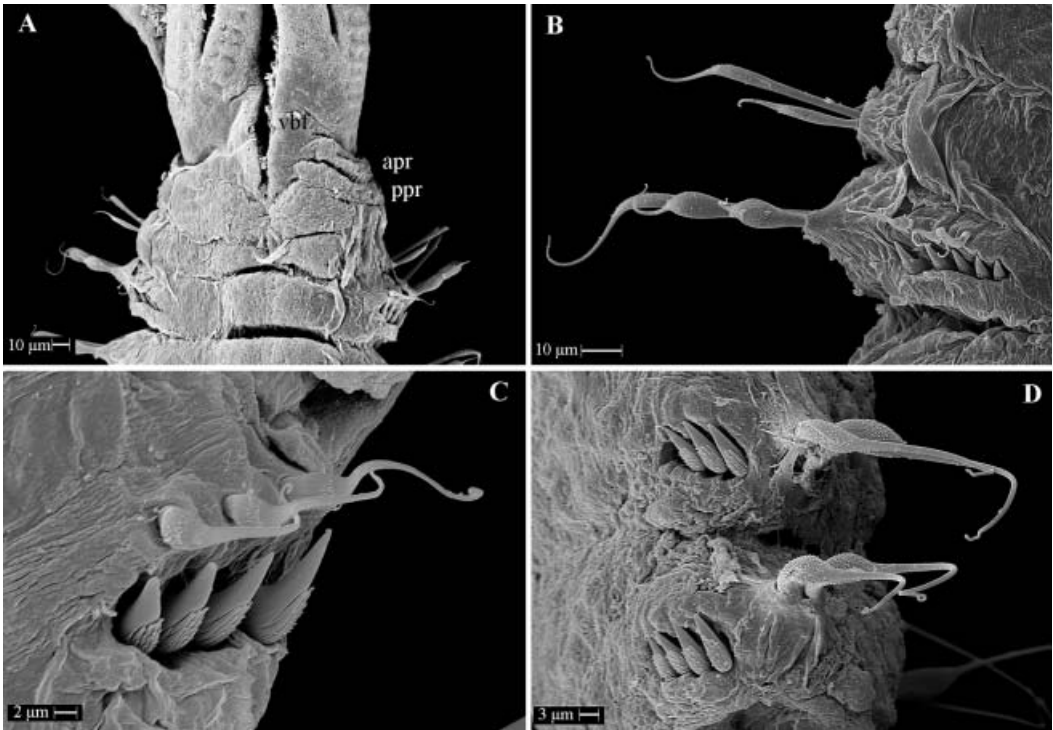


Figure 10. *Amphiglana jimenezzi*. SEM preparation, paratypes, AM W30487. (A) Anterior segments and base of crown, ventral view; (B) second and third thoracic chaetigers, ventral view; (C) uncini and companion chaetae, seventh thoracic segment; (D) mid-abdominal segment, lateral view.

Material examined

Panama, Coiba National Park. Holotype: MNCN 16.01/8880a, Granito de Oro Island, 7°35'30"N, 81°42'30"W, 2 m. Paratypes: MNCN 16.01/8880b, same locality; MNCN 16.01/8882, Rosario Beach, Coiba Island, 7°38'30"N, 81°41'40"W, 2.5 m; MNCN 16.01/8883, Ensenada Maria, Coiba Island, 7°24'20"N, 81°41' W, 6 m. The paratype MNCN 16.01/8881, Canal de Afuera Island, 7°41'50"N, 81°38'25"W, 4 m, is now on a stub for SEM in the Australian Museum (AM W30487).

Remarks

In the original description some important features were not described. Large ventral basal flanges extending posteriorly to the posterior peristomial ring are present (Figure 10A). Anterior peristomial ring (though only visible on one side in Figure 10A) is of even height all around (see Capa and López 2004). Posterior peristomial ring is low and not projected ventrally (Figure 10A). In the cladistic results provided here, this species forms a clade with *A. bondi* sp. nov., *A. magna* sp. nov., *A. lenae* sp. nov., and *A. nathae* based on the number of pinnules in radioles and the small size of the dorsal radiolar appendages.

Amphiglena terebro Rouse, 1993

(Figure 11)

Amphiglena terebro Rouse 1993, p 5–13, Figures 1–45.

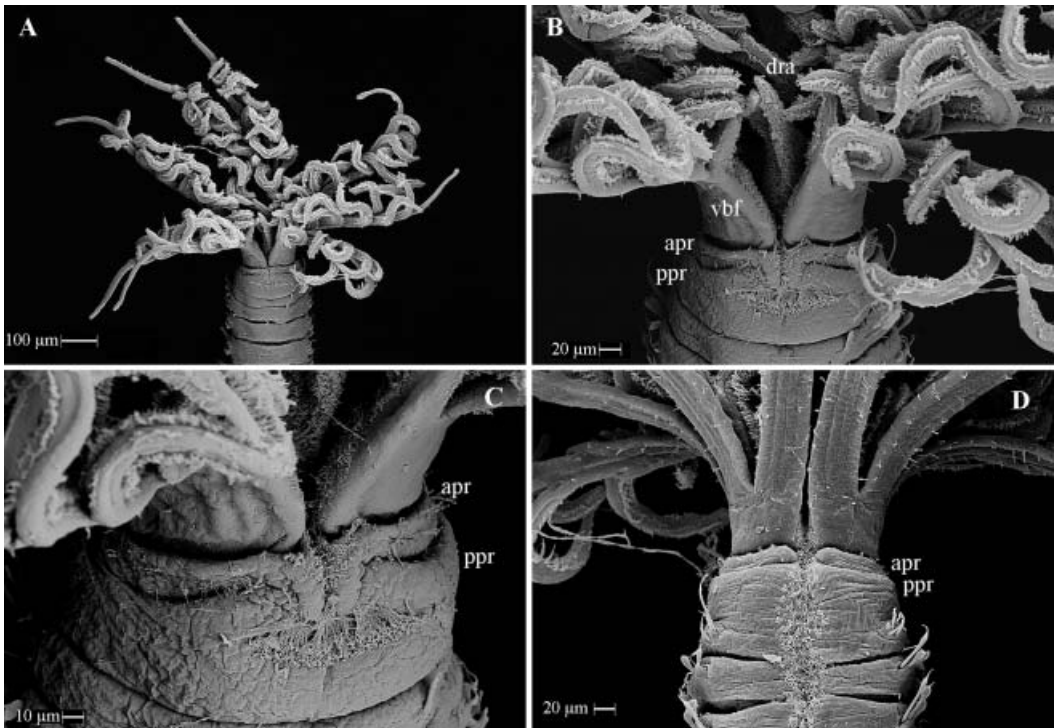


Figure 11. *Amphiglena terebro*. SEM preparation, AM W30482. (A) Anterior segments and crown, ventral view; (B) detail of anterior segments and base of crown; (C) anterior and posterior peristomial rings; (D) anterior segments and base of crown, dorsal view.

Material examined

Australia, New South Wales. Holotype: AM W21357, North Bondi, New South Wales, Australia (33°58' S, 151°00' E), in calcareous base of *Corallina officinalis*, intertidal, 20 February 1992. Paratypes: W21358–60, same locality (three specimens). Additional material: AM W30019, North Maroubra, 33°57' S, 151°15' E, coralline encrusting algae in rock platform, intertidal, 2 July 2005 (six specimens), AM W30482, same sample (one specimen, SEM).

Remarks

This species is very distinctive in that the thoracic uncini have a narrow breast and abdominal uncini have long handles (Rouse 1993). In the original description the ventral basal flanges are described as coming from the posterior peristomial ring (Rouse 1993), but study of new specimens with SEM shows that these paired, narrow lappets are located from the base of the first pinnule of the ventral radioles to the base of the crown and do not continue into the peristomium (Figure 11B, C). The anterior peristomial ring is conspicuous all the way around and divided ventrally by the mid-ventral incision (Figure 11A–D). The posterior peristomial ring, apart from the ventral incision, has a constant height all around (Figure 11B, D).

Key to the Australian and Japanese species of *Amphiglana*

1. Four to six pairs of pinnules, proximal ones reaching the tip of radioles *A. gracilis* sp. nov.
- Six to 10 pairs of pinnules 2
- Ten to 15 pairs of pinnules 3
- Fifteen to 20 pairs of pinnules 5
- 2(1). Short thoracic uncini handles. Ventral basal flanges continuing to peristomium. *A. nishii* sp. nov.
- Medium thoracic uncini handles. Ventral basal flanges continuing to peristomium *A. maiteae* sp. nov.
- Long thoracic uncini with long handles and narrow breasts. Ventral basal flanges reaching the junction of crown and body *A. terebro*
- 3(1). Short thoracic uncini handles *A. lenae* sp. nov.
- Medium thoracic uncini handles 4
- 4(3). Ventral basal flanges reach posteriorly to the junction of crown and thorax *A. magna* sp. nov.
- Ventral basal flanges continue posteriorly to peristomium *A. bondi* sp. nov.
- 5(1). Thoracic uncini with medium length handles and broad breast; abdominal uncini with short handles and broad breast *A. magna* sp. nov.
- Thoracic uncini with long handles and narrow breast; abdominal uncini with long handles and narrow breast *A. pacifica*

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Appendix 1. Characters and states used in the analysis of the relationships among the *Amphiglena* species

See text for discussion of characters.

1. Number of radioles: (0) two pairs; (1) four pairs; (2) five pairs; (3) six pairs; (4) more.
2. Radiolar skeleton axis cells: (0) two cells; (1) four or more cells.
3. Number of pinnules: (0) 4–6; (1) 6–10 pairs; (2) 10–15 pairs; (3) 15–20 pairs.
4. Length of pinnules: (0) longer distally; (1) longer proximally; (2) similar in length all along.
5. Basal membrane: (0) absent or slightly developed; (1) well developed.
6. Ventral basal flanges: (0) absent; (1) present.
7. Length of ventral basal flanges: (0) to the junction of crown and body; (1) continue posteriorly across the peristomium.
8. Ventral lips: (0) absent; (1) present.
9. Parallel lamellae: (0) absent; (1) present.
10. Dorsal lips: (0) radiolar appendages absent; (1) radiolar appendages with branchial skeleton; (2) radiolar appendages without branchial skeleton.
11. Dorsal radiolar appendages: (0) about one-third the length of the crown; (1) about one-quarter the length of the crown; (2) smaller.
12. Dorsal pinnular appendages: (0) absent; (1) present.
13. Anterior peristomial ring: (0) even height all around; (1) not visible laterally.
14. Anterior margin of posterior peristomial ring: (0) horizontal; (1) projected forward ventrally.
15. Posterior peristomial ring collar: (0) absent; (1) present.
16. Peristomial eyes: (0) absent; (1) present.
17. Number of thoracic chaetigers: (0) eight; (1) more; (2) less.
18. Superior thoracic chaetae: (0) narrowly hooded; (1) broadly hooded.
19. Inferior thoracic chaetae: (0) paleate; (1) broadly hooded.
20. Thoracic uncini breast width: (0) broad; (1) narrow.
21. Thoracic uncini breast length: (0) double the space to main fang; (1) similar to the space; (2) shorter.
22. Thoracic uncini handles: (0) short; (1) medium; (2) long; (3) absent.
23. Companion chaetae shape: (0) symmetrical membrane; (1) asymmetrical membrane.
24. Neurochaetae on abdominal chaetae on singles rows: (0) present; (1) absent.
25. Abdominal uncini breast: (0) broad; (1) narrow.
26. Abdominal uncini handles: (0) short; (1) medium; (2) long; (3) absent.
27. Pygidial eyes: (0) absent; (1) present.
28. Eggs located from: (0) mid-abdomen (from 3rd abdominal segment); (1) anterior of abdomen (first or second abdominal segment).
29. Spermathecae: (0) absent; (1) present unpigmented; (2) present pigmented.

30. Spermiogenesis: (0) tetrads; (1) cytophores with more than 100 spermatids; (2) cytophores with less than 100 spermatids.
31. Sperm nucleus morphology: (0) spherical; (1) elongate; (2) cylindrical.
32. Sperm nucleus shape: (0) not spiralled; (1) spiralled distally; (2) spiralled along most length.
33. Sperm acrosome shape: (0) not spiralled; (1) tightly spiralled; (2) loosely spiralled.
34. Sperm nuclear fossa: (0) absent; (1) present.
35. Sperm mitochondria: (0) spherical; (1) elongate and spirals; (2) elongate and straight.
36. Hermaphroditism: (0) absent; (1) present. Sperm and eggs in different segments.
37. Larval development: (0) planktonic; (1) brooded, with larval release before crown development; (2) brooded, with juvenile release after crown development.

Appendix 2. Material studied and references used for scoring the matrix

Abbreviations are explained in the Acknowledgements.

Potamilla neglecta: syntypes ZMB 6796, Knight-Jones (1983).

Kirkia rizo: Rouse (1996).

Laonome albicingillum: Hsieh (1995), Fitzhugh (2003).

Terebrasabella heterouncinta Fitzhugh and Rouse (1999), Murray and Rouse (forthcoming).

Amphiglena jimenezi: holotype MNCN 16.01/8880a, paratypes MNCN 16.01/8880b, MNCN 16.01/8882, MNCN 16.01/8883, AM W30487, Capa and López (2004).

A. lindae: W. 23935, Rouse and Gambi (1997).

A. mediterranea: Rouse and Gambi (1997).

A. nathae: Rouse (1994).

A. pacifica: Annenkova (1934), Rzhavsky (1995).

A. terebro: holotype AM W21357, paratypes AM W21360, AM W21358, AM W21359, Rouse (1993).

A. gracilis: holotype AM W30002, paratypes AM W26004, AM W30483, AM W30484.

A. bondi: holotype SAM E3636, paratypes SAM 3637, AM W30480.

A. lenae: holotype AM W30003, paratypes AM W30004, AM W30478, AM W30479.

A. magna: holotype MV F109494, paratypes MV F91001, AM W30486.

A. nishii: holotype SAM E3639, paratypes SAM E3639, AM W30485.

A. maiteae: holotype AM W30510, paratypes AM W25995, AM W30398–AM W30400.

Appendix 3. Character state distributions among outgroup and ingroup taxa used to determine relationships within *Amphiglena* with other genera

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
<i>Potamilla neglecta</i>	4	1	3	2	1	0	–	1	1	0	–	1	0	0	1	0	0	0	0	0	1	2	1	1	0	1	0	?	?	1	1	?	?	?	?	?	2	
<i>Kirkia rivoi</i>	2	0	3	1	0	0	–	1	1	0	–	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	
<i>Laonome albicingillum</i>	4	0	3	2	1	0	–	1	1	0	–	0	0	0	1	0	0	0	0	0	0	3	–	1	0	3	0	1	?	0	0	0	0	0	0	1	?	
<i>Terebrasabella heterouncinata</i>	0	0	0	1	0	1	0	0	0	2	1	0	0	0	1	0	0	1	1	0/1	0/2	2	1	0	1	2	0	1	0	2	1	0	0	0	0	?	1	
<i>A. jimenezi</i>	1	0	2	0	0	1	1	0	0	2	2	0	0	1	0	1	0	1	0	0	1	1	0	0	1	1	1	0	?	?	?	?	?	?	?	1	?	
<i>A. lindae</i>	1	0	1	1	0	1	1	0	0	2	1	0	1	1	0	1	0	1	0	0	1	2	0	0	0	1	1	0	2	2	1	2	2	1	1	1	?	
<i>A. mediterranea</i>	2	0	1	0	0	1	0	0	0	2	1	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1	2	2	1	2	1	1	1	2	
<i>A. nathae</i>	2	0	2	0	0	1	1	0	0	2	2	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	1	1	1	2	1	1	2	1	1	1	1	
<i>A. pacifica</i>	2/3	0	3	1	0	1	1	0	0	2	?	0	?	?	0	1	0	1	0	1	1	2	0	0	1	2	1	?	?	?	?	?	?	?	?	?	?	
<i>A. terebro</i>	1	0	1	2	0	1	0	0	0	2	2	0	0	0	1	1	1	0	1	2	2	0	0	0	1	1	1	1	0	2	1	2	2	1	2	1	1	
<i>A. maiteae</i> a	1	0	1	0	0	1	1	0	0	2	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	1	?	?	?	?	?	?	?	1	?
<i>A. maiteae</i> b, c	1	0	1	0	0	1	1	0	0	2	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	?	?	?	?	?	?	?	1	?
<i>A. gracilis</i> a, b	1	0	0	1	0	1	0	0	0	2	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	?	?	?	?	?	?	?	?	1	?
<i>A. gracilis</i> c	1	0	0	1	0	1	0	0	0	2	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1	?	?	?	?	?	?	?	1	?
<i>A. lenae</i> a	1	0	2	2	0	1	1	0	0	2	2	0	1	1	0	1	0	1	0	0	2	0	0	0	0	0	0	1	1	?	?	?	?	?	?	?	1	?
<i>A. lenae</i> b, c	2	0	2	2	0	1	1	0	0	2	2	0	1	1	0	1	0	1	0	0	2	0	0	0	0	0	0	1	1	?	?	?	?	?	?	?	1	?
<i>A. bondi</i> a, b, c	3	0	2	2	0	1	1	0	0	2	2	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	0	?	?	?	?	?	?	?	1	?
<i>A. magna</i> a	3	0	2	2	0	1	0	0	0	2	2	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1	?	?	?	?	?	?	?	1	?
<i>A. magna</i> b, c	4	0	2	2	0	1	0	0	0	2	2	0	1	1	0	1	0	1	0	0	1	1	0	0	0	0	0	1	1	?	?	?	?	?	?	?	1	?
<i>A. nishii</i> a	1	0	1	0	0	1	1	0	0	2	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	?	1	2	1	2	2	1	1	2
<i>A. nishii</i> b	2	0	1	0	0	1	1	0	0	2	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	?	1	2	1	2	2	1	1	1	2
<i>A. nishii</i> c	2	0	1	0	0	1	1	0	0	2	1	0	1	0	0	1	2	1	0	0	0	0	0	0	0	0	0	1	?	1	2	1	2	2	1	1	1	2