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Biodiversity and phylogeny of Ammotheidae (Arthropoda: Pycnogonida)

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Abstract. The family Ammotheidae is the most diversified group of the class Pycnogonida, with 297 species described in 20 genera. Its monophyly and intergeneric relationships have been highly debated in previous studies. Here, we investigated the phylogeny of Ammotheidae using specimens from poorly studied areas. We sequenced the mitochondrial gene encoding the first subunit of cytochrome c oxidase (CO1) from 104 specimens. The complete nuclear 18S rRNA gene was sequenced from a selection of 80 taxa to provide further phylogenetic signal. The base composition in CO1 shows a higher heterogeneity in Ammotheidae than in other families, which may explain their apparent polyphyly in the CO1 tree. Although deeper nodes of the tree receive no statistical support, Ammotheidae was found to be monophyletic and divided into two clades, here defined as distinct subfamilies: Achelinae comprises the genera *Achelia* Hodge, 1864, *Ammothella* Verrill, 1900, *Nymphopsis* Haswell, 1884 and *Tanystylum* Miers, 1879; and Ammotheinae includes the genera *Ammothea* Leach, 1814, *Acheliana* Arnaud, 1971, *Cilunculus* Loman, 1908, *Sericosura* Fry & Hedgpeth, 1969 and also *Teratonotum* gen. nov., including so far only the type species *Ammothella stauromata* Child, 1982. The species *Cilunculus gracilis* Nakamura & Child, 1991 is reassigned to *Ammothella*, forming the binomen *Ammothella gracilis* (Nakamura & Child, 1991) comb. nov. Additional taxonomic re-arrangements are suggested for the genera *Achelia*, *Acheliana*, *Ammothella* and *Cilunculus*.

Keywords. Pantopoda, taxonomy, DNA phylogeny, barcode of life, strand-bias.

Sabroux R., Corbari L., Krapp F., Bonillo C., Le Prieur S. & Hassanin A. 2017. Biodiversity and phylogeny of Ammotheidae (Arthropoda: Pycnogonida). *European Journal of Taxonomy* 286: 1–33. <http://dx.doi.org/10.5852/ejt.2017.286>

Introduction

Sea spiders (Arthropoda: Pycnogonida: Pantopoda) represent a small group of exclusively marine arthropods which are distributed worldwide, from the tropical to the polar regions, from littoral to abyssal depths. There are 1385 described species that are classified in 79 genera and 11 families (Bamber *et al.* 2015). They exhibit a typical “spider-like” appearance, with generally four pairs of walking legs attached to a slender trunk. They show a remarkable diversity of forms (from the slender *Nymphon* Fabricius, 1794 to the stout *Pycnogonum* Brünnich, 1764), a variable number of leg pairs (from 4 to 6), a wide size range (leg span from 1 to 700 mm) and a great diversity of colours, ranging from the palest to the most colourful (e.g., the multi-coloured *Anoplodactylus evansi* Clark, 1963) (Arnaud & Bamber 1987; Bamber *et al.* 2015). The position of sea spiders as a class of the subphylum Chelicerata is accepted by most recent molecular studies (Regier *et al.* 2010; Rehm *et al.* 2014; Roeding *et al.* 2009).

The pycnogonid families were distinguished based on the presence/absence and the structure of the three cephalic appendages: chelifores, palps and ovigers (e.g., Hedgpeth 1948). However, the number of families has varied heavily during the taxonomic history of Pycnogonida, from 8 families in Hedgpeth (1948) to 27 in Fry (1978). More recent classifications have recognized between 9 (Arnaud & Bamber 1987) and 11 families (Bamber 2007b). The position of some genera was also highly debated (e.g., *Pallenopsis* Wilson, 1881, *Endeis* Philippi, 1843, *Tanystylum* Miers, 1879). The four previous molecular studies on pycnogonids have questioned the validity of several families (Arango 2003b; Arango & Wheeler 2007; Nakamura *et al.* 2007; Arabi *et al.* 2010). Among them, the most problematic taxon probably remains the family Ammotheidae.

Originally, Dohrn (1881) gave an extensive definition of Ammotheidae by integrating the genera *Ammothea* Leach, 1814, *Barana* Dohrn, 1881 (currently accepted as *Ascorhynchus* Sars, 1878), *Clotenia* Dohrn, 1881 (accepted as *Tanystylum*) and *Trygaeus* Dohrn, 1881. Later during the same year, Hoek (1881) created the family Ascorhynchidae combining *Ascorhynchus*, *Ammothea* and *Tanystylum*, *inter alia*. The question of splitting *Ammothea* and *Ascorhynchus* (and their respective relatives) into two families has always been a matter of debate, because they show puzzling combinations of characters (e.g., the same chelae reduction vs different oviger structures). While most specialists included them in a single family (e.g., Arnaud & Bamber 1987; Dohrn 1881; Hedgpeth 1941; Hoek 1881; Nakamura & Child 1991; Stephensen 1933; Stock 1994), some early authors split them into two families (e.g., Bouvier 1923). Finally, molecular studies (Arango & Wheeler 2007; Nakamura *et al.* 2007; Arabi *et al.* 2010) cast doubt on the hypothesis of a single family (which was Ammotheidae according the priority rule of taxonomic nomenclature). Nakamura *et al.* (2007) suggested the resurrection of the family Ascorhynchidae, a position that was followed by the commonly used classifications of Bamber (2007b; 2015), but Ascorhynchidae and Ammotheidae were grouped into the same superfamily Ascorhynchoidea Hoek, 1881 (and not “Pocock, 1904” as listed by Bamber 2007b and Bamber *et al.* 2015).

Even now, the status of Ammotheidae *sensu* Bouvier (1923) (i.e., excluding Ascorhynchidae) is rather unclear. As previously commented by Bouvier, most of the characters used to describe this reduced taxon show exceptions: for instance, their main character, i.e., the reduction of chelae to small buds, is not constant (for example, adults of *Nymphopsis muscosa* Loman, 1908 bear chelate chelae); the number of palp articles is highly variable (4 to 9); the abdomen can be articulated or not to the trunk, and their development can be direct or larval (Bamber 2007b). The family Ammotheidae *sensu* Bouvier (1923)

was found to be poly- or paraphyletic in the molecular studies of Nakamura *et al.* (2007) and Arango & Wheeler (2007). However, these results may have been caused by the use of unreliable molecular data (carryover DNA contamination and high levels of missing data, inaccurate methods of DNA alignment and the extreme heterogeneity of nucleotide composition in the mitochondrial genes of sea spiders, see Arabi *et al.* 2010). Nevertheless, the taxon was recovered monophyletic in the tree obtained from a concatenation of five mitochondrial and nuclear markers, but based on only ten species (of the 297 described) (Arabi *et al.* 2010). Therefore, we consider that the status of Ammotheidae is an open question that needs to be studied with a better taxonomic sampling, including more species diversity.

Previous studies on sea spiders have mainly focused on species from the Southern Ocean. As a consequence, we have a great amount of knowledge on this fauna in terms of biodiversity (León 2001; Munilla & Soler-Membrives 2009, 2015), integrative taxonomy, population genetics (Arango *et al.* 2011; Dietz *et al.* 2015; Krabbe *et al.* 2010), biogeography (Griffiths *et al.* 2011; Munilla & Soler-Membrives 2009) and parasitology (Schiaparelli *et al.* 2008). Historically, one of the first invertebrate species described from Antarctica was a sea spider (*Decolopoda australis* Eights, 1835) and since then, pycnogonids have been considered as a flagship group in Antarctica. Moreover, taxonomists were particularly interested in Antarctic sea spiders because of their large size and their “extra-legged” representatives (ten legs or more) (e.g., Bouvier 1910). In contrast, species from non-Antarctic regions are often tiny in size, and thus more difficult to collect or study by non-specialists. As a consequence, most of the barcode sequences (5’ fragment of the gene encoding the first subunit of cytochrome c oxidase, CO1) currently available for Ammotheidae in the nucleotide databases come from southern ecoregions (South Australia, South America and Antarctica; Fig. 1).

Here, we investigated the diversity and phylogeny of Ammotheidae by focusing on sea spiders collected in several poorly studied tropical areas (e.g., Papua New Guinea, South Madagascar, Marquesas Islands) during the latest expeditions of the Muséum national d’Histoire naturelle of Paris (MNHN). We generated 104 sequences of the CO1 mitochondrial gene and 80 sequences of the 18S rRNA (18S) nuclear gene. These datasets were analysed to address the following three main questions: (1) Are Ammotheidae monophyletic? (2) Are the “big five” ammotheid genera (*Ammothea*, *Achelia* Hodge, 1864, *Ammothella* Verrill, 1900, *Cilunculus* Loman, 1908 and *Tanystylum*) monophyletic? (3) How heterogeneous is the base composition in CO1 sequences of Ammotheidae?

Material and methods

Sampling

The specimens selected for this study were collected during the following MNHN expeditions and deep sea cruises (Fig. 2), organized under the “*Planète Revisitée*” and “Tropical Deep Sea Benthos” programs (Bouchet *et al.* 2008; Richer de Forges *et al.* 2013): BATHUS 3 (New Caledonia, 1993), SANTO (Vanuatu, 2006), CEAMARC (Antarctica, 2008), MAINBAZA (Mozambique Channel, 2009), ATIMO VATAE (South Madagascar, 2010), BIOPAPUA (Bismarck Sea, Papua New Guinea, 2010), PAPUA NIUGINI (Madang, Papua New Guinea, 2012), PAKAIHI I TE MOANA (Marquesas Islands, 2012), KARUBENTHOS (Guadeloupe, 2012); KAVIENG (Kavieng, Papua New Guinea, 2014), GUYANE 2014 (off French Guiana, 2014), and ILES DU SALUT (French Guiana, 2014). Details of the field operations are available at <http://expeditions.mnhn.fr/>. Some additional specimens have been provided by IFREMER (BICOSE, BIOBAZ, HYDROMAR and FUTUNA 3 cruises). All the geographical locations are reported in Appendix 1. The specimens were collected from littoral shores to hydrothermal vents (3500 m depth).

DNA extraction, amplification and sequencing

Total DNA was extracted from a leg removed on specimens preserved in 80–95% ethanol using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). The samples were lysed in 360 µl of ATL (twice

as much as recommended by the manufacturer) and 40 µl of proteinase K. The volumes of AL and ethanol were also doubled. The rest of the protocol followed the volumes indicated by the manufacturer. Final extract volumes contained between 25 and 100 µl of DNA solution.

Two markers were sequenced for this study: the mitochondrial CO1 gene and the nuclear 18S gene. We used a new set of primers to amplify CO1 sequences (Py-CO1-U: 5'-TCA-ACW-AAT-CAT-AAA-GAY-ATT-GG-3' and Py-CO1-L3: 5'-GGR-TCH-CCH-CCH-GMD-GGR-TC-3') and the three sets used in previous studies for the 18S sequences (see details in Arabi *et al.* 2010). DNA amplification were done using Hot start mix RTG Taq (GE Healthcare, Waukesha, WI, USA) in a 25 µl final volume containing between 1 and 5 µl of DNA and 1µl of each 10 mM primer. Initial denaturation was performed at 94°C for 4 min, then we applied 40 cycles of denaturation-hybridization of 30 s at 94°C, 30 s at hybridization temperature (50°C for CO1, 52°C for 18S), and 1 min at 72°C. Final elongation lasts 10 min at 72°C. Purification and cycle sequencing were performed by Eurofins (Munich, Germany) using the PCR primers detailed above.

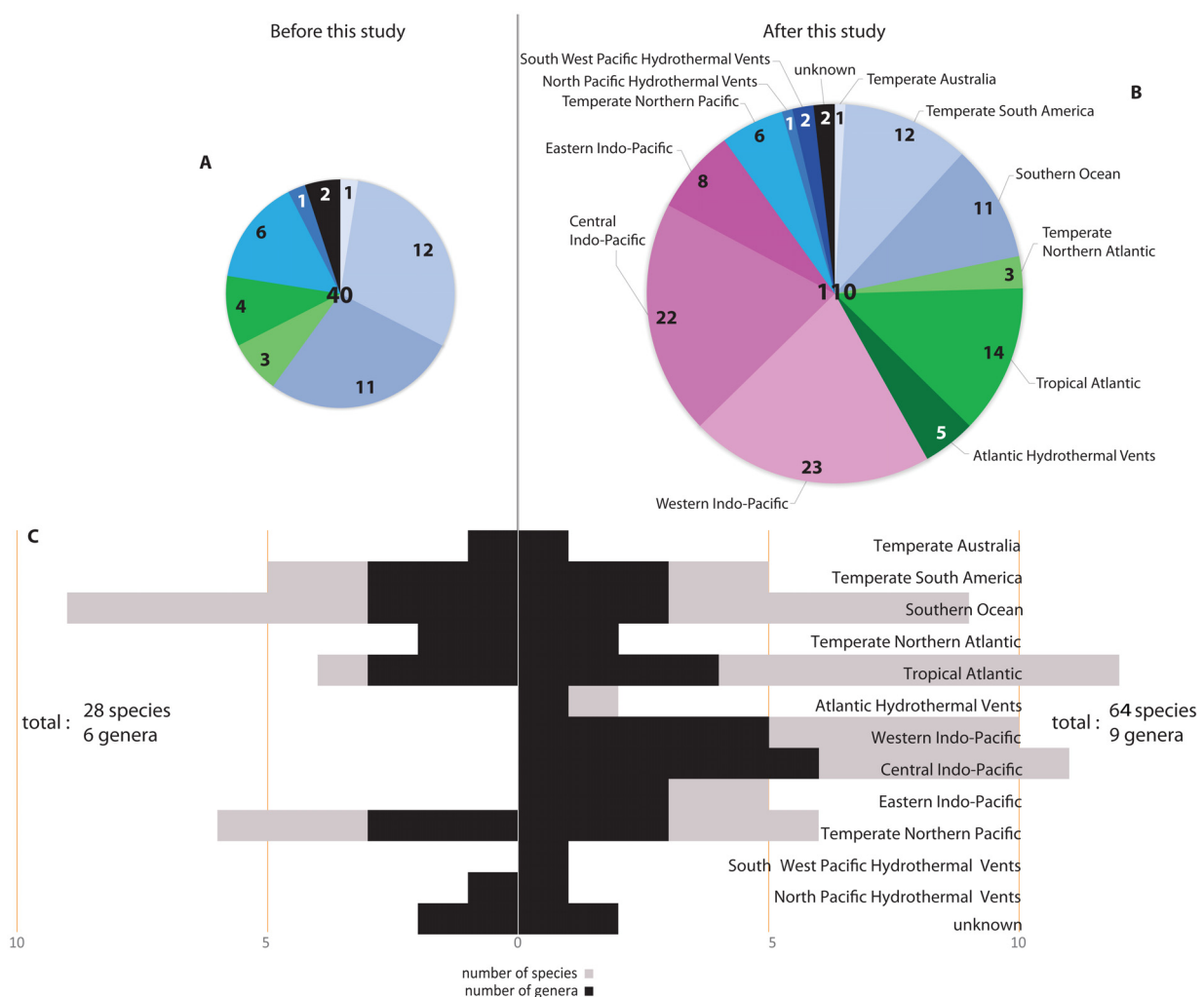


Fig. 1. Barcode richness in GenBank before and after this study. **A–B.** Number of CO1 haplotypes before (**A**) and after (**B**) this study as a function of the origins of the specimens sequenced. **C.** Number of species (grey) and genera (black) represented by a CO1 sequence found in GenBank databases before (left) and after (right) this study, classified by ecoregions. Littoral ecoregions defined according to Spalding *et al.* (2007) and abyssal ecoregions following a simplification of Bachraty *et al.* (2009).

The sequences were cleaned using CodonCode Aligner v. 3.7.1 (CodonCode Corporation, Dedham, MA, USA) by comparing forward and reverse electrophoregrams. Nucleotide ambiguities were coded according to the IUPAC nomenclature. Cleaning was achieved after multiple DNA alignments using Se-AI v. 2.0a11 (Rambaut 2002). Each sequence was compared *via* BLAST methods (NCBI, Benson *et al.* 2015) in order to detect potential contaminations. The sequences were deposited in GenBank under accession numbers KX535346–KX535450 (CO1) and KX536422–KX536501 (18S).

Phylogenetic analyses

The 104 CO1 barcodes and 80 18S sequences generated for this study were compared to all sequences of Ammotheidae (48) and Aschorhynchidae (19) downloaded from GenBank. For other pycnogonid families, we selected only species for which both CO1 and 18S sequences were available. Details on the 75 CO1 and 79 18S sequences from GenBank are provided in Appendix 1. The pycnogonid tree was rooted with 10 outgroup species, including two chelicerates (Uropygi: *Mastigoproctus giganteus* (Lucas, 1835); Xiphosura: *Limulus polyphemus* (Linnaeus, 1785)), six mandibulates (Stomatopoda: *Squilla empusa* Say, 1818; Branchiopoda: *Triops longicaudatus* LeConte, 1846; Chilopoda: *Lithobius variegatus* Leach, 1814 and *L. forficatus* (Linnaeus, 1785); Diplopoda: *Thyropygus* sp. and *Orthoporus* sp.) and two onychophorans (*Opisthopatus cinctipes* Purcell, 1899 and *Peripatooides novaezaelandiae* (Hutton, 1876)). We avoided CO1 sequences characterized by an inverted bias in base composition (e.g., scorpions, spiders, etc.) in order to limit artefacts during phylogenetic reconstruction. This strategy was determined in agreement with previous studies showing that asymmetric mutational constraints occurred during the evolution of the mitochondrial genome of Chelicerata, including sea spiders (Hassanin *et al.* 2005; Hassanin 2006; Arabi *et al.* 2010, 2012).

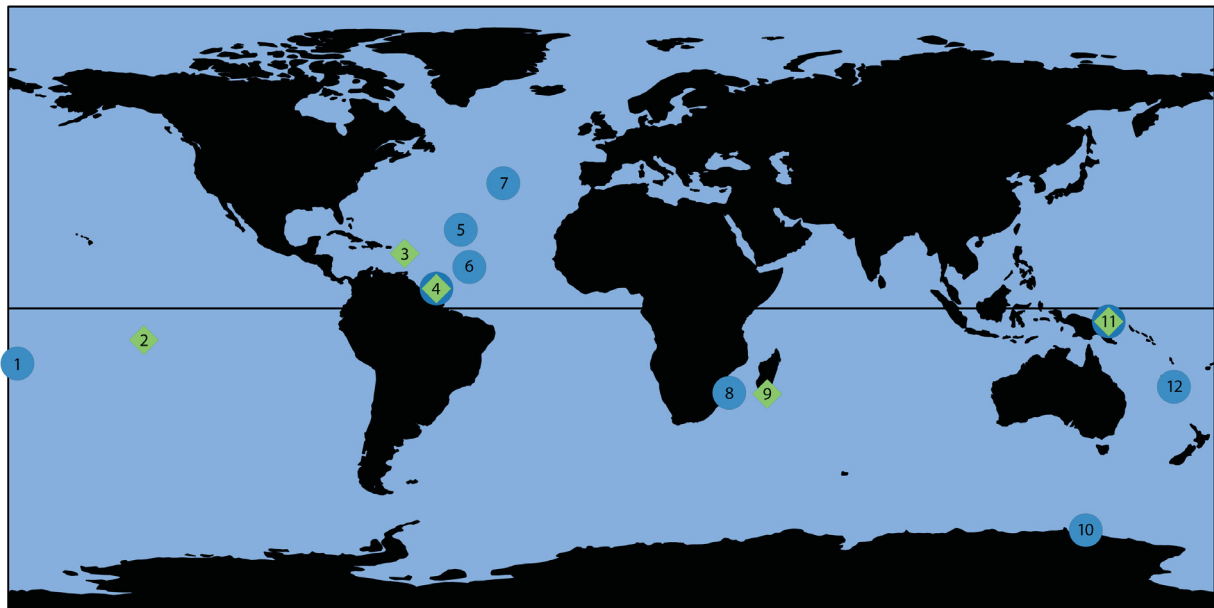


Fig. 2. Regional distribution of the studied specimens. Green diamonds indicate locations where specimens were collected at shallow depths (< 100 m), blue circles those from deep-sea samplings; the combinations of both symbols indicates mixed samplings (below and above 100 m depth). 1: Futuna; 2: Marquesas Islands; 3: Guadeloupe; 4: French Guiana; 5: Mid-Atlantic Ridge, Snake Pit site; 6: Mid-Atlantic Ridge, Logatchev site; 7: Mid-Atlantic Ridge, Lucky Strike site; 8: Mozambique Channel; 9: South Madagascar; 10: Antarctica, Terre Adélie; 11: Papua New Guinea; 12: New Caledonia and Vanuatu.

The 18S alignment was achieved using a first trial with CodonCode Aligner v. 3.7.1, and it was optimized manually on Se-Al v. 2.0a11. Several regions with too many ambiguous positions for homology were removed from the analyses. In other regions that are difficult to align, but which provide phylogenetic information at lower taxonomic levels, we chose a different strategy based on the use of different taxonomic blocks. For instance, the region located at positions 679–693 (with respect to the DQ389932 sequence) was aligned using four shifted blocks corresponding to the following taxa: (1) Ammotheidae Dohrn, 1881, Nymphonidae Wilson, 1878, Callipallenidae Hilton, 1942 and Pallenopsidae Fry, 1978, (2) Ascorhynchidae Hoek, 1881 and Endeidae Norman, 1908, (3) Phoxichilidiidae Sars, 1891, and (4) Colossendeidae Jarvinsky, 1870, Pycnogonidae Wilson, 1878 and Austrodecidae Stock, 1954.

Phylogenetic analyses were performed with MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003), running 4 chains for 10 million generations, and a 25% burn-in. We analysed the 18S and CO1 (partitioned by codon positions or without partition) datasets separately, and the model (i.e., GTR+G+I) was selected using the best AIC score calculated on jModelTest (Posada 2008). For the reduced dataset of 135 taxa used for the concatenation of CO1 and 18S markers, we performed a partitioned analysis using a GTR for each marker.

Analysis of the nucleotide composition in COI sequences

Strand-bias in nucleotide composition was analysed at third codon positions of CO1 sequences. As similar trends were previously found for two- and fourfold degenerate sites (Hassanin *et al.* 2005), we followed the simplified approach previously published in Arabi *et al.* (2010), in which AT and CG skews were calculated on all third codon positions of CO1 sequences using the following formulas: $AT\ skew = (F_A - F_T)/(F_A + F_T)$ and $CG\ skew = (F_C - F_G)/(F_C + F_G)$, where F is the frequency of the considered nucleotide at third codon positions. The skew values were considered as significant if the null hypothesis could be rejected to a confidence level of 5%.

Results

Datasets

The great extent of localities and depth range explored here allowed us to examine a large diversity of pycnogonids from 8 of the 11 families and from the seven main genera of Ammotheidae, except *Ammothea* (i.e., *Achelia*, *Ammothella*, *Cilunculus*, *Nymphopsis* Haswell, 1884, *Sericosura* Fry & Hedgpeth, 1969 and *Tanystylum*). The presently monospecific *Acheliiana* Arnaud, 1971 is also sequenced for the first time with an undescribed species (in prep.). With the sequences extracted from GenBank (see Appendix 1), we included one additional ammotheid genus (*Ammothea*) and two other families (Austrodecidae and Colossendeidae) in the analyses.

We detected several potential errors in the GenBank sequences, involving substitutions in highly conserved regions (e.g., *Ammothea hilgendorfi* DQ389936; *Anoplodactylus batangensis* DQ389918), as well as unexpected indels in the 18S stems, which are very constrained regions (see below) (e.g., *Ascorhynchus castelloides* DQ389905; *Callipallene novaezealandiae* DQ389927; *Anoplodactylus batangensis* DQ389918). However, these potential errors were not so problematic for phylogenetic inferences, since most of them are autapomorphic. More worrying is the misleading effect of the 18S sequence of *Pentapycnon charcoti* (DQ389924) on phylogenetic reconstruction. Indeed, our preliminary analyses revealed its chimeric origin as positions 1 to 923 correspond to an undetermined fungus. In this case, we excluded the fungal part of the sequence from the alignment. Similar problems have previously been described in Arabi *et al.* (2010) for other sea spiders. Other sequences susceptible to generate reconstruction artefacts, due to their poor quality (highly divergent sequences in conserved regions) or their shortness, were removed from the analyses (CO1: *Achelia alaskensis* DQ390093; 18S: *Pallenopsis macronyx* DQ389908).

The CO1 alignment contains 179 sequences (of which 104 are new) and 618 nucleotides representing 376 informative characters (369 without outgroups). It is worth noting that all specimens of the genus *Eurycyde* Schiödte, 1857 share a synapomorphic deletion of two codons in the CO1 gene (positions 472–477 in the DQ390087 sequence of *Achelia assimilis*). There is no significant difference between the CO1 analyses made with or without partition (Fig. 3). The 18S alignment contains 159 sequences (of which 80 are new) and 1750 nucleotides (including gaps due to alignment) representing 321 informative characters (*idem* without outgroups). The 18S tree is shown in Fig. 4. The concatenation of the two markers contains 135 sequences and 2550 nucleotides representing 746 informative characters (602 without outgroups). The tree obtained from the concatenation is illustrated in Fig. 5.

Phylogenetic relationships

The monophyly of Pycnogonida is supported by maximal values of posterior probability (PP = 1) in all analyses. Most families are monophyletic with high support (PP > 0.9) in most analyses: Colossendeidae, Endeidae, Pallenopsidae, Phoxichilidiidae and Pycnogonidae. In the CO1 tree (Fig. 3), however, Pallenopsidae is recovered as paraphyletic (PP = 0.87) and the monophyly of Phoxichilidiidae is less robust (PP < 0.5). The family Ascorhynchidae is found to be monophyletic in the concatenated analysis (PP = 0.9) (Fig. 5) and the 18S analysis based on 135 specimens (PP = 0.72) (Appendix 2), whereas different, but unrobust relationships are shown in other analyses (PP < 0.6). All members of the families Callipallenidae and Nymphonidae are systematically grouped, but these taxa are always found to be either poly- or paraphyletic.

Within pycnogonids, most basal relationships are poorly supported (PP < 0.8), but the families Austrodecidae and Pycnogonidae are found to be the first divergent lineages in most topologies (Figs 3–5, Appendix 2). However, these results only show good support in the combined analysis, where Austrodecidae and Pycnogonidae are the first and second offshoots, respectively (PP > 0.85). The family Colossendeidae is the next taxon to diverge in both combined and 18S analyses, but this has only weak support (PP = 0.57–0.77) (Figs 4–5). In the CO1 analyses (Fig. 3, Appendix 2), Colossendeidae appear as the sister-group of Phoxichilidiidae (PP = 0.69 and 0.93), and they are allied to the clade uniting Nymphonidae and Callipallenidae (PP = 0.82) in the CO1 analysis based on 179 haplotypes (Fig. 3).

The clade uniting Callipallenidae and Nymphonidae is grouped with Ascorhynchidae in the 18S and combined analyses (PP = 0.72–0.93) (Figs 4–5, Appendix 2). In the CO1 analysis based on 135 specimens (Appendix 2), however, the paraphyletic ascorhynchids are grouped with some ammotheids (i.e., excluding the “*Achelia sawayai* group”, see below) and Pallenopsidae (PP = 0.81).

The family Ammotheidae appears to be monophyletic in the 18S and CO1 + 18S trees with good supports (PP = 0.83–0.95) (Figs 4–5, Appendix 2). The CO1 analyses (Fig. 3, Appendix 2), however, support its polyphyly (PP = 0.81–0.9) due to the position of the “*Achelia sawayai* group” (*Achelia sawayai* Marcus, 1940 + *Achelia* sp. 15 + specimen MNHN-IU-2013-18602), which is found to be monophyletic with maximum support in all analyses (PP = 1) and divergent from other Ammotheidae (PP = 0.79).

Two major subfamilies of Ammotheidae can be distinguished: (1) Achelinae Wilson, 1881, which includes *Achelia*, *Nymphopsis*, *Tanystylum*, all *Ammothella* except *A. stauromata* Child, 1982 and *A. biunguiculata* Dohrn, 1881, and *Cilunculus gracilis* Nakamura & Child, 1991 (see 18S analysis in Fig. 4); and (2) Ammotheinae Dohrn, 1881 emend., which is composed of the genera *Ammothea*, *Acheliana*, *Sericosura*, all *Cilunculus* but *C. gracilis*, and the species *Ammothella stauromata* and *A. biunguiculata* (see 18S analysis in Fig. 4). The subfamily Ammotheinae is supported in all analyses, in general with highest support values. The subfamily Achelinae is monophyletic in the 18S and CO1 + 18S trees (PP = 1) (Figs 4–5, Appendix 2), but paraphyletic in the CO1 trees (PP = 0.94–0.98) (Fig. 3, Appendix 2) because the “*Achelia sawayai* group” appears as the sister-group of Endeidae

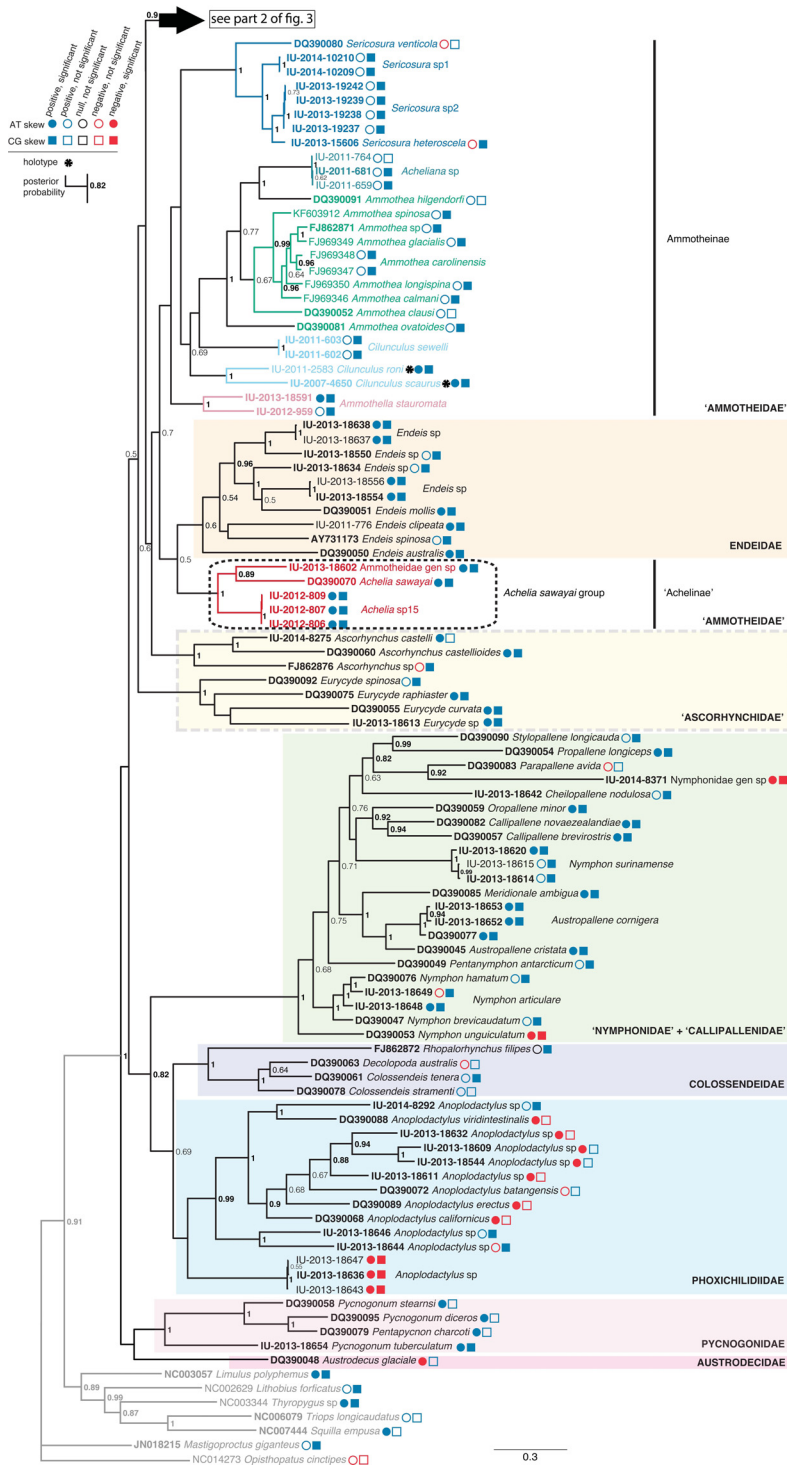


Fig. 3. Bayesian tree of Pycnogonida based on 179 sequences of the mitochondrial CO1 gene (partitioned analysis). Coloured rectangles show non-ammotheid families, and coloured branches discriminate ammotheid genera. The numbers at the nodes indicate posterior probabilities greater than 0.5. Symbols associated with each taxon name indicate the bias in base composition, as expressed by AT (circles) and CG (squares) skews (see main text for details): blue symbols represent a significant positive bias; red symbols indicate a significant negative bias; uncoloured symbols show insignificant values of skews. Asterisks after taxon names indicate holotype specimens. The arrow at the top of the tree shows the connection with Part 2 of the tree (see next page).

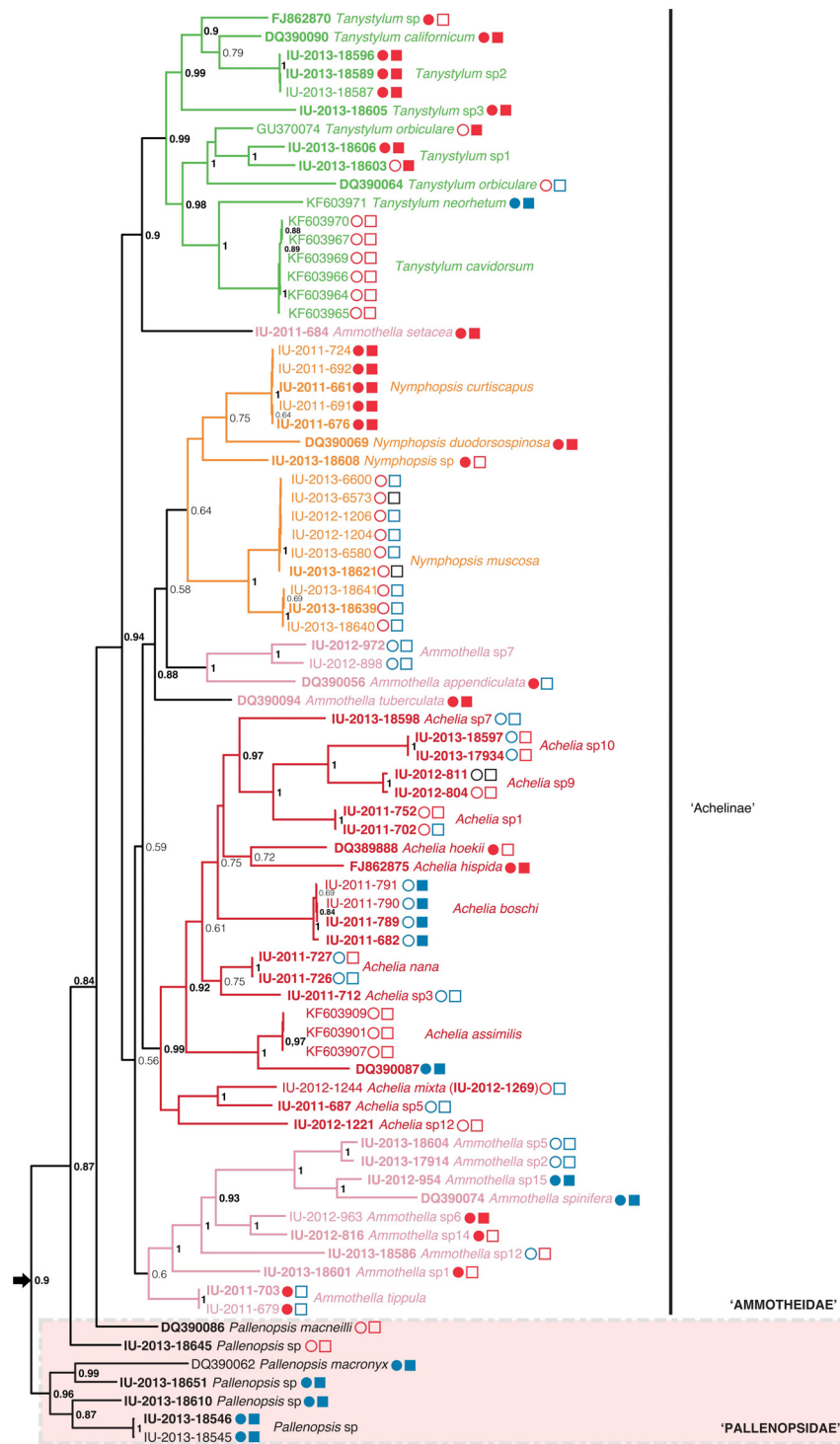


Fig. 3. Part 2, see Part 1 for explanation.

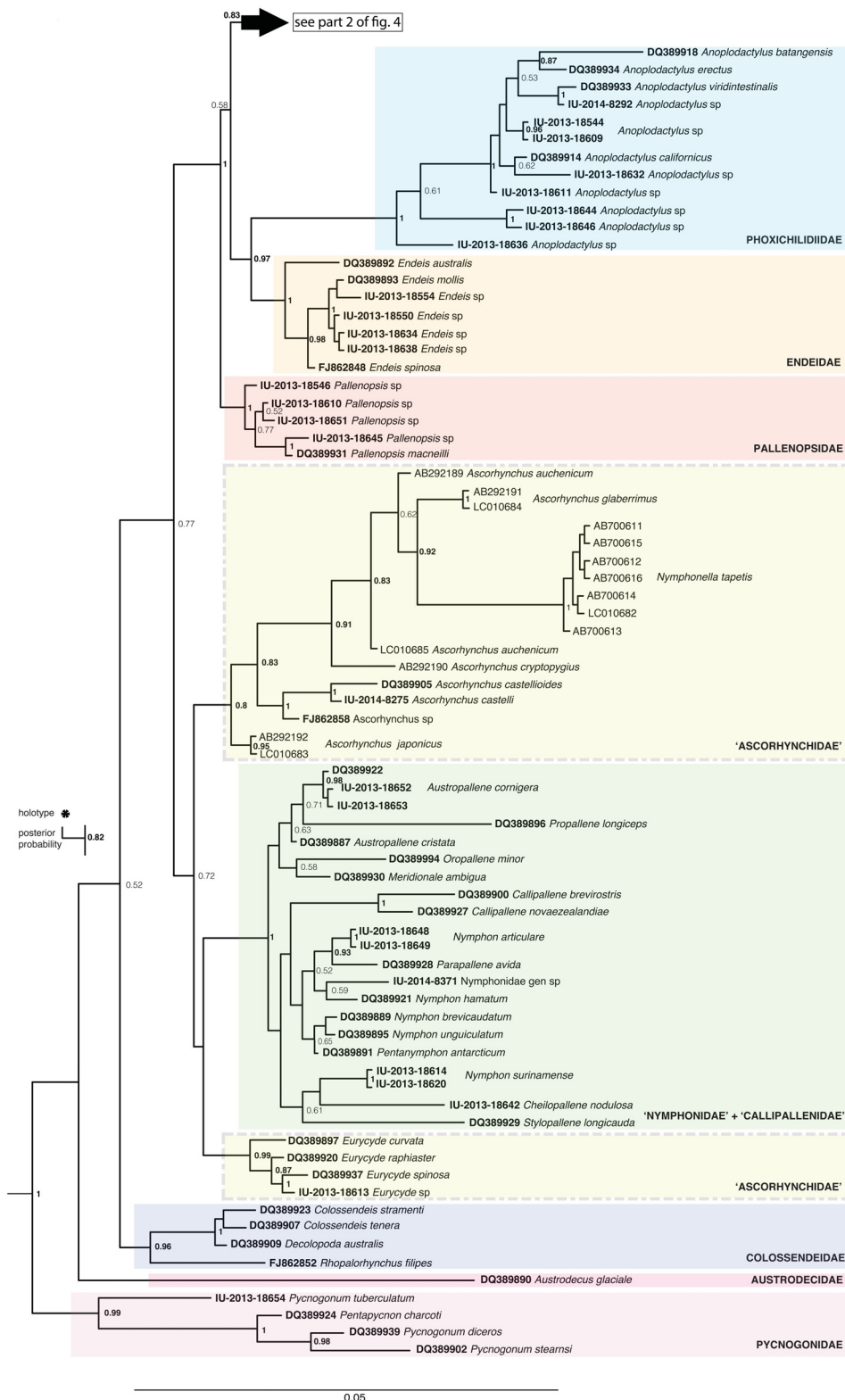


Fig. 4. Bayesian tree of Pycnogonida based on 159 sequences of the nuclear 18S rRNA gene. Coloured rectangles show non-ammotheid families and coloured branches discriminate ammotheid genera. The numbers at the nodes indicate posterior probabilities greater than 0.5. Asterisks after taxon names indicate holotype specimens. Outgroups were removed for better readability. The arrow at the top of the tree shows the connection with Part 2 of the tree (see next page).

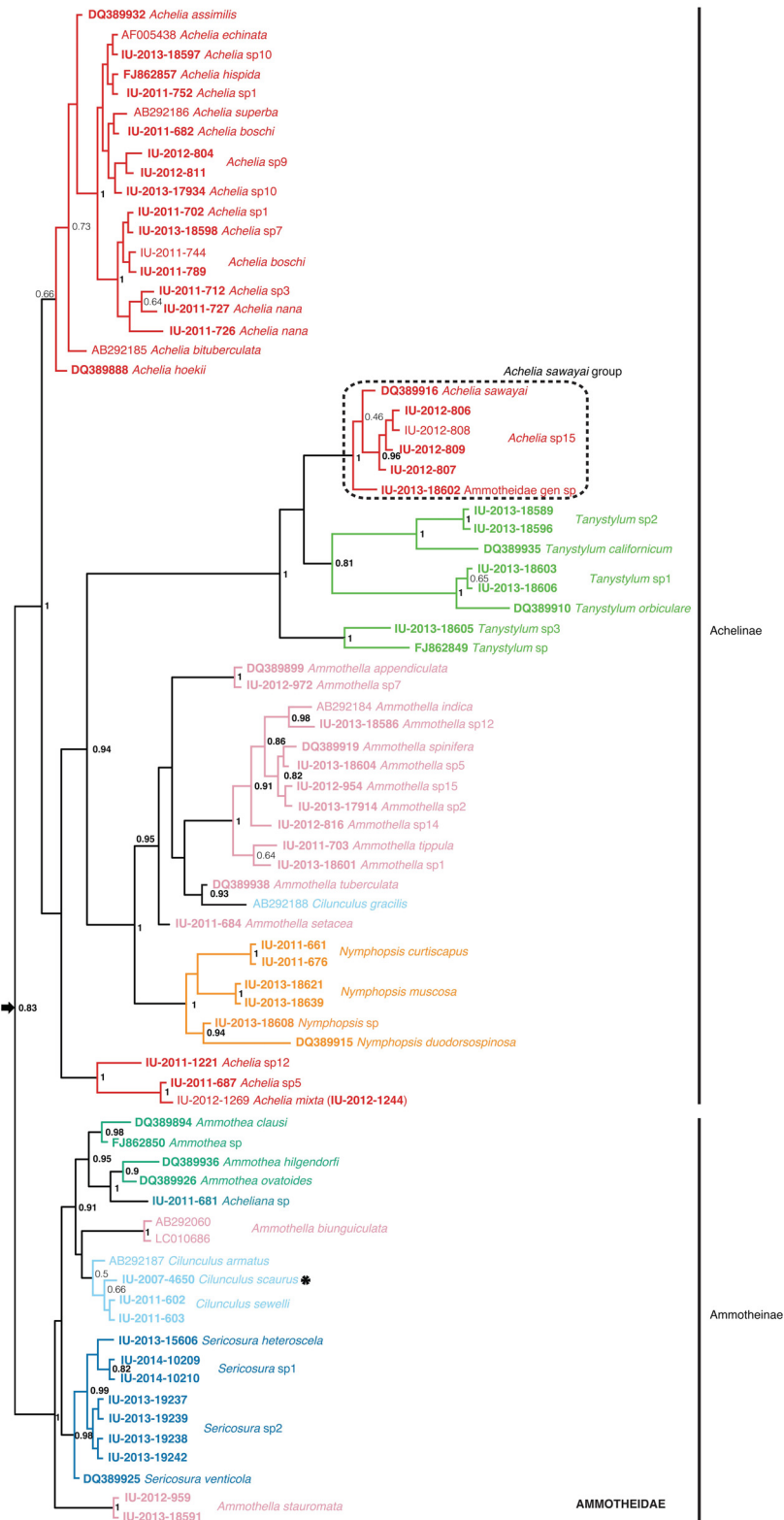


Fig. 4. Part 2, see Part 1 for explanation.

(PP = 0.5–0.74). In the 18S and combined analyses (Figs 4–5, Appendix 2), the “*Achelia sawayai* group” is included within Achelinae, where it constitutes a strongly supported clade with *Tanystylum* (PP = 1).

Within Achelinae, the monophyly of *Nymphopsis* is recovered in all analyses (PP = 0.56–0.64 in CO1 analyses, PP = 1 in 18S and concatenated analyses). In the CO1+18S tree (Fig. 5), *Ammothella* s. str. (excluding *A. stauromata* and *A. biunguiculata*) constitutes a monophyletic clade (PP = 1), but the 18S tree (Fig. 4) shows that *Cilunculus gracilis* (which is absent from the CO1 tree) also falls into this clade (PP = 0.95). In the CO1 analyses (Fig. 3, Appendix 2), *Ammothella* s. str. is found to be polyphyletic: a first group, which includes *Ammothella* sp. 7, *A. tuberculata* Cole, 1904 and *A. appendiculata* (Dohrn, 1881), is related to *Nymphopsis* with rather strong support (PP = 0.6–0.88); a second group, which is only represented by the species *Ammothella setacea* (Helfer, 1938), is close to the genus *Tanystylum* (PP = 0.9); and a third group, which is composed of *Ammothella* sp. 1, 2, 6, 12, 14, 15, *A. spinifera* Cole, 1904 and *A. tippula* Child, 1983 (PP = 0.6), shows an unstable position with respect to other lineages of Achelinae. The monophyly of *Achelia* s. str. (i.e., excluding the “*Achelia sawayai* group”) is well supported in all analyses (PP = 0.92–1), except in the 18S tree of Fig. 5, where its paraphyly is not found to be robust (PP < 0.5).

Within Ammotheinae, *Ammothea* is constantly shown as paraphyletic due to the inclusive position of the genus *Acheliana* (PP = 1) (Figs 3–5, Appendix 2). The genus *Cilunculus* (excluding *C. gracilis*) is found to be monophyletic in the 18S and CO1+18S trees (PP = 0.95–1). The genus *Sericosura* is monophyletic in all analyses (PP = 0.97–1).

Nucleotide composition in CO1 sequences

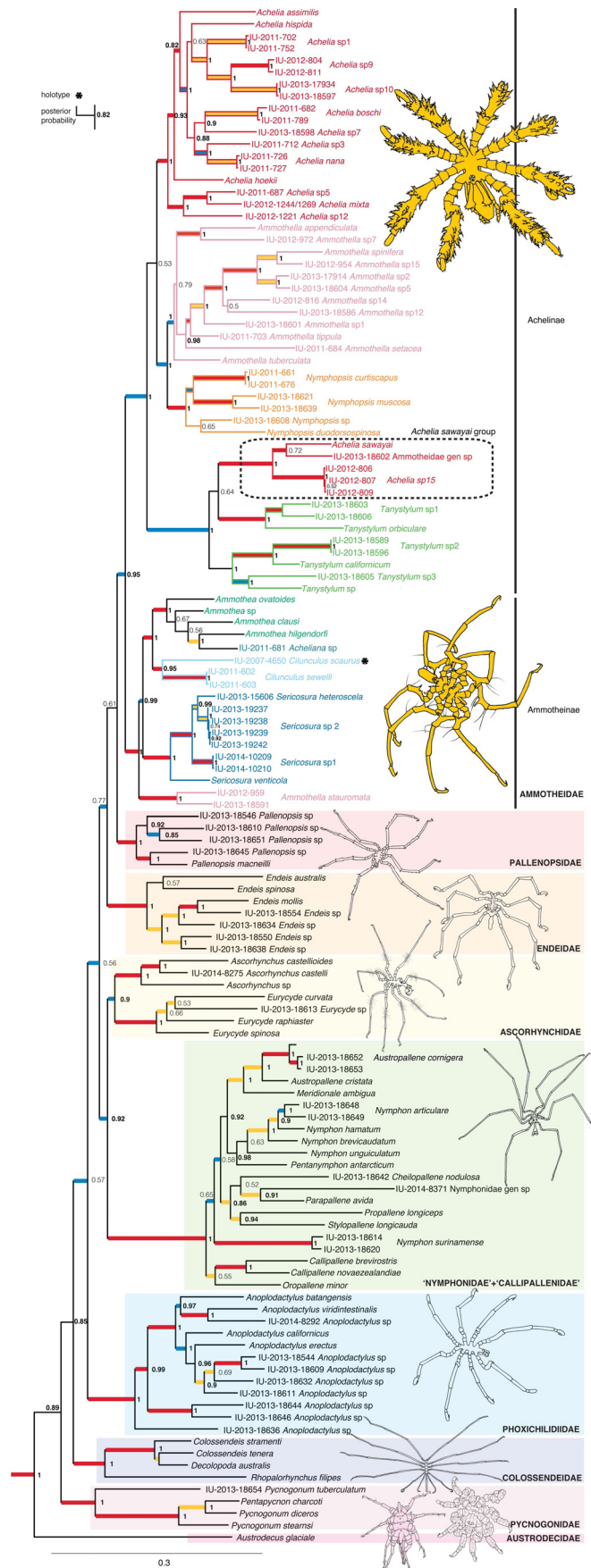
The analysis of third codon positions of the CO1 gene shows that most families of Pycnogonida are characterized by positive values for AT and CG skews (Fig. 3; detailed values in [Supplementary file](#)), which means that their sequences have an excess of A relative to T nucleotides and of C relative to G nucleotides. However, the AT skew is significantly negative in most species of the genus *Anoplodactylus* Wilson, 1878 (10 on 14), and in *Austrodecus glaciale* Hodgson, 1907. In the clade grouping Callipallenidae and Nymphonidae, two unrelated species (MNHN-IU-2014-8371 Nymphonidae gen. sp. and *Nymphon hamatum*) also show negative AT and CG skews. For the family Ammotheidae, our analyses revealed a higher heterogeneity of base composition. The members of the subfamily Achelinae exhibit a strong heterogeneity: for most taxa, the skew values are non-significant (e.g., *Nymphopsis* and *Achelia* excluding the “*Achelia sawayai* group”); *Ammothella*, *Achelia* and *Tanystylum* generally have negative skews, but with some exceptions (*Achelia boschi* Stock, 1992; *A. assimilis* (Haswell, 1885); *Ammothella spinifera* Cole, 1904; *Ammothella* sp. 15; *Tanystylum neorhetum* Marcus, 1940 all show positive skews); the “*Achelia sawayai* group,” however, shows a strong positive bias.

Discussion

Monophyly and low supports of interfamilial relationships

Both our CO1 and 18S analyses supported the monophyly of most pycnogonid families. In contrast, the two families Ammotheidae and Ascorhynchidae were only recovered as monophyletic in the 18S trees. In the CO1 trees, the polyphyly of Ammotheidae *sensu* Bouvier (1923) seems to be robust (but see next paragraph below), whereas the polyphyly of Ascorhynchidae is not highly supported (PP < 0.6). For Ascorhynchidae, the lack of robustness suggests that their basal diversification (i.e., the divergence between *Ascorhynchus* and *Eurycyde*) was more ancient than in other families, excepting, perhaps, Ammotheidae (see below). Indeed, for the deepest nodes of the CO1 trees most of the genuine phylogenetic signal has been erased because of the high saturation of synonymous substitutions in the mitochondrial genome and the low levels of variation observed at non-synonymous sites of the CO1 gene (very high selective pressure). This explains why the CO1 gene did not provide robust support for most interfamilial relationships (Fig. 5). The sole exception concerns Callipallenidae and Nymphonidae,

Fig. 5. Bayesian tree of Pycnogonida obtained from the concatenation of CO1 and 18S genes (135 taxa). Coloured rectangles show non-ammotheid families and coloured branches discriminate ammotheid genera. The numbers at the nodes indicate posterior probabilities greater than 0.5. Bold branches indicate CO1 (yellow), 18S (blue), or both (red) support in the independent analyses of CO1 and 18S genes provided in Appendix 1. Asterisks after taxon names indicate holotype specimens. Outgroups were removed for better readability.



which are grouped together at the end of a very long branch in the CO1 and CO1+18S trees (Figs 3, 5). This result clearly indicates that the divergence between Callipallenidae and Nymphonidae is more recent than that of other families. In agreement with this view, they share many morphological characters, including the structure of the 10-articled ovigers and the presence of well-developed chelae (Bamber 2007b). In addition, our datasets did not provide any evidence for their reciprocal monophyly. On the contrary, our analyses suggested that these two families are reciprocally polyphyletic ($PP < 0.92$). However, we found very different polyphyletic patterns in the combined, CO1 and 18S analyses (Figs 3–5), which suggests that CO1 and/or 18S genes are not the best markers to resolve relationships at this level of the pycnogonid tree (Figs 3–4, Appendix 2).

Even with the 18S dataset, most basal relationships within Pycnogonida were not well-resolved, suggesting that most families of Pycnogonida have diverged rapidly from each other. As pointed out by Charbonnier *et al.* (2007), there is a substantial hiatus in the fossil record of Pycnogonida, between the Devonian (ca 400 Mya) and the Jurassic (ca 160 Mya), and the concomitant appearance of several different families during the Mesozoic is in agreement with a crown group radiation. To explain both the lack of resolution for interfamilial relationships and the long branch that separates outgroups from pycnogonids, Arabi *et al.* (2010) have also suggested that a very long period of time occurred between the origin of Pycnogonida, and the subsequent rapid diversification that led to extant families.

Strong heterogeneity in base composition in the CO1 gene of Ammotheidae

The analyses of CO1 and 18S genes revealed real discordance regarding the phylogeny of Ammotheidae. In the 18S tree (Fig. 4), the family Ammotheidae is found to be monophyletic and it can be divided into two major clades corresponding to the subfamilies Ammotheinae and Achelinae. In the CO1 tree (Fig. 3), the family appears to be polyphyletic: most species of Achelinae fall into the paraphyletic Pallenopsidae, whereas those of the “*Achelia sawayai* group” are related to the Endeidae, the Ammotheinae being their sister group. As exposed in detail below, we suggest that the apparent topological conflict between CO1 and 18S markers is the consequence of the combined effects of mutational saturation and multiple changes in base composition during the mtDNA evolution of Ammotheidae.

Previous studies have shown that in most species of Metazoa, the double-stranded and circular mitochondrial genome presents a typical strand asymmetry, in which synonymous sites of the positive strand are characterized by an excess of A relative to T nucleotides and of C relative to G nucleotides (i.e., positive AT and CG skews), while synonymous sites of the complementary negative strand show a reverse bias. This strand-bias in base composition is the consequence of asymmetric mutational constraints during replication and/or transcription of the mtDNA genome (Hassanin *et al.* 2005; Hassanin 2006). Hassanin *et al.* (2005) have suggested that the orientation of the control region of the mtDNA is crucial in the establishment of asymmetric mutational constraints, because this region contains both replication and transcription origins. In support of that hypothesis, they showed that two kinds of mitogenomic inversions can lead to a reversal in nucleotide composition: (1) inversion of the control region can result in a global reversal of asymmetric mutational constraints; (2) inversion of a genomic fragment can result in a local reversal of asymmetric mutational constraints. Several reversals of strand specific bias were identified in CO1 sequences of Chelicerata (Arabi *et al.* 2010, 2012) including the common ancestors of Scorpiones and Opisthothelae spiders, as well as several taxa within Acari, Opiliones, Pseudoscorpiones and Pycnogonida. Within Pycnogonida, Arabi *et al.* (2010, 2012) have revealed a strong heterogeneity in base composition, particularly among ammotheids, with taxa characterized by positive AT and CG skews (*Achelia assimilis*), positive CG skew but no significant bias for AT (*Ammothea*), negative AT and CG skews (e.g., *Achelia hispida* Hodge, 1864, *Ammothella tuberculata* and *Nymphopsis duodorsospinosa* Hilton, 1942) and negative AT skew but no significant bias for CG (*Ammothella appendiculata*). Our analyses showed that the situation is even more complex with the detection of two additional categories: taxa with no significant strand-bias (e.g., *Nymphopsis*)

and taxa with negative CG skew but no significant bias for AT (e.g., *Tanystylum orbiculare* Wilson, 1878). More importantly, our analyses of base composition in CO1 sequences suggest that the apparent polyphyly of Ammotheidae and that of Achelinae resulted from artefacts in tree reconstruction, because Ammotheinae, Achelinae s. str. (excluding the “*Achelia sawayai* group”) and the “*Achelia sawayai* group” exhibit clear differences in base composition. Indeed, many species of Achelinae excluding the “*Achelia sawayai* group” are characterized by one or two negative skews in their CO1 sequence. Only four species of this large clade appear to be characterized by positive AT and CG skews (*Achelia assimilis*, *Ammothella* sp. 15 + *A. spinifera*, and *Tanystylum neorhetum*), and all of them occupy a derived position within the subfamily. As a consequence, we can infer that the CO1 sequence of the most recent common ancestor of Achelinae was characterized by a negative AT skew and a negative or non-significant CG skew. In contrast, all species of the “*Achelia sawayai* group” clade have positive AT and CG skews, and those of the Ammotheinae clade generally show a positive CG skew associated with a non-significant AT skew. As pointed out in previous studies, important differences in base composition of mtDNA genes may be misleading for phylogenetic inferences, because they can produce artefacts such as Long Branch Attraction (LBA), when distantly related taxa with similar base composition tend to group together, or the opposite, Long Branch Repulsion (LBR), when closely related taxa with reverse strand-specific biases do not group together (Hassanin *et al.* 2005; Hassanin 2006; Arabi *et al.* 2010, 2012).

Here, we suggest that the CO1 polyphyly of Achelinae and Ammotheidae can be explained by both LBA and LBR artefacts, because two groups of Achelinae have very different base compositions, and because each of them tends to be attracted by the taxa with the most similar strand-specific bias, i.e., Endeidae for the “*Achelia sawayai* group” and *Pallenopsis macneilli* Clark, 1963 for all other species of Achelinae, resulting in the apparent polyphyly of Achelinae and Ammotheidae. This effect is particularly misleading for Ammotheidae because their diversification is assumed to be ancient, as revealed by their remarkable morphological diversity and by our analyses of 18S and CO1 genes (up to 25% divergence between ammotheid CO1 sequences). In this context, and given that the 18S gene is more appropriate for studying the deepest nodes of arthropod classes (e.g., Mallatt *et al.* 2004; Arabi *et al.* 2012), we conclude that our nuclear gene gave accurate information for the monophyly of Ammotheidae, Ammotheinae and Achelinae. Morphologically, all representatives of Ammotheidae *sensu* Bouvier (1923) share the structure of the ovigers with feeble strigilis, without rows of compound spines, and generally without terminal claw. The grouping of *Achelia*, *Ammothella* s. str., *Nymphopsis* and *Tanystylum* in the subfamily Achelinae also makes sense from a purely morphological perspective. Indeed, *Ammothella* s. str. and *Nymphopsis*, which are shown as sister-groups in our study, share a lot of characters (long abdomen, eventually bi-articulated and trumpet-like scapes, long ocular tubercle), while *Achelia* and *Tanystylum* share a discoidal body outline.

Taxonomic implications

Only two ammotheid genera, *Nymphopsis* and *Sericosura*, are found to be monophyletic. Interestingly, specimens of *Sericosura* from the Pacific (*Sericosura* sp. 1 and *S. venticola* Child, 1987) occupy a paraphyletic position with respect to the Atlantic specimens (*Sericosura* sp. 2 and *S. heteroscela* Child & Segonzac, 1996). This pattern is coherent with the biogeographic model proposed by Bachraty *et al.* (2009), in which they suggested that most hydrothermal vent taxa have dispersed from the Pacific into the North Atlantic Ocean by a deep-sea corridor that stayed open until the closure of the Panama Isthmus around 3 million years ago.

The genus *Achelia* is polyphyletic: most species are grouped into a robust clade, whereas the “*Achelia sawayai* group” is placed within the genus *Tanystylum*. However, the paraphyly of *Tanystylum* was not highly supported by the 18S dataset, suggesting that the hypothesis of monophyly cannot be excluded. Although *Achelia* and *Tanystylum* share some superficial similarities, the latter genus shows a typical morphology characterized by palps with a reduced number of articles. Therefore, we consider that

further studies are needed to decide whether a new genus should be described for members of the “*Achelia sawayai* group”.

The inclusive position of *Acheliana* within the genus *Ammothea* suggests that *Acheliana* should be synonymized with *Ammothea*. This result was partially perceived by Arnaud (1971a), who noted the close relationship between the two genera. However, the type species of *Acheliana*, *A. tropicalis* Arnaud, 1971, must be re-examined to provide a definitive conclusion.

The genus *Cilunculus* is polyphyletic in the 18S tree, because the species *C. gracilis* is included within *Ammothella*. Misidentification is rather unlikely, as the 18S sequence of *C. gracilis* was produced by Nakamura *et al.* (2007), who described the species with Child in 1991 (Nakamura & Child 1991). A taxonomic issue is a more plausible explanation. Indeed, *C. gracilis* is one of the seven species of *Cilunculus* presenting a two-jointed scape, a characteristic shared with all species of the genus *Ammothella*. Nakamura & Child (1991) themselves recognized that *C. gracilis* was closely allied to *Ammothella*. Its assignation to the genus *Cilunculus* was based on the presence of a (shallow) hood-like extension of the head above the chelifores and the proboscis (Loman 1908). However, according to Child (1994), this is the only character to “temporarily” support a genus (*Cilunculus*) which “hangs rather precariously over the pit of synonymy”, and Nakamura *et al.* (2007) also expressed the unclarity of this character for several species. Now, it is becoming obvious that a revision of the genus *Cilunculus* is urgently needed, as our results confirmed that the presence of a cephalic hood is not a reliable character for diagnosing members of this taxon. As there is no reason to wait to reassign the currently discussed species to the genus *Ammothella*, we refer to it as *Ammothella gracilis* (Nakamura & Child, 1991) comb. nov. The status of other species of *Cilunculus* with two-jointed scapes and shallow hoods will probably follow the same reassignment in forthcoming revisions.

The genus *Ammothella* is polyphyletic in all analyses, as most species are clustered into the Achelinae, whereas *A. stauromata*, and potentially *A. biunguiculata* (according to 18S data), are robustly placed in the Ammotheinae. From a morphological point of view, both latter species possess the typical characters of the genus *Ammothella*, but *A. biunguiculata* shows a very original profile with short chelifores and short abdomen (see Dohrn 1881; Bouvier 1923; Hedgpeth 1941), and *A. stauromata* is easily recognizable, with its characteristic dorso-median tubercles (see Child 1982; Fig. 6A, C). Therefore, both molecular and morphological data suggest that the taxonomic status of these two species must be revised. For *A. biunguiculata*, no taxonomic change can be proposed here because we do not have morphological material and the CO1 sequence is not available. For *A. stauromata*, however, we can describe a new genus, maybe provisional, as there is no evidence for a relationship with any other described genus. Thus, this taxon must receive due attention in future studies.

Family Ammotheidae Dohrn, 1881
Subfamily Ammotheinae Dohrn, 1881 emend.

Teratonotum gen. nov.

urn:lsid:zoobank.org:act:CD6D82C7-E0EC-4E46-A2C6-559678E48B29

Fig. 6

Type species

Ammothella stauromata Child, 1982: 270 (in list), 271–273, fig. 1.

Ammothella stauromata – Arango 2003a: 2730–2731. — Bamber 2004: 2–3, 21 (in tab.); 2007a: 256 (in list). — Child 1987: 180 (in list); 1988: III (in list), 5–7, 30–31 (in list); 1990: 316 (in description); 1996: 544; 1998: 290–291. — Müller 1989: 125; 1990a: 66; 1990b: 106. — Nakamura & Child 1988: 809–810. — Stock 1994: 29.

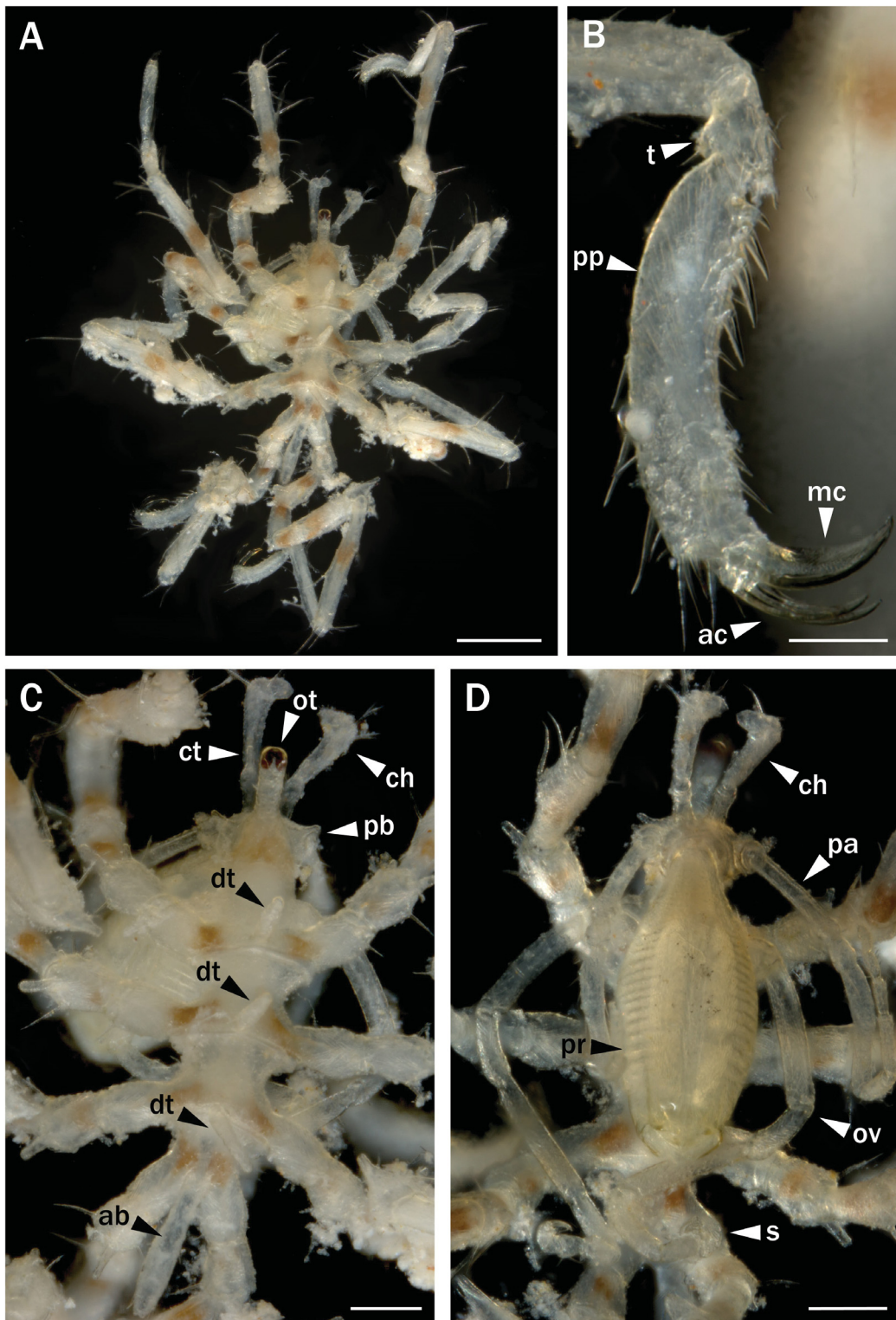


Fig. 6. *Teratonotum stauromatum* (Child, 1982) gen. et comb. nov. (MNHN-IU-2013-17964). **A.** Dorsal view. **B.** Propodus of third leg. **C.** Dorsal view of body. **D.** Ventral view of body. Abbreviations: *ab* = abdomen; *ac* = auxiliary claw; *ch* = chelifore; *ct* = chelifore tubercle on the anterior tip of the first scape; *dt* = dorsal tubercle; *mc* = main claw; *ot* = ocular tubercle; *ov* = oviger; *pa* = palp; *pb* = bulbous tubercle bearing the palp; *pp* = propodus; *pr* = proboscis; *s* = strigilis; *t* = tarsus. Scale bars: A = 0.5 mm; B = 0.1 mm; C–D = 0.2 mm.

Diagnosis

Trunk slender, totally segmented; ocular tubercle present, with three long, slender dorso-median tubercles; abdomen long, straight, almost vertical; scapes bi-articled, more proximal article with dorso-distal slender tubercle, chelae atrophied in adult; palps 9-articulated, originating from bulbous tubercles (with slender tubercle) placed anterolaterally on cephalic segment; oviger 10-articulated, originating ventral to first lateral processes, strigilis with denticulate spines; third leg with a single cement tube at anterior tip in males, tarsus short, propodus curved, auxiliary claws present.

Etymology

τέρας (*téras*): monster, and νῶτον (*nōton*): back (ancient Greek); referring to its remarkable back tubercles. Gender neutral.

An underestimated biodiversity

The CO1 data generated in this study on specimens collected during the recent MNHN expeditions indicate that the diversity of Ammotheidae was poorly represented in the nucleotide databases, such as GenBank and BOLD (Ratnasingham & Hebert 2007). Indeed, all our new CO1 sequences show at least 7% of nucleotide divergence with the ca 430 pycnogonid sequences available in GenBank, and even up to 11% if the genus *Sericosura* is excluded from the comparisons. This means that none of the ammotheids analysed here can be identified at the species level using molecular barcoding. Actually, this result is not surprising if we consider that most of the pycnogonids previously registered in the GenBank and BOLD databases were collected along the coastlines of temperate South America (Chile and Argentina) and Antarctica (Fig. 1), whereas our specimens come from widely spread geographic areas, i.e., French Guiana, Guadeloupe, Madagascar, Marquesas Islands, New Caledonia, Papua New Guinea, Vanuatu, and Atlantic and Pacific vents (Fig. 2). Besides, another issue for molecular taxonomy is the fact that most of the barcode sequences deposited in BOLD were not identified to the family level (958 of 1315, 72.85%). Beyond a problem of molecular taxonomy, ammotheids, and more generally pycnogonids, suffer from a lack of knowledge. For instance, our specimens collected along the coastlines of southern Madagascar show a far more rich diversity than previously recorded in the literature: five different species of *Achelia* were identified, whereas only two species were previously known from Madagascar; none of the species of *Ammothella* and *Endeis* studied here were known in the region; and a new species of *Acheliana* was found (Arnaud 1971a, 1971b, 1972, 1973; Stock 1974). In a more general way, it seems that the large collection of sea spiders assembled during the MNHN expeditions represents an important input to our knowledge of this group and promises the description of numerous new species (in prep.).

Acknowledgements

The studied specimens were collected during MNHN expeditions of the programs “Tropical Deep Sea Benthos” and “Planète Revisitée” (<http://laplaneterevisitee.org>). Deep-sea specimens were mainly collected during the many cruises organized by P. Bouchet, S. Samadi and L.C. of the MNHN and *Institut de Recherche pour le Développement* (IRD). Recent expeditions were funded by the Total Foundation, the Prince Albert II of Monaco Foundation, the Sloan Foundation, the Stavros Niarchos Foundation and conducted by the MNHN and Pro-Natura International. The KARUBENTHOS expedition was sponsored via European funding (FEDER), the *Port Autonome de la Guadeloupe* (PAG). Specimens from hydrothermal vents were provided by the IFREMER: BICOSE and FUTUNA 3 cruises (financial support was provided by the Eramet and Technip companies). The CEAMARC cruise on RSV Aurora Australis was funded by the Australian Antarctic Division, the French polar institute IPEV, CNRS and the ANR project ANTFLOCKS (G. Lecointre). Special thanks to S. Hourdez for the specimens from the Hydromar cruise, to N. Schnell for photography and to the editor and the two anonymous reviewers for useful comments on the first version of the manuscript. R.S. acknowledges the French-Taiwanese

grant TF-DeepEvo co-funded by the ANR and the Ministry of Science and Technology of Taiwan for supporting his visit to Germany. This work was supported by the project “*Taxonomie moléculaire: DNA Barcode et Gestion durable des Collections*”, funded by the MNHN and the “*Bibliothèque du Vivant*” network funded by the CNRS, MNHN, INRA and CEA (Genoscope).

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Manuscript received: 15 February 2016

Manuscript accepted: 29 October 2016

Published on: 24 February 2017

Guest editors: Line Le Gall, Frédéric Delsuc, Stéphane Hourdez, Guillaume Lecointre and Jean-Yves Rasplus

Desk editor: Danny Eibye-Jacobsen

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Appendix 1. CO1 and 18S sequences used for this study, including GenBank accession numbers.

Family	Genus	Species	MNHN code	CO1	18S	Geographical origin	Reference
Ammotheidae	–	Ammotheidae gen sp	IU-2013-18602	KX535450	KX536501	Iles du Salut	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia assimilis</i>	–	DQ390087	DQ389932	Victoria, Australia	Arango & Wheeler 2007
Ammotheidae	<i>Achelia</i>	<i>Achelia assimilis</i>	–	KF603901	–	Chile, Region de los Lagos	Weis & Melzer 2002
Ammotheidae	<i>Achelia</i>	<i>Achelia assimilis</i>	–	KF603907	–	Chile, Region de los Lagos	Weis & Melzer 2002
Ammotheidae	<i>Achelia</i>	<i>Achelia assimilis</i>	–	KF603909	–	Chile, Region de los Lagos	Weis & Melzer 2002
Ammotheidae	<i>Achelia</i>	<i>Achelia bituberculata</i>	–	–	AB292185	Manazuru, Kanagawa	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Achelia</i>	<i>Achelia boschi</i>	IU-2011-682	KX535401	KX536462	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia boschi</i>	IU-2011-744	–	KX536436	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia boschi</i>	IU-2011-789	KX535415	KX536473	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia boschi</i>	IU-2011-790	KX535370	–	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia boschi</i>	IU-2011-791	KX535438	–	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia echinata</i>	–	–	AF005438	?	Giribet & Ribera 2000
Ammotheidae	<i>Achelia</i>	<i>Achelia hispidula</i> voucher	–	FJ862875	FJ862857	Brittany	Arabi <i>et al.</i> 2010
Ammotheidae	<i>Achelia</i>	<i>Achelia hoeki</i>	–	DQ389888	DQ389888	Palmer S Antarctica	Arango & Wheeler 2007
Ammotheidae	<i>Achelia</i>	<i>Achelia mixta</i>	IU-2012-1244	KX535421	–	Madang	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia mixta</i>	IU-2012-1269	–	KX536478	Madang	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia nana</i>	IU-2011-726	KX535354	KX536425	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia nana</i>	IU-2011-727	KX535359	KX536430	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia sawyayi</i>	–	DQ390070	DQ389916	Colombian Caribbean	Arango & Wheeler 2007
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 1	IU-2011-702	KX535360	KX536431	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 1	IU-2011-752	KX535355	KX536426	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 3	IU-2011-712	KX535349	KX536423	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 5	IU-2011-687	KX535419	KX536476	south Madagascar	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 7	IU-2013-18598	KX535449	KX536500	Kavieng	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 9	IU-2012-804	KX535362	KX536433	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 9	IU-2012-811	KX535383	KX536451	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 10	IU-2013-17934	KX535440	KX536493	Kavieng	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 10	IU-2013-18597	KX535372	KX536441	Kavieng	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 12	IU-2012-1221	KX535422	KX536479	Madang	this study

Family	Genus	Species	MNHN code	COI	18S	Geographical origin	Reference
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 15	IU-2012-806	KX535371	KX536440	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 15	IU-2012-807	KX535369	KX536439	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 15	IU-2012-808	–	KX536446	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia</i> sp. 15	IU-2012-809	KX535378	KX536448	Marquesas	this study
Ammotheidae	<i>Achelia</i>	<i>Achelia superba</i>	–	–	AB292186	Shimoda, Izu, Shizuoka	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Acheliana</i>	<i>Acheliana</i> sp.	IU-2011-659	KX535416	–	south Madagascar	this study
Ammotheidae	<i>Acheliana</i>	<i>Acheliana</i> sp.	IU-2011-681	KX535418	KX536475	south Madagascar	this study
Ammotheidae	<i>Acheliana</i>	<i>Acheliana</i> sp.	IU-2011-764	KX535400	–	south Madagascar	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea calmani</i>	–	FJ969346	–	Ross Sea	Nielsen <i>et al.</i> 2009
Ammotheidae	<i>Ammothea</i>	<i>Ammothea carolinensis</i>	–	FJ969347	–	Ross Sea	Nielsen <i>et al.</i> 2009
Ammotheidae	<i>Ammothea</i>	<i>Ammothea carolinensis</i>	–	FJ969348	–	Ross Sea	Nielsen <i>et al.</i> 2009
Ammotheidae	<i>Ammothea</i>	<i>Ammothea clausi</i>	–	DQ300052	DQ389894	Palmer S Antarctica	Arango & Wheeler 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea glacialis</i>	–	FJ969349	–	Ross Sea	Nielsen <i>et al.</i> 2009
Ammotheidae	<i>Ammothea</i>	<i>Ammothea hilgendorffi</i>	–	DQ390091	DQ389936	California, USA	Arango & Wheeler 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea longispina</i>	–	FJ969350	–	Ross Sea	Nielsen <i>et al.</i> 2009
Ammotheidae	<i>Ammothea</i>	<i>Ammothea ovatoides</i>	–	DQ390081	DQ389926	California, USA	Arango & Wheeler 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp.	–	FJ862871	FJ862850	?	Arabi <i>et al.</i> 2010
Ammotheidae	<i>Ammothea</i>	<i>Ammothea spinosa</i>	–	KF603912	–	Chile	Weis & Melzer 2002
Ammotheidae	<i>Ammothea</i>	<i>Ammothea appendiculata</i>	–	DQ390056	DQ389899	Colombian Caribbean	Arango & Wheeler 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea biunguiculata</i>	–	–	AB292060	Shimoda, Izu, Shizuoka	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea biunguiculata</i>	–	–	LC010686	?	Tamaoki <i>et al.</i> (unpubl.)
Ammotheidae	<i>Ammothea</i>	<i>Ammothea gracilis</i> (ex <i>Cilunculus gracilis</i>)	–	–	AB292188	off Hokkaido	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea indica</i>	–	–	AB292184	Manazuru, Kanagawa	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Ammothea</i>	<i>Ammothea setacea</i>	IU-2011-684	KX535350	KX536424	south Madagascar	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 1	IU-2013-18601	KX535377	KX536447	Kavieng	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 2	IU-2013-17914	KX535436	KX536490	Iles du Salut	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 5	IU-2013-18604	KX535428	KX536483	Iles du Salut	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 6	IU-2012-963	KX535424	–	Marquesas	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 7	IU-2012-898	KX535380	–	Guadeloupe	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 7	IU-2012-972	KX535368	KX536438	Guadeloupe	this study
Ammotheidae	<i>Ammothea</i>	<i>Ammothea</i> sp. 12	IU-2013-18586	KX535382	KX536450	Madang	this study

Family	Genus	Species	MNHN code	COI	18S	Geographical origin	Reference
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla</i> sp. 14	IU-2012-816	KX535443	KX536495	Marquesas	this study
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla</i> sp. 15	IU-2012-954	KX535374	KX536443	Guadeloupe	this study
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla spinifera</i>	-	DQ390074	DQ389919	Colombian Caribbean	Arango & Wheeler 2007
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla tippula</i>	IU-2011-679	KX535385	-	south Madagascar	this study
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla tippula</i>	IU-2011-703	KX535429	KX536484	south Madagascar	this study
Ammotheidae	<i>Ammotheilla</i>	<i>Ammotheilla tuberculata</i>	-	DQ390094	DQ389938	California, USA	Arango & Wheeler 2007
Ammotheidae	<i>Cilunculus</i>	<i>Cilunculus armatus</i>	-	-	AB292187	Shimoda, Izu, Shizuoka	Nakamura <i>et al.</i> 2007
Ammotheidae	<i>Cilunculus</i>	<i>Cilunculus roni</i>	IU-2011-2583	KX535367	-	Manus Island	this study
Ammotheidae	<i>Cilunculus</i>	<i>Cilunculus scaurus</i>	IU-2007-4650	KX535357	KX536428	New Caledonia	this study
Ammotheidae	<i>Cilunculus</i>	<i>Cilunculus sewelli</i>	IU-2011-602	KX535417	KX536474	Mozambic Channel	this study
Ammotheidae	<i>Cilunculus</i>	<i>Cilunculus sewelli</i>	IU-2011-603	KX535356	KX536427	Mozambic Channel	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis curtiscapus</i>	IU-2011-661	KX535363	KX536434	south Madagascar	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis curtiscapus</i>	IU-2011-676	KX535366	KX536437	south Madagascar	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis curtiscapus</i>	IU-2011-691	KX535347	-	south Madagascar	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis curtiscapus</i>	IU-2011-692	KX535348	-	south Madagascar	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis curtiscapus</i>	IU-2011-724	KX535353	-	south Madagascar	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis duodorospinosa</i>	-	DQ390069	DQ389915	Colombian Caribbean	Arango & Wheeler 2007
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2012-1204	KX535352	-	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2012-1206	KX535351	-	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-18621	KX535448	KX536499	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-18639	KX535399	KX536461	Kavieng	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-18640	KX535393	-	Kavieng	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-18641	KX535394	-	Kavieng	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-6573	KX535411	-	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-6580	KX535414	-	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-6600	KX535392	-	Madang	this study
Ammotheidae	<i>Nymphopsis</i>	<i>Nymphopsis muscosa</i>	IU-2013-18608	KX535390	KX536456	Iles du Salut	this study
Ammotheidae	<i>Sericosura</i>	<i>Sericosura heteroscela</i>	IU-2013-15606	KX535444	KX536496	N Medio-Atlantic Ridge, Snake Pit site	this study
Ammotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 1	IU-2014-10209	KX535439	KX536492	Futuna	this study
Ammotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 1	IU-2014-10210	KX535442	KX536494	Futuna	this study
Ammotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 2	IU-2013-19237	KX535437	KX536491	N Medio-Atlantic Ridge, Logatchev site	this study
Ammotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 2	IU-2013-19238	KX535384	KX536452	N Medio-Atlantic Ridge, Logatchev site	this study

Family	Genus	Species	MNHN code	COI	18S	Geographical origin	Reference
Ammonotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 2	IU-2013-19239	KX535346	KX536422	N Medio-Atlantic Ridge, Lucky Strike site	this study
Ammonotheidae	<i>Sericosura</i>	<i>Sericosura</i> sp. 2	IU-2013-19242	KX535373	KX536442	N Medio-Atlantic Ridge, Lucky Strike site	this study
Ammonotheidae	<i>Sericosura</i>	<i>Sericosura venticola</i>	–	DQ390080	DQ389925	North Pacific vents	Arango & Wheeler 2007
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum californicum</i>	–	DQ3900990	DQ389935	California, USA	Arango & Wheeler 2007
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603964	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603965	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603966	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603967	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603969	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum cavidorsum</i> voucher	–	KF603970	–	Chile, Region de los Rios	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum neorhetum</i> voucher	–	KF603971	–	Chile, Region de Magallanes y de la Antartica Chilena	Weis & Melzer 2002
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum orbiculare</i>	–	DQ390064	DQ389910	Mar del Plata, Argentina	Arango & Wheeler 2007
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum orbiculare</i>	–	GU370074	–	Massachusetts	Masta <i>et al.</i> 2010
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp.	–	FJ862870	FJ862849	Australia	Arabi <i>et al.</i> 2010
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 1	IU-2013-18603	KX535445	KX536497	Iles du Salut	this study
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 1	IU-2013-18606	KX535386	KX536453	Iles du Salut	this study
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 2	IU-2013-18587	KX535446	–	Kavieng	this study
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 2	IU-2013-18589	KX535358	KX536429	Kavieng	this study
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 2	IU-2013-18596	KX535379	KX536449	Kavieng	this study
Ammonotheidae	<i>Tanystylum</i>	<i>Tanystylum</i> sp. 3	IU-2013-18605	KX535396	KX536458	Iles du Salut	this study
Ammonotheidae	<i>Teratonotum</i>	<i>Teratonotum stauromatum</i> (ex. <i>Ammothella stauromata</i>)	IU-2012-959	KX535420	KX536477	Marquesas	this study
Ammonotheidae	<i>Teratonotum</i>	<i>Teratonotum stauromatum</i> (ex. <i>Ammothella stauromata</i>)	IU-2013-18591	KX535361	KX536432	Kavieng	this study
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus auchenicum</i>	–	–	AB292189	Shimoda, Izu, Shizuoka	Nakamura <i>et al.</i> 2007
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus auchenicum</i>	–	–	LC010685	?	Tamaoki <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus castelli</i>	–	FJ862876	FJ862858	Salomon Islands	Arabi <i>et al.</i> 2010
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus castelli</i>	IU-2014-8275	KX535408	KX536468	Iles du Salut	this study
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus castellioides</i>	–	DQ390070	DQ389905	Colombian Caribbean	Arango & Wheeler 2007
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus cryptopygus</i>	–	–	AB292190	off Taito-zaki, Chiba	Nakamura <i>et al.</i> 2007

Family	Genus	Species	MNHN code	COI	18S	Geographical origin	Reference
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus glaberrimus</i>	-	-	AB292191	Shimoda, Izu, Shizuoka	Nakamura <i>et al.</i> 2007
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus glaberrimus</i>	-	-	LC010684	?	Tamaoki <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus japonicus</i>	-	-	AB292192	Tosa Bay, Kochi	Nakamura <i>et al.</i> 2007
Ascorhynchidae	<i>Ascorhynchus</i>	<i>Ascorhynchus japonicus</i>	-	-	LC010683	?	Tamaoki <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Eurycyde</i>	<i>Eurycyde curvata</i>	-	DQ390055	DQ389897	Colombian Caribbean	Arango & Wheeler 2007
Ascorhynchidae	<i>Eurycyde</i>	<i>Eurycyde raphiaester</i>	-	DQ390075	DQ389920	Caribbean	Arango & Wheeler 2007
Ascorhynchidae	<i>Eurycyde</i>	<i>Eurycyde</i> sp.	IU-2013-18613	KX535388	KX536454	French Guiana	this study
Ascorhynchidae	<i>Eurycyde</i>	<i>Eurycyde spinosa</i>	-	DQ390092	DQ389937	California, USA	Arango & Wheeler 2007
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB790611	Japan	Chows <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB700615	Japan	Chows <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB700612	Japan	Chows <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB700616	Japan	Chows <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB700614	Japan	Chows <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	LC010682	?	Tamaoki <i>et al.</i> (unpubl.)
Ascorhynchidae	<i>Nymphonella</i>	<i>Nymphonella tapetis</i>	-	-	AB700613	Japan	Chows <i>et al.</i> (unpubl.)
Austrodecidae	<i>Austrodecus</i>	<i>Austrodecus glaciale</i>	-	DQ390048	DQ389890	Palmer S Antarctica	Arango & Wheeler 2007
Callipallenidae	<i>Austropallene</i>	<i>Austropallene cornigera</i>	-	DQ390077	DQ389922	Palmer S Antarctica	Arango & Wheeler 2007
Callipallenidae	<i>Austropallene</i>	<i>Austropallene cornigera</i>	IU-2013-18652	KX535376	KX536445	Antarctica	this study
Callipallenidae	<i>Austropallene</i>	<i>Austropallene cornigera</i>	IU-2013-18653	KX535364	KX536435	Antarctica	this study
Callipallenidae	<i>Austropallene</i>	<i>Austropallene cristata</i>	-	DQ390045	DQ389887	Palmer S Antarctica	Arango & Wheeler 2007
Callipallenidae	<i>Callipallene</i>	<i>Callipallene brevisstris</i>	-	DQ390057	DQ389900	Colombian Caribbean	Arango & Wheeler 2007
Callipallenidae	<i>Callipallene</i>	<i>Callipallene novaezealandiae</i>	-	DQ390082	DQ389927	Victoria, Australia	Arango & Wheeler 2007
Callipallenidae	<i>Cheilopallene</i>	<i>Cheilopallene nodulosa</i>	IU-2013-18642	KX535430	KX536485	Kavieng	this study
Callipallenidae	<i>Meridionale</i>	<i>Meridionale ambigua</i>	-	DQ390085	DQ389930	Victoria, Australia	Arango & Wheeler 2007
Callipallenidae	<i>Oropallene</i>	<i>Oropallene minor</i>	-	DQ390059	DQ389994	New South Wales, Australia	Arango & Wheeler 2007
Callipallenidae	<i>Parapallene</i>	<i>Parapallene avida</i>	-	DQ390083	DQ389928	Victoria, Australia	Arango & Wheeler 2007
Callipallenidae	<i>Propallene</i>	<i>Propallene longiceps</i>	-	DQ390054	DQ389896	Japan	Arango & Wheeler 2007
Callipallenidae	<i>Sylopallene</i>	<i>Sylopallene longicauda</i>	-	DQ390090	DQ389929	Victoria, Australia	Arango & Wheeler 2007
Colossendeidae	<i>Colossendeis</i>	<i>Colossendeis stramenti</i>	-	DQ390078	DQ389923	Antarctica, Polarsten Exp.	Arango & Wheeler 2007
Colossendeidae	<i>Colossendeis</i>	<i>Colossendeis tenera</i>	-	DQ390061	DQ389907	North Pacific	Arango & Wheeler 2007
Colossendeidae	<i>Decolopoda</i>	<i>Decolopoda australis</i>	-	DQ390063	DQ389909	Livingstone, Antarctica	Arango & Wheeler 2007
Colossendeidae	<i>Rhopalorhynchus</i>	<i>Rhopalorhynchus filipes</i>	-	FJ862872	FJ862852	New Caledonia	Arabi <i>et al.</i> 2010

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Endeidae	<i>Endeis</i>	<i>Endeis australis</i>	–	DQ390050	DQ389892	Colombian Caribbean	Arango & Wheeler 2007
Endeidae	<i>Endeis</i>	<i>Endeis clipeata</i>	IU-2011-776	KX535441	–	south Madagascar	this study
Endeidae	<i>Endeis</i>	<i>Endeis mollis</i>	–	DQ390051	DQ389893	Palmer S Antarctica	Arango & Wheeler 2007
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18550	KX535406	KX536466	Iles du Salut	this study
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18554	KX535447	KX536498	Madang	this study
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18556	KX535381	–	Madang	this study
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18634	KX535405	KX536465	Kavieng	this study
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18637	KX535426	–	Kavieng	this study
Endeidae	<i>Endeis</i>	<i>Endeis</i> sp.	IU-2013-18638	KX535427	KX536482	Kavieng	this study
Endeidae	<i>Endeis</i>	<i>Endeis spinosa</i>	–	–	F1862848	Brittany	Arabi <i>et al.</i> 2010
Endeidae	<i>Endeis</i>	<i>Endeis spinosa</i>	–	AY731173	–	?	Hassanin <i>et al.</i> 2005
Nymphonidae	–	Nymphonidae gen. sp.	IU-2014-8371	KX535433	KX536487	Iles du Salut	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon articulare</i>	IU-2013-18648	KX535398	KX536460	Antarctica	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon articulare</i>	IU-2013-18649	KX535397	KX536459	Antarctica	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon brevicaudatum</i>	–	DQ390087	DQ389889	Palmer S Antarctica	Arango & Wheeler 2007
Nymphonidae	<i>Nymphon</i>	<i>Nymphon hamatum</i>	–	DQ390076	DQ389921	Antarctica, Polarsten Exp.	Arango & Wheeler 2007
Nymphonidae	<i>Nymphon</i>	<i>Nymphon surinamense</i>	IU-2013-18614	KX535404	KX536464	French Guiana	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon surinamense</i>	IU-2013-18615	KX535387	–	French Guiana	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon surinamense</i>	IU-2013-18620	KX535434	KX536488	French Guiana	this study
Nymphonidae	<i>Nymphon</i>	<i>Nymphon unguiculatum</i>	–	DQ390053	DQ389895	Palmer S Antarctica	Arango & Wheeler 2007
Nymphonidae	<i>Pentanympion</i>	<i>Pentanympion antarcticum</i>	–	DQ390049	DQ389891	Palmer S Antarctica	Arango & Wheeler 2007
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis macnelli</i>	–	DQ390086	DQ389931	Victoria, Australia	Arango & Wheeler 2007
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis macronyx</i>	–	DQ390062	–	Livingston, Antarctica	Arango & Wheeler 2007
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis</i> sp. 1	IU-2013-18651	KX535435	KX536489	Terre Adélie	this study
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis</i> sp. 2	IU-2013-18645	KX535409	KX536469	Kavieng	this study
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis</i> sp. 3	IU-2013-18545	KX535402	–	Iles du Salut	this study
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis</i> sp. 3	IU-2013-18546	KX535407	KX536467	Iles du Salut	this study
Pallenopsidae	<i>Pallenopsis</i>	<i>Pallenopsis</i> sp. 4	IU-2013-18610	KX535432	KX536486	French Guiana	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus californicus</i>	–	DQ390068	DQ389914	Colombian Caribbean	Arango & Wheeler 2007
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus viridintestinalis</i>	–	DQ390088	DQ389933	California, USA	Arango & Wheeler 2007
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus batagensis</i>	–	DQ390072	DQ389918	Colombian Caribbean	Arango & Wheeler 2007
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus erectus</i>	–	DQ390089	DQ389934	California, USA	Arango & Wheeler 2007

Family	Genus	Species	MNHN code	COI	18S	Geographical origin	Reference
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18544	KX535391	KX536457	Iles du Salut	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18609	KX535413	KX536472	French Guiana	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18611	KX535412	KX536471	French Guiana	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18632	KX535403	KX536463	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18636	KX535425	KX536481	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18643	KX535395	-	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18644	KX535389	KX536455	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18646	KX535410	KX536470	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2013-18647	KX535431	-	Kavieng	this study
Phoxichilidiidae	<i>Anoplodactylus</i>	<i>Anoplodactylus</i> sp.	IU-2014-8292	KX535423	KX536480	Iles du Salut	this study
Pycnogonidae	<i>Pentapycnon</i>	<i>Pentapycnon charcoi</i>	-	DQ390079	DQ389924	Antarctica, Polarsten Exp.	Arango & Wheeler 2007
Pycnogonidae	<i>Pycnogonum</i>	<i>Pycnogonum diceros</i>	-	DQ390095	DQ389939	Antarctica, Polarsten Exp.	Arango & Wheeler 2007
Pycnogonidae	<i>Pycnogonum</i>	<i>Pycnogonum steamsi</i>	-	DQ390058	DQ389902	California, USA	Arango & Wheeler 2007
Pycnogonidae	<i>Pycnogonum</i>	<i>Pycnogonum tuberculatum</i>	IU-2013-18654	KX535375	KX536444	Vanuatu	this study
OUTGROUP	<i>Limulus</i>	<i>Limulus polyphemus</i>	-	-	L81949	-	Spears & Abele 1998
OUTGROUP	<i>Limulus</i>	<i>Limulus polyphemus</i>	-	NC003057	-	-	Lavrov et al. 2000a
OUTGROUP	<i>Lithobius</i>	<i>Lithobius forficatus</i>	-	NC002629	-	-	Lavrov et al. 2000b
OUTGROUP	<i>Lithobius</i>	<i>Lithobius variegatus</i>	-	-	AF000773	-	Giribet & Ribera 1998
OUTGROUP	<i>Mastigoproctus</i>	<i>Mastigoproctus giganteus</i>	-	JN018215	JN018311	-	Arabi et al. 2010
OUTGROUP	<i>Opisthopatus</i>	<i>Opisthopatus cinctipes</i>	-	NC014273	-	-	Braband et al. 2010
OUTGROUP	<i>Orthoporus</i>	<i>Orthoporus</i> sp.	-	-	AY210829	-	Mallatt et al. 2004
OUTGROUP	<i>Peripatoides</i>	<i>Peripatoides novaezealandiae</i>	-	-	AF342794	-	Mallatt & Winchell 2002
OUTGROUP	<i>Squilla</i>	<i>Squilla empusa</i>	-	-	L81946	-	Spears & Abele 1998
OUTGROUP	<i>Squilla</i>	<i>Squilla empusa</i>	-	NC007444	-	-	Swinstrom 2009
OUTGROUP	<i>Thyropygus</i>	<i>Thyropygus</i> sp.	-	NC003344	-	-	Lavrov et al. 2002
OUTGROUP	<i>Triops</i>	<i>Triops longicaudatus</i>	-	-	AF144219	-	Spears & Abele 2000
OUTGROUP	<i>Triops</i>	<i>Triops longicaudatus</i>	-	NC006079	-	-	Cook et al. 2005

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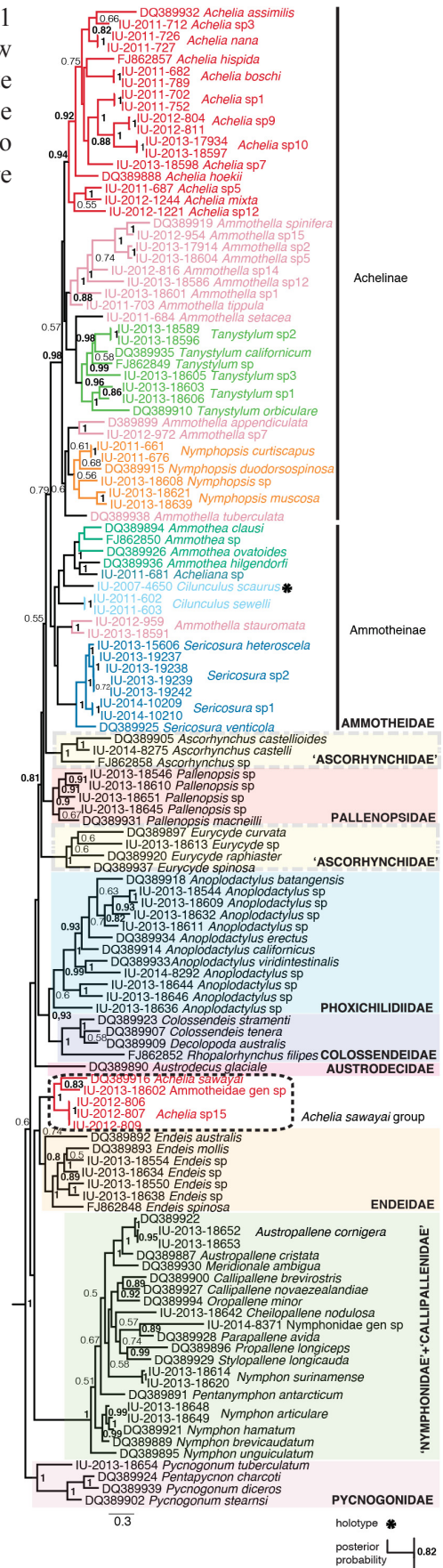
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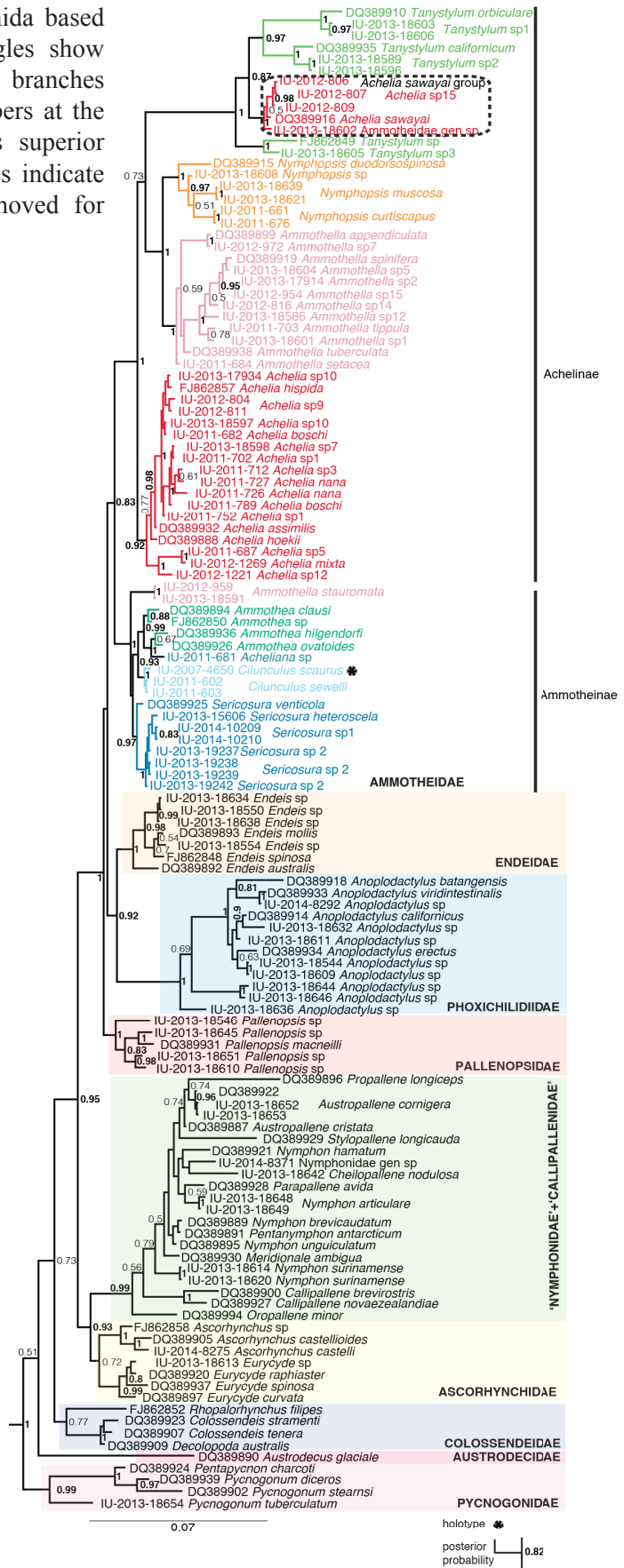
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Appendix 2A. Bayesian tree of Pycnogonida based on 135 CO1 sequences (un-partitioned analysis). Coloured rectangles show non-ammotheid families, and coloured branches discriminate ammotheid genera. The numbers at the nodes indicate the posterior probabilities superior to 0.5. Asterisks associated to taxon names indicate holotype specimens. Outgroups were removed for better readability.



Appendix 2B. Bayesian tree of Pycnogonida based on 135 18S sequences. Coloured rectangles show non-ammotheid families, and coloured branches discriminate ammotheid genera. The numbers at the nodes indicate the posterior probabilities superior to 0.5. Asterisks associated to taxon names indicate holotype specimens. Outgroups were removed for better readability.



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Zeitschrift/Journal: [European Journal of Taxonomy](#)

Jahr/Year: 2017

Band/Volume: [0286](#)

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