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Phylogeny of the sea spiders (Arthropoda, Pycnogonida) based on direct optimization of six loci and morphology

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Abstract

Higher-level phylogenetics of Pycnogonida has been discussed for many decades but scarcely studied from a cladistic perspective. Traditional taxonomic classifications are yet to be tested and affinities among families and genera are not well understood. Pycnogonida includes more than 1300 species described, but no systematic revisions at any level are available. Previous attempts to propose a phylogeny of the sea spiders were limited in characters and taxon sampling, therefore not allowing a robust test of relationships among lineages. Herein, we present the first comprehensive phylogenetic analysis of the Pycnogonida based on a total evidence approach and Direct Optimization. Sixty-three pycnogonid species representing all families including fossil taxa were included. For most of the extant taxa more than 6 kb of nuclear and mitochondrial DNA and 78 morphological characters were scored. The most parsimonious hypotheses obtained in equally weighted total evidence analyses show the two most diverse families Ammotheidae and Callipallenidae to be non-monophyletic. Austrodecidae + Colossendeidae + Pycnogonidae are in the basal most clade, these are morphologically diverse groups of species mostly found in cold waters. The raising of the family Pallenopsidae is supported, while *Eurycyde* and *Ascorhynchus* are definitely separated from Ammotheidae. The four fossil taxa are grouped within living Pycnogonida, instead of being an early derived clade. This phylogeny represents a solid framework to work towards the understanding of pycnogonid systematics, providing a data set and a testable hypothesis that indicate those clades that need severe testing, especially some of the deep nodes of the pycnogonid tree and the relationships of ammotheid and callipallenid forms. The inclusion of more rare taxa and additional sources of evidence are necessary for a phylogenetic classification of the Pycnogonida.

Pycnogonids or sea spiders (Arthropoda; Pycnogonida) are one of the most extraordinary and intriguing arthropods. A prominent proboscis, the ovigers and the extreme reduction of the abdomen are some of the peculiar characteristics making them easily recognizable and immediately striking. Pycnogonida contains more than 1300 species described, some relatively common at particular habitats and locations, but infrequent and many quite rare. They exhibit a broad range of sizes, forms and habits, from tiny, short-legged intertidal species to large, and long-legged abyssal forms. They inhabit all marine benthic environments worldwide but tend to be cryptic and infrequent thus generally neglected in marine studies. A revision of the biology of sea spiders is in Arnaud and Bamber (1987).

The unresolved phylogenetic affinities of Pycnogonida have been controversial for many years (earlier literature in Hedgpeth, 1947, 1954; Snodgrass, 1952). They are generally regarded as sister group of Euchelicerata or as the sister group to all extant euarthropods (see revision in Dunlop and Arango, 2005; more recent discussion in Giribet et al., 2005; Maxmen et al., 2005; Jager et al., 2006; Manuel et al., 2006). The uncertain origin of the ''obscure'' sea spiders, is part of, as recently expressed, ''one of the most controversial of all zoological topics'' (Budd and Telford, 2005). This controversy is continuing and being fueled by a flow of recent data such as phylogenetic data sets (Arango, 2003a), the

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characterization and interpretation of larval anatomy and neurobiology (Vilpoux and Waloszek, 2003; Maxmen et al., 2005), the description of new fossils (Siveter et al., 2004; Poschmann and Dunlop, 2006), and more recently, hox gene expression data (Jager et al., 2006; Manuel et al., 2006). Maxmen et al. (2005) provided an interpretation of novel neuroanatomical data from an *Anoplodactylus* species (i.e., *A. eroticus*, see details in Arango and Maxmen, 2006), suggesting chelifores are innervated by the protocerebrum and are not homologous to chelicerae, which are deutocerebral. According to their interpretation, chelifores might be homologous to the "great appendage" of Cambrian stem-arthropods, implying pycnogonids could be the most primitive of all extant arthropods. However, Maxmen et al.'s interpretation has been refuted (Jager et al., 2006; Manuel et al., 2006) with the first data on hox gene expression in sea spiders (*Endeis spinosa* and *Nymphon gracile*), which provides strong evidence that chelifores and chelicerae are homologous. Jager et al. (2006) and Manuel et al. (2006) support classical embryological studies showing that chelifores ganglia first appear postorally and then migrate closer to the protocerebrum (Sanchez, 1959 and earlier literature therein). These recent results seem to contribute strongly to a sister group relationship of Pycnogonida and Chelicerata. Such patterns of relationships are yet to be tested in a phylogenetic analysis using an enlarged taxon sampling for Pycnogonida that reflects the variation within the group, and that includes diverse sources of data that can explain the affinities of Pycnogonida in Arthropoda.

On a lower level, there is very little analytical work on the phylogenetic affinities or diversification of sea spiders, and basically no systematic reviews at family or genus level are available. Testable phylogenies of Pycnogonida based on morphological cladistic analysis and molecular data were produced only recently (see References of previous non-cladistic attempts in Arango, 2002, 2003a). These analyses gave a preliminary indication of possible evolutionary patterns in the group such as the parallelism in the reduction and loss of cephalic appendages (chelifores, palps and ovigers) challenging traditional taxonomic classifications (revision in Hedgpeth, 1947; Arnaud and Bamber, 1987; Child, 1998). The inclusion of fragments of ribosomal DNA (18S and 28S) in a first attempt of a total evidence analysis provided some further indications of Austrodecidae as possibly early derived taxon, and the non-monophyly of Ammotheidae (Arango, 2002). However, too-low variation in the 18S fragment and limited taxon sampling reduced the robustness of hypotheses of interfamilial relationships. The need for an improved taxon sampling, the addition of informative characters, and the use of multiple molecular markers analyzed under more exhaustive and consistent analytical procedures was recognized in Arango (2003a).

The aim of the present study was to test the higher-level phylogeny of the families of Pycnogonida using multiple molecular markers and morphological characters simultaneously. We substantially increase the body of morphological and molecular data from previous studies by (i) the use of six loci adding more than 6 kb; (ii) including 78 morphological characters [42 characters added to the previous morphological matrix in Arango (2002)]; (iii) adding four pycnogonid fossils described in the literature to test their affinities to extant taxa; and (iv) adding data of nine non-pycnogonid taxa as outgroup information. This is the largest phylogenetic data set for Pycnogonida available so far, representing all families and taxa from a broad range of latitudes and habitats. Most of the numerous genera are included, except some for which molecular data could not be obtained (e.g., *Cilunculus;* see Table 1). In Pycnogonida, 22% of the known genera (18 of 80) are monospecific (many of them monotypic), and a similar number have only two or three species described. Generally these taxa are known from deep sea or other remote areas and the chances of collecting fresh material are low. In this study, we were able to include some of the rare or remotely found taxa, such as the Antarctic *Decolopoda australis*, *Pentanymphon antarcticum* and *Pentapycnon charcoti*, and the hydrothermal vents species *Sericosura venticola* found at more than 1000 m depth. Thus, although more of the rare taxa are needed for a complete test of affinities, the proposed hypothesis of relationships here constitutes a solid framework for posterior definition of a classification of Pycnogonida, and a revision of the monophyly of the more numerous and complex taxa.

Methods

Taxon sampling

An exemplar approach was implemented at least for the ingroup taxa, scoring morphological characters for all the species of pycnogonids for which DNA sequences were obtained, without assuming monophyly at higher levels (Yeates, 1995; Prendini, 2001). Data were compiled for 29 genera and a total of 59 species (Tables 1 and 3). We included species from a wide range of depths and latitudes, as our main aim was to cover as many representative genera from all the families as possible. Most of the species were collected by the first author in a variety of intertidal and subtidal habitats (e.g., coral and rocky reefs, seagrass and algal beds, rocky platforms, fouling docks, low tide sandy beaches) in tropical and subtropical locations (e.g., Caribbean, Pacific USA, east and south-east Australia). Other material was kindly provided by collaborators sampling diverse areas, as remote as

Table 1
Classification of sea spiders based on Hedgpeth (1954), Stock (1994), Child (1979, 1982, 1998), Arnaud and Bamber (1987), Bamber and El Naga (unpublished data). Number of species in each genera in parentheses. This account indicates a total 1334 species of pycnogonids known. Geographical distribution complemented based on Müller (1993). Genera included in this study marked with *

Phylum ARTHROPODA Siebold, 1848
Class PYCNOGONIDA Latreille, 1810
EXTINCT TAXA
Cambropycnogonon klausmuelleri Waloszek & Dunlop, 2003 Upper Cambrian Orsten, Sweden
**Haliestes dasos* Siveter et al., 2004 Silurian of Herefordshire, United Kingdom
**Palaeisopus problematicus* Broili, 1928 Lower Devonian Hunsrück Slate, Germany
**Palaeopantopus maucheri* Broili, 1929 Lower Devonian Hunsrück Slate, Germany
**Palaeothea devonica* Bergström et al., 1980 Lower Devonian Hunsrück slate, Germany
Flagellopantopus blocki Poschmann and Dunlop, 2006 Lower Devonian Hunsrück slate, Germany
LIVING TAXA
AMMOTHEIDAE Dohrn, 1881
**Achelia* Hodge, 1864 (70 spp.) tropical and subtropical shallow waters in both hemispheres
Acheliana Arnaud, 1971 (1) Madagascar
**Ammothea* Leach, 1814 (41) North and South East Pacific, Antarctica, Kenya
**Ammothella* Verrill, 1900 (45) Tropical East and West Pacific, Caribbean, Africa
**Ascorhynchus* Sars, 1877 (77) Africa, Antarctica, West Pacific, North Atlantic, Caribbean, Indian Ocean
Austroraptus Hodgson, 1907 (5) Antarctica
Bathyzetes Stock, 1955 (3) Indonesia, New Caledonia
Boehmia Hoek, 1881(4) South Africa, Antarctica
Calypsopycnon Hedgpeth, 1948 (1) uncertain locality
Chonothea Nakamura & Child, 1983 (2) Japan
Cilunculus Loman, 1908 (30) Deep-sea; Africa, Antarctica, West Pacific, Philippines, North Pacific (Japan)
Dromedopycnon Child, 1982 (1) South West Atlantic
Elassorhis Child, 1982 (1) Deep-sea; Brazil
Ephyrogymna Hedgpeth, 1943 (1) Martinique
**Eurycyde* Schioedte, 1857 (21) East Atlantic (Cape Verde, Senegal), Antarctica, Pacific, Caribbean, Norway
Hedgpethius Child, 1974 (3) Caribbean
Hemichela Stock, 1954 (2) Indo-West Pacific, Japan
Heterofragilia Hedgpeth, 1943 (6) New Caledonia, Japan, St Vincent & the Grenadines
Megarhethus Child, 1982 (1) Atlantic abyssal, North American Basin
Nymphonella Oshima, 1933 (2) SouthWest Africa, Mediterranean, Japan
**Nymphopsis* Haswell, 1884(13) Africa, Indo-West Pacific, Caribbean, Mid-Pacific, Japan, Oman
Paranymphon Caullery, 1896 (3) Caribbean, Japan
Proboehmia Stock, 1991 (1) New Caledonia
Prototrygaeus Stock, 1975 (3) Mid Pacific, Guyana
Scipiolus Loman, 1908 (4) South Africa, Indo-west Pacific, North Pacific (Japan, Russia)
**Sericosura* Fry & Hedgpeth, 1969 (7) Southwest Africa, Antarctica, Pacific
**Tanystylum* Miers, 1879 (46) Africa, Antarctica, Indo-West Pacific, North Pacific, Caribbean, Japan, Oman
Trygaeus Dohrn, 1881 (1) Mediterranean
AUSTRODECIDAE Stock, 1954
**Austrodecus* Hodgson, 1907 (42) Southern Hemisphere
**Pantopipetta* Stock, 1963 (15) Antarctica and Northern Atlantic
CALLIPALLENIDAE Hilton, 1942
Anoropallene Stock, 1956 (3) Pacific Ocean
**Austropallene* Hodgson, 1915 (11) Antarctica
Bradypallene Kim & Hong, 1987 (1) Korea
Bango Bamber, 2004 (1) Gabon, Equatorial Guinea
**Callipallene* Flynn, 1929 (33) Cosmopolitan
Cheilopallene Stock, 1955 (7) Antarctica, Pacific, Caribbean, Maldives
Decachela Hilton, 1939 (2) North Pacific
Hannonia Hoek, 1881 (2) Spain, Somalia
Mimipallene Child, 1982 (1) Argentina
Neopallene Dohrn, 1881 (3) New Zealand, Mediterranean
**Oropallene* Schimkewitsch, 1930 (5) Pacific
**Parapallene* Carpenter, 1892 (21) Madagascar, Australia, Indo-west Pacific, Caribbean
Pigrogromitus Calman, 1927 (1) Pantropical
**Propallene* Schimkewitsch, 1909 (12) East Africa, Australia, North Pacific (syn. *Metapallene*, Staples, 1982).
**Pseudopallene* Wilson, 1878 (17) Polar, Australia, Philippines (syn. *Spasmopallene*, Staples 2005)
Pycnothea Loman, 1920 (2) Madagascar, Western Australia, Chile
Quebus Barnard, 1946 (1) South Africa
Safropallene Arnaud & Child, 1988 (1) South Africa
Seguapallene Pushkin, 1975 (6) Sub-Antarctic, Indo-west Pacific, North Australia, Saudi Arabia
**Stylopallene* Clark, 1963 (4) Southern Australia
PALLENOPSIDAE Fry, 1978
**Pallenopsis* Wilson, 1881 (102) Cosmopolitan
COLOSSENDEIDAE Hoek, 1881
**Colossendeis* Jarzinsky, 1870 (67) Cosmopolitan
**Decolopoda* Eights, 1835 (1) Antarctica
Dodecolopoda Calman & Gordon, 1933 (1) Antarctica
**Hedgpethia* Turpaeva, 1973 (11) Pacific
Pentacolossendeis Hedgpeth, 1943 (1) Caribbean
Rhopalorhynchus Wood-Mason, 1873 (11) South Africa, Australia, Caribbean, Indian Ocean, Philippines
NYMPHONIDAE Wilson, 1878
Boreonymphon Sars, 1888 (4) Palearctic region
Heteronymphon Gordon, 1932 (7) Antarctica, Costa Rica, North West Pacific, North Atlantic
Neonymphon Stock, 1955 (1) Virgin Islands
**Nymphon* Fabricius, 1794 (269) Cosmopolitan
**Pentanymphon* Hodgson, 1904 (1) Antarctica
Sexanymphon Hedgpeth & Fry, 1964 (1) Antarctica
PHOXICHILIDIIDAE Sars, 1891
**Anoplodactylus* Wilson, 1878 (136) Cosmopolitan
**Phoxichilidium* Milne-Edwards, 1840 (11) Subtropical and temperate: South Africa, Australia, North America
Pycnosomia Losina-Losinsky, 1961 (3) Alaska, Russia, Philippines
ENDEIDAE Norman, 1908
**Endeis* Philippi, 1843 (17) Cosmopolitan
PYCNOGONIDAE Wilson, 1878
**Pentapycnon* Bouvier, 1910 (3) Antarctica, Puerto Rico to Brazil
**Pycnogonum* Brunnich, 1764 (69) Cosmopolitan
RHYNCHOTHORACIDAE
**Rhynchothorax* Costa, 1861 (18) Antarctica, Atlantic, Mediterranean, Australia, Indo-West Pacific, California.

Antarctic deep-sea and hydrothermal vents fauna among others (list on Table 3).

Outgroups

Given the uncertain position of pycnogonids in Arthropoda and the uniqueness of their morphology, there is no clear most appropriate sister group, consequently a broad range of taxa was included as outgroups. Nine non-pycnogonid taxa are included as outgroups representing Onychophora, Tardigrada, Chelicerata, Myriapoda and Xiphosura. Pycnogonida have been proposed as sister group to chelicerates or to euarthropods (see Dunlop and Arango, 2005) and this selection of outgroups aims to cover both alternatives. The scoring of outgroups that could test the plesiomorphic state of the pycnogonid characters is not straightforward (Arango, 2002), most of the morphological characters informative for internal pycnogonid phylogeny are not scored in outgroups given the uncertain homologies for most of the key structures (e.g., proboscis, ovigers) of pycnogonids, and in most cases these uncertainties are coded as inapplicable. For some outgroup taxa, a ground-plan approach had to be followed combining DNA data for different species under a supraspecific taxon (see Table 3). Sequences of non-pycnogonid taxa were mostly found in GenBank, unpublished sequences of Scorpiones were kindly made available by L. Prendini.

Fossil taxa

Fossils described so far as pycnogonids are spare and some of them problematic (Hedgpeth, 1954; Bergström et al., 1980). Morphological characters for *Paleoisopus problematicus*†, *Paleopantopus maucheri*† and *Paleothea devonica*† were scored according to the known descriptions based on X-ray images (Bergström et al., 1980). The Silurian sea spider *Haliestes dasos*† recently described based on three-dimensional imaging (Siveter et al., 2004), was also scored in the morphological matrix (note that the *Haliestes* male attribution in Siveter et al. is not necessitated by the presence of ovigers; in fact, females of all lineages have ovigers except Phoxichilidiidae including *Endeis*, and Pycnogonidae). The larval fossil *Cambropycnogon klausmuelleri*†, from the Upper Cambrian "Orsten" of Sweden (Waloszek and Dunlop, 2002) was not included because only adult characters were considered in this data set, and due to enormous uncertainty when proposing homologies of the larval structures. Molecular data for the extinct species included were entered as missing.

Living taxa

Our criterion for using ingroup living taxa was to include species from representative genera from all families, of which tissue suitable for DNA analysis could be obtained (see Table 1 for taxonomic classification of the group and the genera included in the analysis). Interestingly, 10-legged Antarctic species *Pentapycnon charcoti* [only few specimens known (Child, 1995b), the one used in this study recently collected at 3213 m depth by the German Expedition "Polarsten" 42, ANTXIV/2, Museum für Naturkunde Berlin], *Pentanymphon antarcticum* and *Decolopoda australis* were available in this study together with other representatives of Colossendeidae, Nymphonidae and Pycnogonidae, to test for the affinities of these polymerous forms (Hedgpeth, 1954).

Finding and collecting specimens suitable for analysis can be difficult; most of the genera can only be represented by one, two or three species in the analysis, however, speciose, relatively common genera such as *Anoplodactylus*, *Nymphon* and *Achelia* are represented by more species. Our living ingroup taxa resulted in a set of 59 species in 29 living genera scored with morphological characters and six loci in most cases (Table 3).

Character sampling

Morphology

Description of some of the morphological characters mostly follows the character evaluation presented in Arango (2002). Modifications and the descriptions of new characters included in this study are shown on the list of characters (Appendix 2). Description of characters and terminology follow general pycnogonid references (e.g., Arnaud and Bamber, 1987; Child, 1998). A total of 78 characters were scored across 72 terminal taxa. Forty-two characters are added based on additional observations of external morphology including arrangement of eggs and types of larva. Characters were scored from direct observation of the specimens using light microscopy and imaging on a Hitachi S4700 Field Emission Scanning Electron Microscope at the American Museum of Natural History (AMNH). When availability of gender or life stage prevented those observations, literature descriptions were sought. Characters relevant to the relationships among other arthropods and pycnogonids are based on data sets in Giribet et al. (2001) and Edgecombe (2004) as indicated in Appendix 2. Two of these characters are ground-plan coded for Pycnogonida as well as characters related to eye ultrastructure according to Heß et al. (1996) (See Appendix 2).

DNA

Molecular work was carried out at the Molecular Systematics Laboratory of the AMNH. Genomic DNA was extracted from absolute ethanol-preserved specimens using the Qiagen Dneasy Tissue Kit: Dneasy Protocol for animal tissues (Qiagen, Valencia, CA). Depending on the size of the specimen a piece of leg was cut off and macerated with a plastic pestle after adding the lysis buffer and proteinase K. For a few small individuals ($\approx$ 0.6 mm) the whole specimen was used for

Table 2
Primers used in the amplification and sequencing of Pycnogonida

Locus	5′-3′	Reference
12S mt rRNA	12Sai: AAACTAGGATTAGATACCCTATTAT	Kocher et al. (1989)
	12Sbi: AAGAGCGACGGGCGATGTGT	Kocher et al. (1989)
16S mt rRNA	LR-N-13398 (ar): CGCCTGTTTATCAAAAACAT	Simon et al. (1994)
	LR-J-12887 (br): CTCCGGTTTGAACTCAGATCA	Simon et al. (1994)
Cytochrome C oxidase I	LCO1490: GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCOOUTOUT: GTAAATATATGRTGDGCTC	AMNH laboratory
	HCOEXTERNA: AAGTTTATATTTTAATTTTACCTGG	AMNH laboratory
	HCOEXTERNB: CCTATTGAWARAACATARTGAAAATG	AMNH laboratory
18S nuclear rRNA	1F: TACCTGGTTGATCCTGCCAGTAG	Giribet et al. (1996)
	5R: CTTGGCAAATGCTTTCGC	Giribet et al. (1996)
	3F: GTTCGATTCCGGAGAGGGA	Giribet et al. (1996)
	Bi: GAGTCTCGTTCGTTATCGGA	Whiting (2002)
	A2.0: ATGGTTGCAAAGCTGAAAC	Whiting (2002)
	9R: GATCCTTCCGCAGGTTCACCTAC	Whiting (2002)
28S nuclear rRNA	28SRD1A: CCCSCGTAAYTTAGGCATAT	AMNH laboratory
	28SB: TCGGAAGGAACCAGCTACTA	AMNH laboratory
	28SRD3A: AGTACGTGAAACCGTTCAGG	AMNH laboratory
	28SRD4B: CCTTGGTCCGTGTTTCAAGAC	AMNH laboratory
	D3A (28SA): GACCCGTCTTGAAGCAACG	Whiting (2002)
	D3B (28SBOUT): CCCACAGCGCCAGTTCTGCTTACC	Nunn et al. (1996)
	28SRD4.5a: AAGTTTCCCTCAGGATAGCTG	Whiting (2002)
	28SRD7B1: GACTTCCCTTACCTACAT	Whiting (2002)
	AS6/8: ACAAAGAAAAGACAACTCT	Mallatt and Sullivan (1998)
	AS7: GGTCAGTCGGTCCTAAGA	Mallatt and Sullivan (1998)
	OP2: CAGACTAGAGTCAAGCTCAACAGG	Mallatt and Sullivan (1998)
Histone 3	H3AF: ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)
	H3AR: ATATCCTTRGGCATRATRGTGAC	Colgan et al. (1998)

the extraction; in most cases duplicates were kept as vouchers and deposited at corresponding collections (Museum Victoria, Australian Museum, Queensland Museum, and Instituto de Investigaciones Marinas Invemar, Colombia). Certain DNA fragments were obtained for museum specimens (e.g., *Pantopipetta* sp. at AMNH). Extracted DNA aliquots for most of the species used are kept at the Ambrose Monell Cryo Collection at the AMNH (nos 131594–131673).

Double-stranded DNA template suitable for sequencing was prepared by polymerase chain reaction (PCR) using the combination of primers in Table 2. Amplifications were made in a 25-μL volume reaction adding 1 μL of each 10 μM primer, 23 μL of dH_2O and 2 μL of template DNA to the Ready-to-Go PCR beads (Amersham Pharmacia Biotech, Pittsburg, PA). The PCR programs ran on DNA Engine Dyad Thermal Cyclers (MJ Research, Waltham, MA) and Eppendorf Mastercyclers® and consisted of an initial denaturing step at 94 °C for 3 min, 40 amplification cycles (94 °C for 1 min, 48–56 °C for 1 min 15 s, 72 °C for 1 min) and a final step at 72 °C for 5 min. Annealing for 12S and Cytochrome Oxidase I (COI) fragments conducted at lower temperatures (44–46 °C). PCR products were checked on a 1% Agarose/TBE electrophoretic gel and purified using cleaning buffer on the Biomek 2000 Automation Workstation following the manufacturer's protocol. Single-stranded sequencing was conducted using automated Applied Biosystems Inc. Prism 3700 and 3730XL DNA sequencers and the Dye Terminator Sequence Kit (Applied Biosystems, Foster City, CA). Each cycle of sequencing reaction was carried out in a 8 μL reaction containing 2 μL of BDTmix, 1 μL of 3.2 ρM primer and 10 ng/mL of PCR product. The products of the cycling sequencing reactions were cleaned by precipitation with isopropanol and ethanol (40 μL 70% isopropanol added, centrifuged at 2750 ***g*** for 30 min, microplate inverted and spun for 1 min at 43 ***g***; then repeated procedure but instead of isopropanol used 70% ethanol), air-dried for 20 min and resuspended in 10 μL formamide to be loaded on to the sequencer.

Sequences were verified by independently producing the complementary strands for each sample for all fragments. Chromatograms were examined and edited using Sequencher ver. 4.1.4 (Gene Codes Corp., Ann Arbor, MI) and BioEdit (http://www.mbio.ncsu.edu/BioEdit).

A total of 755 final sequences were generated for this study. The nuclear loci used were 18S (1767–1806 bp in sea spiders; 1746–2133 bp in outgroups), 28S (2747–2860 bp in sea spiders; 2510–2949 bp in outgroups) and Histone 3 (H3) (327 bp in all). The mitochondrial loci were 12S (331–359 bp in sea spiders, 333–351 bp in outgroups), 16S (447–517 bp in sea spiders and 442–522 in outgroups) and COI (1081 bp in all). Some fragments (e.g., H3) could not be sequenced successfully for particular genera (e.g., *Austropallene*) or for some of

the species (e.g., 12S for two *Anoplodactylus*, COI for *Hedgpethia dofleini*). For specimens of *Achelia echinata*, *Phoxichilidium femoratum*, and *Rhynchothorax australis* only two of six loci were sequenced. Sequences obtained in this study are deposited in GenBank (accession numbers in Table 3).

Given the dynamic nature of the analysis with Direct Optimization (DO), which is quite demanding computationally when dealing with large data sets, 18S, 28S and COI were split into smaller, recognizably homologous segments within the matrix. The splitting was guided by conserved areas in which known motifs (primers) are located (segments separated by "#" according to POY guidelines) (Wheeler *et al*., 1996–2003; Giribet, 2005). Thus, 28S was segmented into 12 fragments, 18S into nine fragments and COI into four fragments. Targeted fragments of 12S, H3 and 16S were shorter so they were amplified as a single segment and were not split into smaller fragments. The resulting POY-formatted file of the combined data in the form of an implied alignment can be obtained from the authors by request.

Analysis

Phylogenetic reconstruction was performed with parsimony analysis of a combined data set of DNA sequences and morphological characters, following the idea that a simultaneous analysis of all available evidence maximizes explanatory power (Kluge, 1989; Nixon and Carpenter, 1996). The data were analyzed under DO (Wheeler, 1996) as implemented in the program POY, ver. 3.0.11a. (Wheeler *et al*., 1996), run on the AMNH parallel computing cluster using 25–50 processors. POY seeks the cladogram–alignment combination (i.e., the optimal tree alignment) that minimizes the total number of hypothesized transformation events required to explain the data. POY does a one-step minimization (instead of the traditional two-step analysis doing a multiple alignment first and then submitting it as a fixed character matrix to tree searching) of total changes on a trial topology, directly assessing the number of evolutionary events or DNA sequence transformations and taking into account insertion/deletion events (indels, gaps) as historical evidence (Wheeler, 1996, 2002). All data, that is both, morphology and DNA, were treated as non-additive and analyzed all under equal weights (for DNA a cost ratio of indels, transversions and transitions 1 : 1 : 1) providing the simplest minimization of transformations (but see Faivovich et al., 2005).

For the sequence character optimization, we employed two DO algorithms of different degree of exhaustiveness: Optimization Alignment or generally called Direct Optimization (DO; Wheeler, 1996), and Iterative Pass Optimization (Wheeler, 2003b). With the optimization alignment or DO (Wheeler, 1996), hypothetical ancestral sequences are optimized based only on descendant sequences, while Iterative Pass (IP) optimizes hypothetical ancestral sequences based on both descendant and ancestral sequences. IP is based on a three-sequence DO with iterative improvement and it is much more computationally demanding, but it can find more parsimonious cladograms (Wheeler, 2003b). The IP routine is being included as part of the search strategy for final rounds of tree branching and reconnecting (TBR) applied to different types of data sets (see Giannini and Simmons, 2003; Sparks and Smith, 2004; Faivovich et al., 2005; Frost et al., 2006; among others).

For purposes of the analyses, Onychophora was selected as outgroup. A total of 50 random addition sequences were swapped with TBR and altered using ratchet (Nixon, 1999). Resulting topologies were improved by subsequent rounds of ratcheting with different percentages (20 and 40) and severity (2 and 4) and tree fusing (Goloboff, 1999). Based on the resulting 57 most parsimonious topologies, another search using TBR, ratchet and tree fusing was performed under iterative pass optimization (Wheeler, 2003b) and the exact command (De Laet and Wheeler, 2003). For the analysis of only extant taxa, the resulting most parsimonious trees (MPTs) for the complete data set were used as starting point for the search under IP. The implied alignments (Wheeler, 2003a) (see Giribet, 2005) and topologies were visualized and exported from the Winclada platform (Nixon, 2000). Implied alignment was used to calculate support for clades indicated by values from jackknife resampling analyses (1000 replications, 10 random additions per replication) and to generate the list of synapomorphies, all performed in TNT (Goloboff et al., 2000). Morphological synapomorphies are mapped on to the optimal topology in Fig. 8 and molecular synapomorphies for the pycnogonid clades are listed in Appendix 3.

The morphological data were analyzed separately solely for the discussion of differences in the interpretation of character transformations based on a simultaneous analysis and a separate morphological analysis, and for comparison with previous results in Arango (2002). We did heuristic searches in TNT and NONA ver. 2.0 (Goloboff, 1997), performing TBR branch swapping on 100 random addition replicates, holding 10 000 trees and 10 starting trees. Additional swapping on 1000 trees that are up to 5% longer than optimal was run to move between local optima. Finally, these trees were TBR swapped retaining only optimal trees.

Results

Total evidence analysis

Simultaneous analysis of morphological and molecular data (10 902 characters based on implied alignment)

Table 3
Taxa and GenBank accesion numbers for each of the sequences used. Pycnogonid sequences were obtained by the authors at the AMNH laboratory (LP are unpublished outgroup sequences by L. Prendini, AMNH)

Species	Locality	28S	18S	H3	16S	COI	12S
Pycnogonida							
Austrodecidae							
Austrodecus glaciale	Palmer S. Antarctica	DQ390100	DQ389890	DQ390154	DQ389994	DQ390048	DQ389944
Pantopipetta sp.	Antarctica	DQ390112	DQ389903	DQ390167	DQ390006		
Ammotheidae							
Achelia assimilis	Victoria, Australia	DQ390143	DQ389932	DQ390196	DQ390036	DQ390087	DQ389981
Achelia alaskensis	California, USA			DQ390202	DQ390041	DQ390093	DQ389987
Achelia hoekii	Palmer S. Antarctica	DQ390098	DQ389888	DQ390152	DQ389992	DQ390046	DQ389942
Achelia spicata	Palmer S. Antarctica			DQ390176			DQ389963
Achelia echinata	California, USA			DQ390177		DQ390096	
Achelia sawayai	Colombian Caribbean	DQ390126	DQ389916	DQ390180	DQ390019	DQ390070	DQ389966
Ammothella appendiculata	Colombian Caribbean	DQ390109	DQ389899	DQ390163	DQ390003	DQ390056	DQ389953
Ammothella spinifera	Colombian Caribbean	DQ390130	DQ389919	DQ390184	DQ390023	DQ390074	DQ389969
Ammothella tuberculata	California, USA	DQ390149	DQ389938	DQ390203	DQ390042	DQ390094	DQ389988
Eurycyde raphiaster	Caribbean	DQ390131	DQ389920	DQ390185	DQ390024	DQ390075	DQ389970
Eurycyde curvata	Colombian Caribbean	DQ390107	DQ389897	DQ390161	DQ390001	DQ390055	DQ389951
Eurycyde spinosa	California, USA	DQ390148	DQ389937	DQ390201	DQ390040	DQ390092	DQ389986
Ammothea ovatoides	California, USA	DQ390137	DQ389926	DQ390190	DQ390030	DQ390081	DQ389976
Ammothea clausi	Palmer S. Antarctica	DQ390104	DQ389894	DQ390158	DQ389998	DQ390052	DQ389948
Ammothea hilgendorfi	California, USA	DQ390147	DQ389936	DQ390200	DQ390039	DQ390091	DQ389985
Ascorhynchus castelli	Colombian Caribbean	DQ390123	DQ389913		DQ390016	DQ390067	
Ascorhynchus castellioides	Colombian Caribbean	DQ390114	DQ389905	DQ390169	DQ390008	DQ390060	DQ389957
Ascorhynchus japonicum	Brit. Columbia, Canada			DQ390151	DQ389990	DQ390044	DQ389940
Nymphopsis duodorsospinosa	Colombian Caribbean	DQ390125	DQ389915	DQ390179	DQ390018	DQ390069	DQ389965
Sericosura venticola	North Pacific vents	DQ390136	DQ389925	DQ390189	DQ390029	DQ390080	DQ389975
Tanystylum orbiculare	Mar del Plata, Argentina,	DQ390120	DQ389910	DQ390174	DQ390013	DQ390064	DQ389962
Tanystylum californicum	California, USA	DQ390146	DQ389935	DQ390199		DQ390090	DQ389984
Callipallenidae							
Austropallene cornigera	Palmer S. Antarctica	DQ390133	DQ389922		DQ390026	DQ390077	DQ389972
Austropallene cristata	Palmer S. Antarctica	DQ390097	DQ389887		DQ389991	DQ390045	DQ389941
Callipallene brevisrostris	Colombian Caribbean	DQ390110	DQ389900	DQ390164	DQ390004	DQ390057	DQ389954
Callipallene novaezealandiae	Victoria, Australia	DQ390138	DQ389927	DQ390191	DQ390031	DQ390082	DQ389977
Oropallene minor	New South Wales, Australia	DQ390113	DQ389904	DQ390168	DQ390007	DQ390059	DQ389956
Parapallene avida	Victoria, Australia	DQ390139	DQ389928	DQ390192	DQ390032	DQ390083	DQ389978
Propallene longiceps	Japan	DQ390106	DQ389896	DQ390160	DQ390000	DQ390054	DQ389950
Pseudopallene ambigua	Victoria, Australia	DQ390141	DQ389930	DQ390194	DQ390034	DQ390085	DQ389979
Stylopallene longicauda	Victoria, Australia	DQ390140	DQ389929	DQ390193	DQ390033	DQ390084	
Nymphonidae							
Nymphon brevicaudatum	Palmer S. Antarctica	DQ390099	DQ389889	DQ390153	DQ389993	DQ390047	DQ389943
Nymphon floridanum	Colombian Caribbean	DQ390129		DQ390183	DQ390022	DQ390073	
Nymphon hamatum	Antarctica, Polarsten Exp.	DQ390132	DQ389921	DQ390186	DQ390025	DQ390076	DQ389971
Nymphon uniunguiculatum	Palmer S. Antarctica	DQ390105	DQ389895	DQ390159	DQ389999	DQ390053	DQ389949
Pentanymphon antarcticum	Palmer S. Antarctica	DQ390101	DQ389891	DQ390155	DQ389995	DQ390049	DQ389945
Colossendeidae							
Colossendeis stramenti	Antarctica, Polarsten Exp.	DQ390134	DQ389923	DQ390187	DQ390027	DQ390078	DQ389973
Colossendeis tenera	North Pacific	DQ390116	DQ389907	DQ390171	DQ390010	DQ390061	DQ389959
Decolopoda australis	Livingston, Antarctica	DQ390118	DQ389909	DQ390172	DQ390012	DQ390063	DQ389961
Hedgpethia dofleini	Alaska, USA	DQ390108	DQ389898	DQ390162	DQ390002		DQ389952
Phoxichilidiidae							
Anoplodactylus batangensis	Colombian Caribbean	DQ390128	DQ389918	DQ390182	DQ390021	DQ390072	DQ389968
Anoplodactylus californicus	Colombian Caribbean	DQ390124	DQ389914	DQ390178	DQ390017	DQ390068	DQ389964
Anoplodactylus erectus	California, USA	DQ390145	DQ389934	DQ390198	DQ390038	DQ390089	DQ389983
Anoplodactylus evansi	New South Wales, Australia	DQ390115	DQ389906	DQ390170	DQ390009		DQ389958
Anoplodactylus lentus	North Atlantic	AF062972	DQ389912	DQ390175	DQ390015	DQ390066	DQ389912
Anoplodactylus petiolatus	Mar del Plata, Argentina	DQ390121	DQ389911		DQ390014	DQ390065	
Anoplodactylus sp. (*pygmaeus*-group)	Colombian Caribbean	DQ390127	DQ389917	DQ390181	DQ390020	DQ390071	DQ389967

Table 3
Continued

Species	Locality	28S	18S	H3	16S	COI	12S
Anoplodactylus viridintestinalis	California, USA	DQ390144	DQ389933	DQ390197	DQ390037	DQ390088	DQ389982
Endeis australis	Palmer S. Antarctica	DQ390102	DQ389892	DQ390156	DQ389996	DQ390050	DQ389946
Endeis mollis	Colombian Caribbean	DQ390103	DQ389893	DQ390157	DQ389997	DQ390051	DQ389947
Phoxichilidium femoratum	California, USA		DQ389901	DQ390165			
Rhynchothoracidae							
Rhynchothorax australis	Livingston, Antarctica	DQ390119		DQ390173			
Pycnogonidae							
Pycnogonum diceros	Antarctica, Polarsten Exp.	DQ390150	DQ389939	DQ390204	DQ390043	DQ390095	DQ389989
Pycnogonum stearnsi	California, USA	DQ390111	DQ389902	DQ390166	DQ390005	DQ390058	DQ389955
Pentapycnon charcoti	Antarctica, Polarsten Exp.	DQ390135	DQ389924	DQ390188	DQ390028	DQ390079	DQ389974
Pallenopsidae							
Pallenopsis macneilli	Victoria, Australia	DQ390142	DQ389931	DQ390195	DQ390035	DQ390086	DQ389980
Pallenopsis macronyx	Livingston, Antarctica	DQ390117	DQ389908		DQ390011	DQ390062	DQ389960
Onychophora							
Euperipatoides rowelli/ leuckarti			U49910	AF110849			AF338016
Ruhbergia bifalcata						AF337996	
Peripatus		AY210836					
Tardigrada							
Macrobiotus/Milnesium/ Hypsibius		AY210826	U32393	CF544678			
Xiphosura							
Limulus polyphemus		AF212167	U91490	AF370813	AF216203	AF216203	AF216203
Amblypygida							
Damon annulatipes		AY829931	AY829910	AY829971	AY829889	AY829950	AY829870
Araneae							
Atrax sp.				AF110877	AF370857		
Aphonopelma hentzi		AY210803					
Nesticus cellulanus			AF005447				
Zorocrates sp.						ARA00012	ARA00012
Scorpiones							
Centruroides exilicauda			LP2142A	LP2142A	LP2142A	LP2142A	LP2142A
Pandinus imperator		AY210830					
Uropygi							
Typopeltis kasnakowi		LP1468	LP1468	LP1468	LP1468	LP1468	LP1468
Chilopoda							
Lithobius forficatus/obscurus			AF334271	AF110853	AF373608	AF309492	AF309492
Cormocephalus hartmeyeri		AY210812					
Diplopoda							
Polyxenus fasciculatus			AF173235				
Orthoporus sp.		AY210828					
Unixenus mjobergi				AF110859			
Narceus annular						AY055727	AY055727

by DO all under equal costs, resulted in 12 MPTs 28 726 steps long. The 12 MPTs are due to the unresolved position of the fossil *Paleothea devonica*†. *Paleothea devonica*† appears either related to ammotheid taxa, in the callipallenid–nymphonid clade or in the Pycnogonidae clade. The instability of *P. devonica*† causes some deep nodes to collapse (see strict consensus tree Fig. 1). When the fossil taxa were removed from the analysis, a heuristic search and swapping produced a single MPT (Length = 28699), which maintains the groupings from the complete data set and the basic topology remained the same showing most of the groups well supported (Fig. 2). Although the position of fossil taxa is not strongly supported or unambiguous, they are apparently related to extant taxa and do not constitute an early derived "fossil clade". *H dasos*†, *P. problematicus*† and *P. maucheri*† appear related to the segregated ammotheid clade *Ascorhynchus* + *Eurycyde*.

A single most-parsimonious polytomous tree obtained with non-additive, equally weighted morphological and molecular characters (one of the 12 trees obtained with the full data set, which is exactly the same topology of the single MPT obtained after the exclusion of fossils) is presented as the basis to describe the phylogeny of Pycnogonida (Fig. 3). This cladogram definitely supports monophyly of the sea spiders as expected. Monophyly of Austrodecidae, Colossendeidae, Phoxichilidiidae (except *Phoxichilidum femoratum*

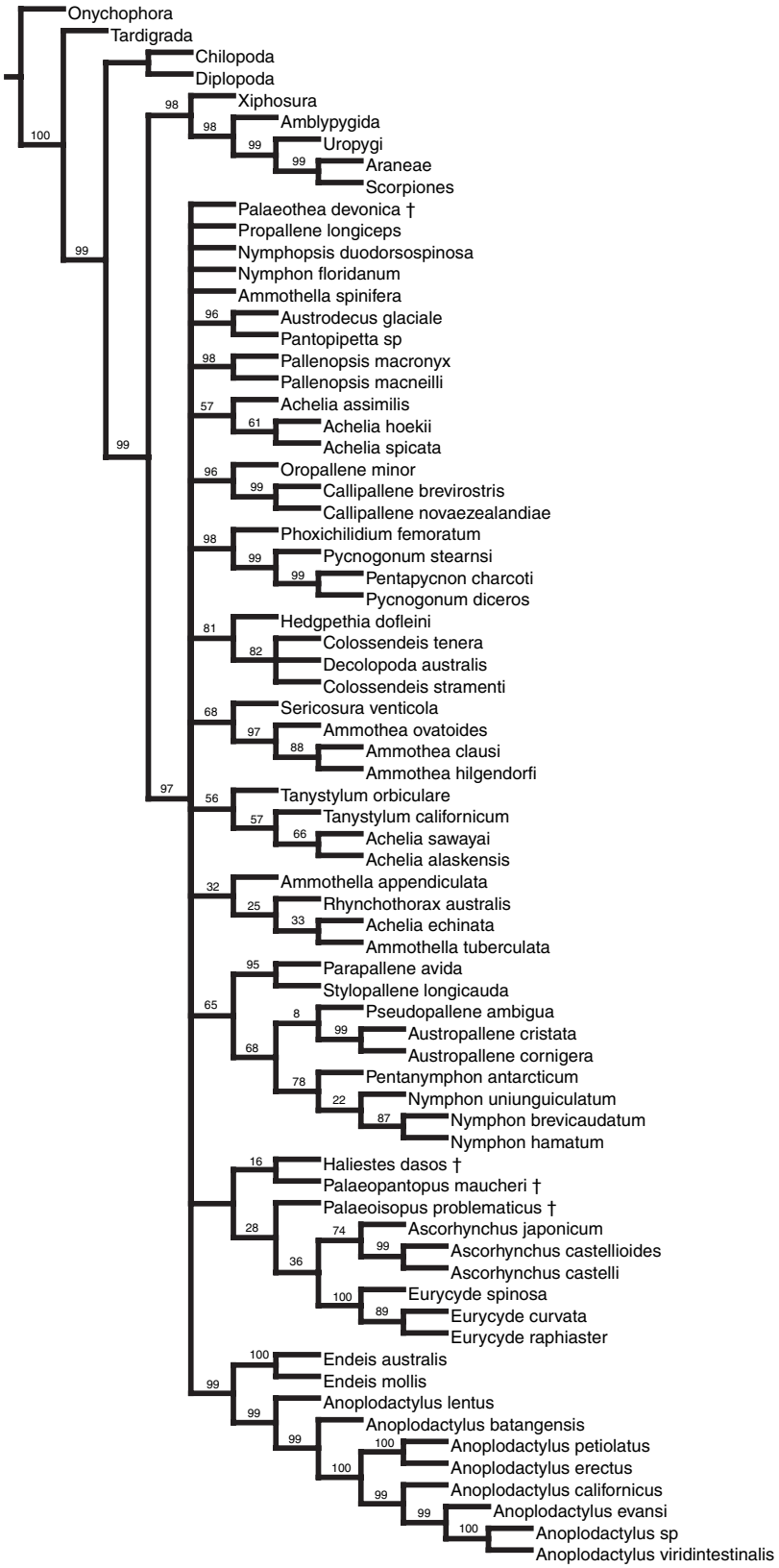

Fig. 1. Strict consensus of 12 most parsimonious trees resulting from total evidence analysis of 10 902 aligned nucleotide characters and morphology (L = 28726). Jackknife values (rep = 1000, $P = 36$) on the nodes.

Fig. 2. Single most parsimonious tree resulting from the same total evidence analysis as in Fig. 1 after excluding the four fossil taxa (L = 28 699). Jackknife values (rep = 1000, $P = 36$) on the nodes.

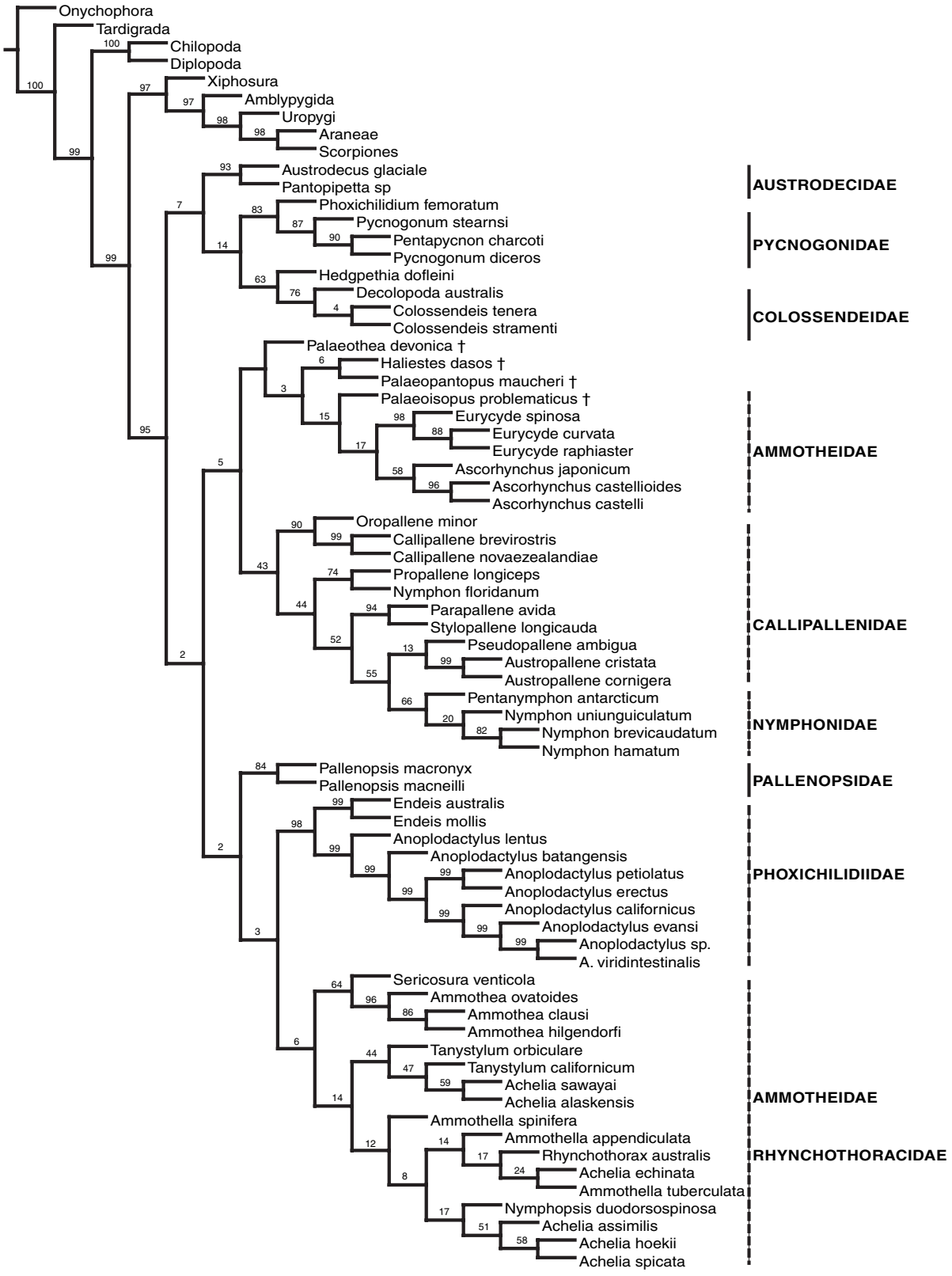

Fig. 3. Most parsimonious tree resulting from total evidence analysis of 10 902 aligned nucleotide characters and morphology. (L = 28 726, CI = 0.44, RI = 0.58). Jackknife values (rep = 1000, $P = 36$) on the nodes. Monophyletic and non-monophyletic groups obtained are indicated by solid and dashed vertical lines, respectively.

placed outside the Phoxichilidiidae clade) and Pycnogonidae was supported by these data, while Ammotheidae was polyphyletic and Callipallenidae and Nymphonidae were not recovered either, due to a Nymphonidae species grouped with callipallenid genera (Fig. 3).

Three main clades of Pycnogonida are proposed according to this topology:

1 Austrodecidae + Pycnogonidae + Colossendeidae. This early derived clade is a robust clade relating three quite diverse and widespread lineages that tend to be of Antarctic and temperate distribution. It includes *Phoxichilidium femoratum* basal to the Pycnogonidae, although well supported by resampling and support values (Figs 2 and 3) this node is based on much fewer DNA sequence characters given unavailability of *P. femoratum* sequences.

2 ((*Haliestes*† + *Paleopantopus*†) + (*Paleoisopus*† + (*Eurycyde* + *Ascorhynchus*)) + (Callipallenidae – Nymphonidae). This node shows very low support in the total analysis (according to the 12 MPTs obtained), but when fossil taxa are excluded the clade ((*Eurycyde* + *Ascorhynchus*) + (callipallenids + nymphonids)) is well supported according to jackknife values (Fig. 2). *Eurycyde* and *Ascorhynchus* two ammotheid genera with strong strigilis and ovigers terminal claw are segregated from Ammotheidae. Their sister group affinities with callipallenids + nymphonids had not been made explicit before.

3 Pallenopsidae + (Phoxichilidiidae + (Ammotheids + Rhynchothoracidae). *Pallenopsis* is solidly positioned at the base of Phoxichilidiidae and the rest of Ammotheids (species with feeble strigilis) supporting the raising of Pallenopsidae (in Fry, 1978) to family rank. *Endeis* is strongly supported as the sister taxon of *Anoplodactylus*. Within the Ammotheidae + Rhynchothoracidae clade, *Ammothea* was putatively monophyletic but other genera were not, being *Achelia* one of the most problematic. The position of the Rhynchothoracidae related to *Achelia* and *Ammothella* is weakly supported, only few informative characters were available for this rare lineage (less than 800 bp being sequenced).

Analysis of morphological data

Separate analysis of the 78 morphological characters equally weighted, resulted in 1344 MPTs (L = 338, consistency index = 0.36, retention index = 0.74). The strict consensus tree (Fig. 4) shows only few clades strongly supported (jackknife values > 60), these are mostly shallow nodes relating species within a genus, in the case of *Eurycyde* and *Endeis*, and the two genera of Pycnogonidae: *Pentapycnon* and *Pycnogonum*. Deeper nodes in the phylogenetic tree are not well supported except the early derived position of *P. problematicus*† (jackknife value = 90). Ammotheidae is shown as polyphyletic again with many of the terminals unplaced (Fig. 4), while Callipallenidae appears monophyletic and sister group to Nymphonidae. *Pallenopsis* species do not form a group and appear related to Phoxichilidiidae, although weakly supported.

Discussion

Pycnogonida relationships

Fossil taxa

A good account of the different classification schemes used for pycnogonid fossils is in Waloszek and Dunlop (2002). The controversial *Paleoisopus problematicus*† defined by a plesiomorphic five-segmented abdomen has been generally regarded as the most primitive known adult form of sea spiders, an earlier form than the Devonian *Paleopantopus maucheri* with a three-segmented abdomen assumed to be sister taxon of the rest of Pycnogonida (Pantopoda; see Hedgpeth, 1978; Bergström et al., 1980). This scheme assumes the evolutionary trend applied to the classification of sea spiders, implying they have gradually changed from more segmented to less segmented or assuming the absence of body parts as the apomorphic state (Bergström et al., 1980; Stock, 1994; Munilla, 1999; Waloszek and Dunlop, 2002). In the present simultaneous analysis, the four fossil taxa were grouped within the crown group not following a reductive trend for the whole group, thus differing from previous interpretations (Bergström et al., 1980; Munilla, 1999; Waloszek and Dunlop, 2002). Similarly, Siveter et al. (2004) analyzed the position of *Haliestes* within Pycnogonida by adding it together with other fossil taxa to the matrix in Arango (2002). Their final topologies, although poorly supported, showed *Haliestes*†, *Paleopantopus*† and *Paleothea*† in a crown-group position (Siveter et al., 2004). However, in Siveter et al. *Paleoisopus*† appears as stem lineage of the Pycnogonida, a pattern only obtained here when the present morphological matrix is analyzed separately (Fig. 4).

In general, these results indicate that fossils could be more derived than thought before. *Paleothea devonica*† is so poorly documented there is no clear indication of its affinities. In this analysis the position of pycnogonid fossils close to the ammotheid *Ascorhynchus* and *Eurycyde* is due to characters of the proboscis (characters 64 and 73) and the segmentation of palps (characters 30 and 32) (Fig. 8). Stock (1994) suggested an early condition of these ammotheid taxa because of the two-segmented proboscis in *Eurycyde* and tripartition marks of the *Ascorhynchus* proboscis, seen as remains of a primitive condition. Also, the bent abdomen in *Eurycyde* with similar armature to that of *Haliestes dasos*†

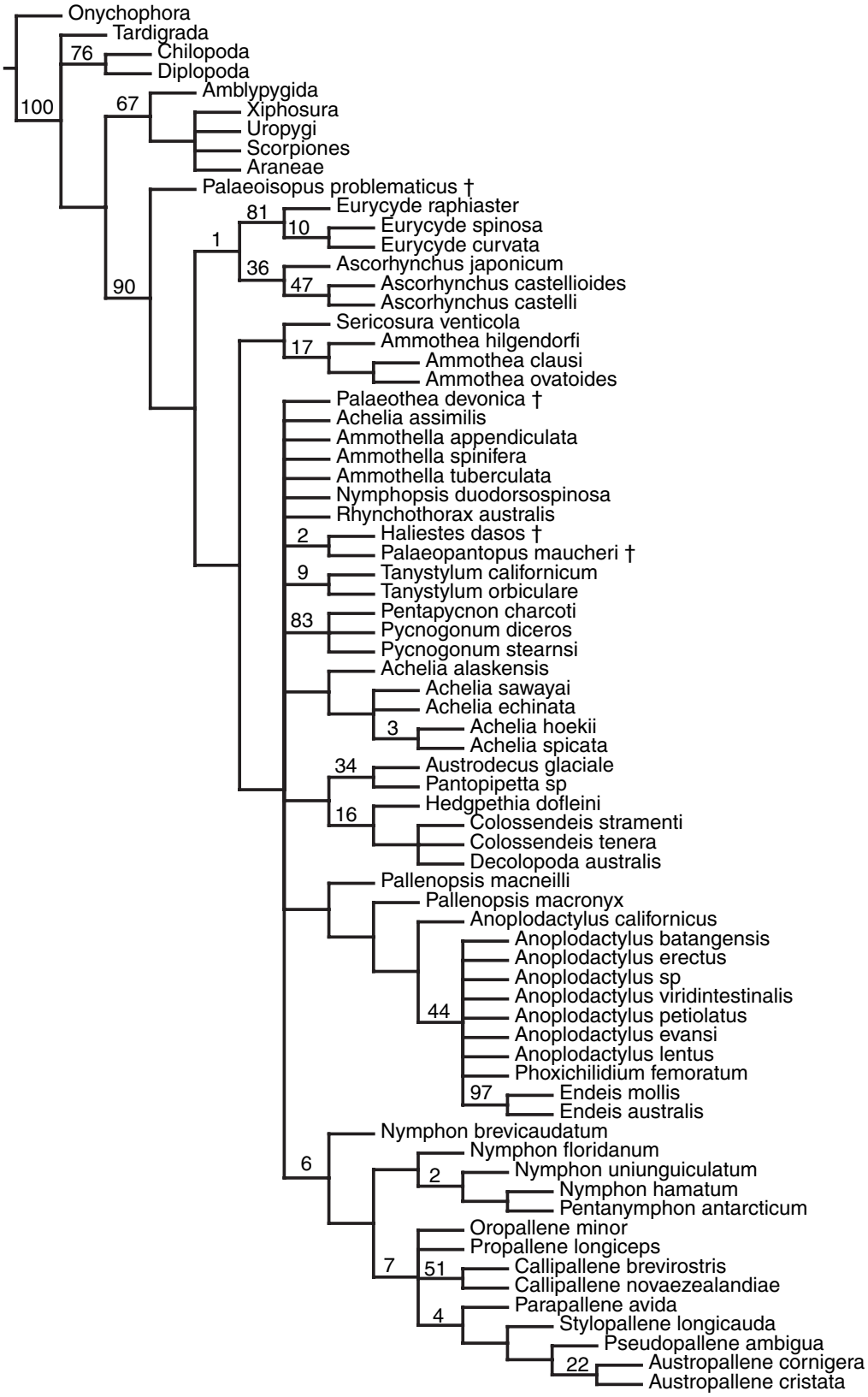

Fig. 4. Strict consensus of 1344 MPTs (Length = 338; CI = 0.36; RI = 0.74) resulting from the Parsimony analysis of the 78 morphological characters equally weighted and considered non-additive. Jackknife values (rep = 1000, $P = 36$) on the nodes.

(Siveter et al., 2004) could agree with this pattern of relationships. This total evidence analysis does not agree with the notion of fossil taxa determining plesiomorphic conditions for the whole Pycnogonida, instead, places them within the extant clade close to ammotheid taxa, therefore it does not support fossils to be considered outgroups (as in Arango, 2002). The sister group relationships of fossil pycnogonids with the *Ascorhynchus* + *Eurycyde* clade are not strongly supported though, thus no definite classification is to be made, but the results suggest the possibility of more plesiomorphic conditions in living taxa compared with the extinct taxa known so far. Pycnogonid fossils known so far and those waiting to be described might be more diverse and of more derived condition than previously recognized (Poschmann and Dunlop, 2006). The inclusion of a recently described Devonian pycnogonid with a flagelliform telson (Poschmann and Dunlop, 2006) in future analyses might challenge this hypothesis and will surely provide alternative hypothesis regarding homologies in abdomen segmentation and the relationship among fossil taxa.

Austrodecidae + Pycnogonidae + Colossendeidae

Austrodecus and *Pantopipetta*, the two Austrodecidae genera, form a monophyletic group within an early derived clade, which agrees with previous results of combined analysis (Arango, 2003a) and with a modified version of the matrix in Arango (2002), which places *Haliestes*† within Pycnogonida (Siveter et al., 2004). Previously, Austrodecidae had been grouped within ammotheids based on morphology (Arango, 2002). Morphologically, Austrodecidae is a special lineage due to the remarkable specialization of the proboscis and mouth. They bear a unique down-curved pipette-like annulated proboscis with a ventrodistal mouth bilaterally symmetrical, not tripartite as in the rest of the taxa (characters 68, 69; Fig. 6A,C). Species of Austrodecidae are extremely small and slender when compared with sister groups Pycnogonidae and Colossendeidae (see Fig. 5B,E,F), but in all three lineages chelifores are completely absent in adults (character 24) (Fig. 5), while palps are present in Austrodecidae and Colossendeidae only (characters 29 and 31). Stock (1994) and Munilla (1999), although not in an explicit analysis, related Colossendeidae to Austrodecidae and Rhynchothoracidae based on the absence of chelifores and presence of palps, while Pycnogonidae had been related to Phoxichilidiidae due to the absence of ovigers in females in both lineages (Stock, 1994; Arango, 2002) but dissolved in the present analysis of an extended morphological matrix (Fig. 4). No males carrying eggs or larvae are known for Colossendeidae or Austrodecidae (Stock, 1957), thus a different mode of reproduction could be suspected, although nothing is known about reproductive traits in these groups (Stock, 1957; Arnaud and Bamber, 1987). In a previous total evidence analysis (Arango, 2003a) Colossendeidae was related to Nymphonidae, but this grouping is not supported by this extended data set, nor are the Colossendeidae grouped with *Ascorhynchus* + *Eurycyde* as proposed before based solely on morphology (Arango, 2002). According to the present topology, the multiple rows of spines on distal segments of ovigers (strigilis; Fig. 7A) have appeared independently in the ammotheids *Ascorhynchus* and *Eurycyde* and in Colossendeids. The position of *Phoxichilidium femoratum*, a phoxichilidiid species has not been debated before. Morphologically, it shares the absence of female ovigers and larval characteristics with Pycnogonidae, but some of these transformations are also found at the Phoxichilidiidae node (Fig. 8); the expected position according to traditional classifications. However, the 18S and 16S sequences suggest a closer position to Pycnogonidae and Colossendeidae, respectively (cladograms not shown). As all loci could not be made available for *P. femoratum*, the synapomorphies at that node *Phoxichilidium* + Pycnogonidae are relatively few (see Appendix 3) and its position out of Phoxichilidiidae should be tested further with a more complete molecular data set and other sources of evidence when available.

The clade Austrodecidae + (Colossendeidae + (*Phoxichilidium* + Pycnogonidae)) has no morphological synapomorphies in the total evidence analysis (Fig. 8), but the list of their molecular synapomorphies is in Appendix 3. From the ecological point of view, the three main lineages are cosmopolitan tending to occur in deep cold waters. Colossendeidae and Austrodecidae have predominantly Antarctic and sub-Antarctic distribution (excepting few species known from North temperate seas), and Pycnogonidae have some more representatives in other shallow and warmer locations. In both, Colossendeidae and Pycnogonidae there is an occurrence of polymeric species, or species with extra trunk somites (here included *Decolopoda australis*, Colossendeidae and *Pentapycnon charcoti*, Pycnogonidae), but the phenomenon also occurs in Nymphonidae, an apparently unrelated lineage according to this topology.

(Paleothea† *+ (Haliestes*† *+ Paleopantopus*†*) + Paleoisopus*† *+ (Ascorhynchus + Eurycyde)) + (Callipallenidae–Nymphonidae)*

The separation of *Ascorhynchus* and *Eurycyde* from Ammotheidae is supported by total evidence here and also by morphological data sets (Fig. 4; Arango, 2002). The clade formed by *Ascorhynchus*, *Eurycyde* and the fossil taxa except *P. devonica*† is stable among the MPTs obtained. It is supported by strict synapomorphies related to palps and proboscis (see Fig. 8). After excluding the fossil taxa the node for *Ascorhynchus* + *Eurycyde* has maximum support as well as in

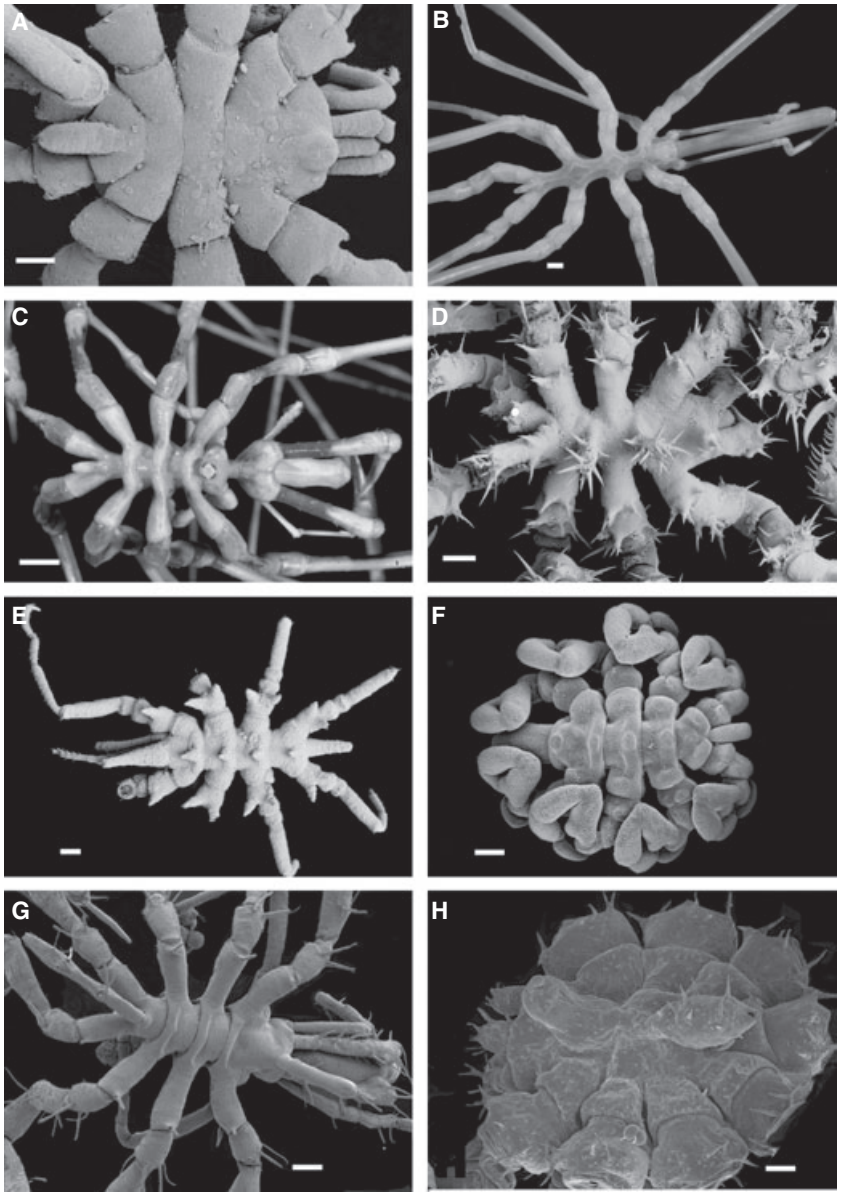

Fig. 5. Dorsal view of the trunk of six different species from different families of Pycnogonida. (A) *Achelia hoekii*, Ammotheidae, chelifores with atrophied chelae seen on the right hand side margin. Scale 100 μm. (B) *Colossendeis* sp., Colossendeidae. Note the complete absence of chelifores. Scale 200 μm. (C) *Nymphon uniuniguiculatum*, Nymphonidae. Scale 200 μm. (D) *Nymphopsis duodorsospinosa*. Note the two dorsal spinose tubercles flanked by the ocular tubercle on the right, and the abdomen, also with a spination pattern, on the left. Scale 100 μm. (E) *Austrodecus glaciale*, Austrodecidae. Ocular tubercle and palps near the left margin. Scale 200 μm. (F) *Pycnogonum stearnsi*, Pycnogonidae. Proboscis part covered by the first pair of legs on the left hand side. Note the complete absence of chelifores and palps. Scale 200 μm. (G) *Ammothella appendiculata*, Ammotheidae. Long abdomen pointing towards the upper left-hand corner. Scale 80 μm. (H) *Tanystylum orbiculare*, Ammotheidae. Chelifores are tiny knobs near the left hand margin (only first coxae of all legs shown). Scale 70 μm.

the separate morphological analysis, showing 100% frequency (values not shown) in the resulting MPTs (consensus in Fig. 4). The oviger distal segments or strigilis (character 56), the type of mouth (character 70; Fig. 6B) and the shape of proboscis (character 62; Fig. 6D), are synapomorphies for the group. *Eurycyde* is notably peculiar due to a clear segmentation of the proboscis, the anterior segment is a unique stalk or pedestal articulating with the pyriform part (Fig. 6D) and in the genotypic data, COI has a deletion of two codons at position 468 in the three species of *Eurycyde*, appearing as a molecular autapomorphy for the genus. The affinities of other ammotheid genera (rarer forms such as *Bathyzetes* or *Heterofragilia*) that could potentially relate to this clade remain to be tested. This result confirms the non-monophyly of Ammotheidae

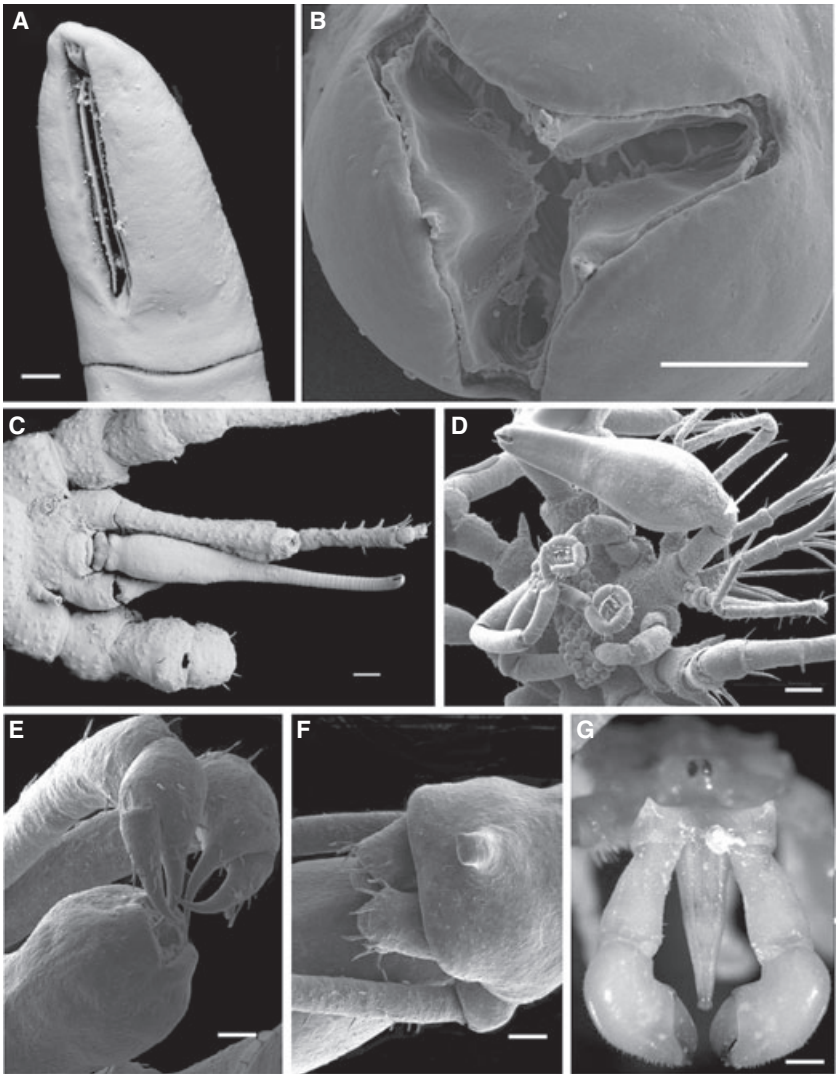

Fig. 6. (A) Mouth of *Austrodecus glaciale*. Ventral view of the distal part of the proboscis bearing the bilobate mouth, autapomorphy of the Austrodecidae. Note the annulated pattern of the proboscis (also C). Scale 10 μm. (B) Mouth of *Achelia hoekii*, frontal view showing a tripartite configuration, characteristic of all pycnogonid families except Austrodecidae. Note the deep incisures of the mouth common to the Ammotheidae. Scale 50 μm. (C) Ventral view of the cephalic segment of *Austrodecus glaciale* showing the thin, down-curved proboscis with an annulated or segmented pattern. Scale 100 μm. (D) Ventrolateral view of *Eurycyde raphiaster*, Ammotheidae, the arrow indicates the segmentation line of the proboscis, the mouth points towards the upper left corner, the ovigers with strong strigilis carrying eggs, seen at the center of the image. Scale 200 μm. (E) Mouth and chelae of *Anoplodactylus californicus*. Scale 100 μm. (F) Reduced chelifores of *Sericosura venticola*. Scale 400 μm. (G) *Austropallene cristata* frontal view of proboscis and chelifores. Scale 60 μm.

suggested in Arango (2002) and implies that the reduction or loss of chelae could be a yet unexplained event occurring in unrelated taxa, instead of being one of the main diagnostic characters of the family Ammotheidae as proposed by traditional taxonomy. Characters related to the proboscis and mouth such as the deep incisures (e.g., characters 66 and 70) (Fig. 6B) are also shared by the two groupings of ammotheids. On the other hand, *Ascorhynchus* and *Eurycyde* species tend to have a more plain and simple propodus configuration (of the type in Fig. 7E), whereas generally small ammotheid taxa such as *Ammothella* and *Achelia* show more complex propodi (Fig. 7D).

With the addition of terminals, and phenotypic and genotypic data, a monophyletic Callipallenidae is not recovered here, similar to Arango (2002) that showed a pectinate pattern of relationships among callipallenid genera. However, the extended morphological data set supports the Callipallenidae node with four synapomorphies, two of them strict (characters 30, 31, 59, 63; Fig. 4). In the total analysis the group formed by callipallenids and nymphonids is the strongest clade

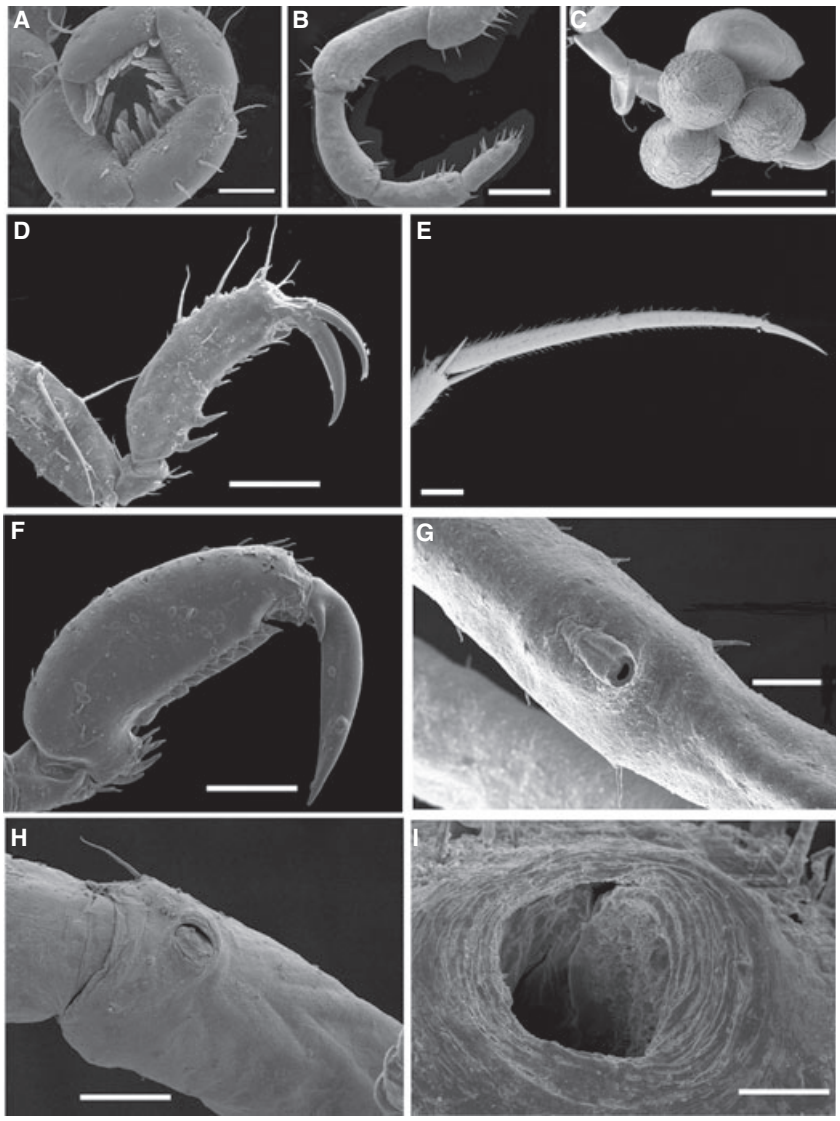

Fig. 7. (A) Detail of the strigilis of *Eurycyde raphiaster* showing the compound spines and the terminal claw on the distal segment. Scale 60 μm. (B) Distal segments of the oviger of *Pallenopsis macronyx*, Pallenopsidae, with no strigilis present. Scale 500 μm. (C) Fifth segment of the oviger of a male *Callipallene californiensis*, Callipallenidae with four eggs attached and the apophysis on the distal end of the segment. This type of apophysis occurs in most Callipallenidae and Nymphonidae species. Scale 300 μm. (D) Lateral view of the distal leg segments of *Ammothella spinifera*, Ammotheidae showing two propodus heel spines and prominent auxiliary claws dorsal to the main claw. Scale 100 μm. (E) Lateral view of the distal leg segments of *Nymphon australe* with tarsus and propodus equally slender, auxiliary claws completely absent. Scale 200 μm. (F) Propodus of *Anoplodactylus viridintestinalis* with reduced, endal auxiliary claws, typical of the genus. Scale 100 μm. (G) Cement gland tube of *Anoplodactylus californicus* male, located dorsally on the femora. Scale 100 μm. (H) Genital pore of an adult female of *Ammothella spinifera*, located ventrodistally on the second coxae of all legs. Scale 70 μm. (I) Detail of a genital pore of an adult male of *Pallenopsis macronyx*. Scale 100 μm.

supported by a large number of character transformations (see Fig. 7 and Appendix 3). In traditional classifications their morphological similarities have served to consider them relatives (Stock, 1994), although Nymphonidae have also been regarded as primitive due to their ''completeness'' (see Hedgpeth, 1947 and discussion in Arango, 2002). Morphological synapomorphies for the Callipallenidae-Nymphonidae group include the presence of an apophysis on the male oviger (character 59) illustrated in Fig. 7(C) and the type of larvae (character 77). Internally, *Oropallene* is sister taxon of *Callipallene* instead of *Propallene* (as in Arango, 2002), which unexpectedly joins *Nymphon floridanum*, the only shallow water, tropical *Nymphon* included here. The diversity in species ($\approx$ 300 species described, Table 1) and morphology patterns in *Nymphon* needs to be considered further in future studies. For instance, *N. floridanum* and other species have

auxiliary claws and heel spines on propodi while other complex of species (e.g., *N. australe*) have the simple type of propodus (Fig. 7E). This, and other combinations of characteristics have been used by taxonomists to define species complexes (for example see Child, 1988). Species-level revision of the cosmopolitan Nymphonidae and the systematic revision of callipallenid genera [21 or 22 depending on synonymies, see Staples, 2005) are essential for further testing of affinities among nymphonid and callipallenid forms. The major clade of Pycnogonida including fossil taxa, *Ascorhynchus*, *Eurycyde* and callipallenid–nymphonid taxa is supported in the total evidence analysis (Figs 3 and 8) by several synapomorphies from all partitions, but mostly from the 28S sequences (see Appendix 3). The morphological characters supporting the clade are the multiple, scattered cement glands (character 45) and the position of chelae opposing to each other (character 61) typical of Callipallenidae and Nymphonidae and here coded for *P. problematicus*† according to Bergström et al., (1980) description. The sister group relationship between the ammotheid genera and the callipallenid–nymphonids is very low supported according to jackknife resampling in the total analysis (although maximum support was obtained after excluding the fossils, Fig. 2), and the affinities of the Callipallenidae–Nymphonidae group to the other extant pycnogonid taxa remain to be tested by additional sources of non-genotypic evidence.

Pallenopsidae + (Phoxichilidiidae + ammotheids)

The position of *Pallenopsis* supports raising the familial rank Pallenopsidae following a numerical taxonomy analysis by Fry (1978) and suggested in taxonomic studies (Child, 1992; see Discussion in Bamber, 2004), and the previous cladistic analyses (Arango, 2002) of morphology, although the combined results placed *Pallenopsis* as sister taxon to *Anoplodactylus* (Arango, 2003a). The genus *Anoplodactylus* is a robust monophyletic group also well supported as sister group to *Endeis*. The *Endeis–Anoplodactylus* grouping is supported by the absence of palps (characters 29, 31), simplicity of ovigers in males (character 38), complete absence of ovigers in females (character 35) and the lack of developed strigilis (character 56). On the other hand, *Endeis* shows a remarkable set of morphological autapomorphies (Fig. 7), related to the size and position of proboscis (characters 64, 67, 76), the configuration of cement glands (characters 42–45) and especially the total absence of chelifores in adulthood. The familial rank of Endeidae Norman (Hedgpeth, 1947; Fry, 1978; Endeididae in Child 1992, 1998), although generally used in taxonomy, has been questioned before (Stock, 1965; Arango, 2002). In this study the lack of support for *Phoxichilidium* within Phoxichilidiidae, and the absence of other Phoxichilidiidae genera (Table 1) makes it difficult to propose a definite position for *Endeis* within Phoxichilidiidae. The number of autapomorphies evident for this genus could be taken as support for its familial rank, but Endeidae can only be clarified with better taxon sampling and an increased data set for the Phoxichilidiidae.

Sericosura (Fig. 6F) and *Ammothea* are at the base of the second ammotheid clade, *Ammothea* putatively monophyletic. More problematic genera are *Tanystylum* (Fig. 5H) and *Achelia*, which are again, not recovered as monophyletic even after the addition of taxa and data to previous data sets (Arango, 2002, 2003a). Rhynchothoracidae appears related to species of *Ammothella* and *Achelia*, as obtained before solely with morphological characters (Arango, 2002), although this position is supported by fewer molecular data available and this extended morphological data fails to position *R. australis* unambiguously (see strict consensus Fig. 4). Pallenopsidae + (Phoxichilidiidae + ammotheids) is supported by reduction of palps (characters 30 and 32), the absence of oviger terminal claw (character 37) (see Fig. 7) and a number of nuclear transformations mostly in 28S and 16S sequences (Appendix 3).

Implications and remarks

Novel relationships proposed in this topology are the deep nodes relating the main lineages. Based on a supposed reductive trend, earlier taxonomists had suggested Ammotheidae or Nymphonidae as primitive taxa from which more derived groups have appeared, showing a gradual reduction of appendages (e.g., Hedgpeth, 1947; Stock, 1994; Munilla, 1999). The hypothesis of the reductive trend is not supported here (nor was it in Arango, 2002, 2003a). The resulting most parsimonious hypothesis of relationships shows nymphonids strongly related to callipallenids in a quite derived position (Fig. 3). Additionally, the occurrence of species with extra trunk somites seems to be an independent event occurring at least in Nymphonidae (the 10-legged *Pentanymphon antarcticum* included here), unrelated to Colossendeidae and Pycnogonidae. According to this result the polymeric forms cannot be traced as a shared feature between the three different families. The occurrence of the extra segments remains unstudied from genetic, functional or structural perspectives (see discussion in Hedgpeth, 1954).

The phylogeny we present here, implies that chelifores have been lost three times in the Pycnogonida, a more parsimonious hypothesis than previously proposed (five losses) based on smaller data sets of morphology and DNA (Arango, 2002, 2003a). In the present phylogeny the loss of chelifores in *Endeis* could be a case of parallelism not explained, but also, the process of the loss of chelifores in *Endeis* could be different to that of Austrodecidae, Colossendeidae and Pycnogonidae (e.g., reduction or loss at a different larval stage). On the other

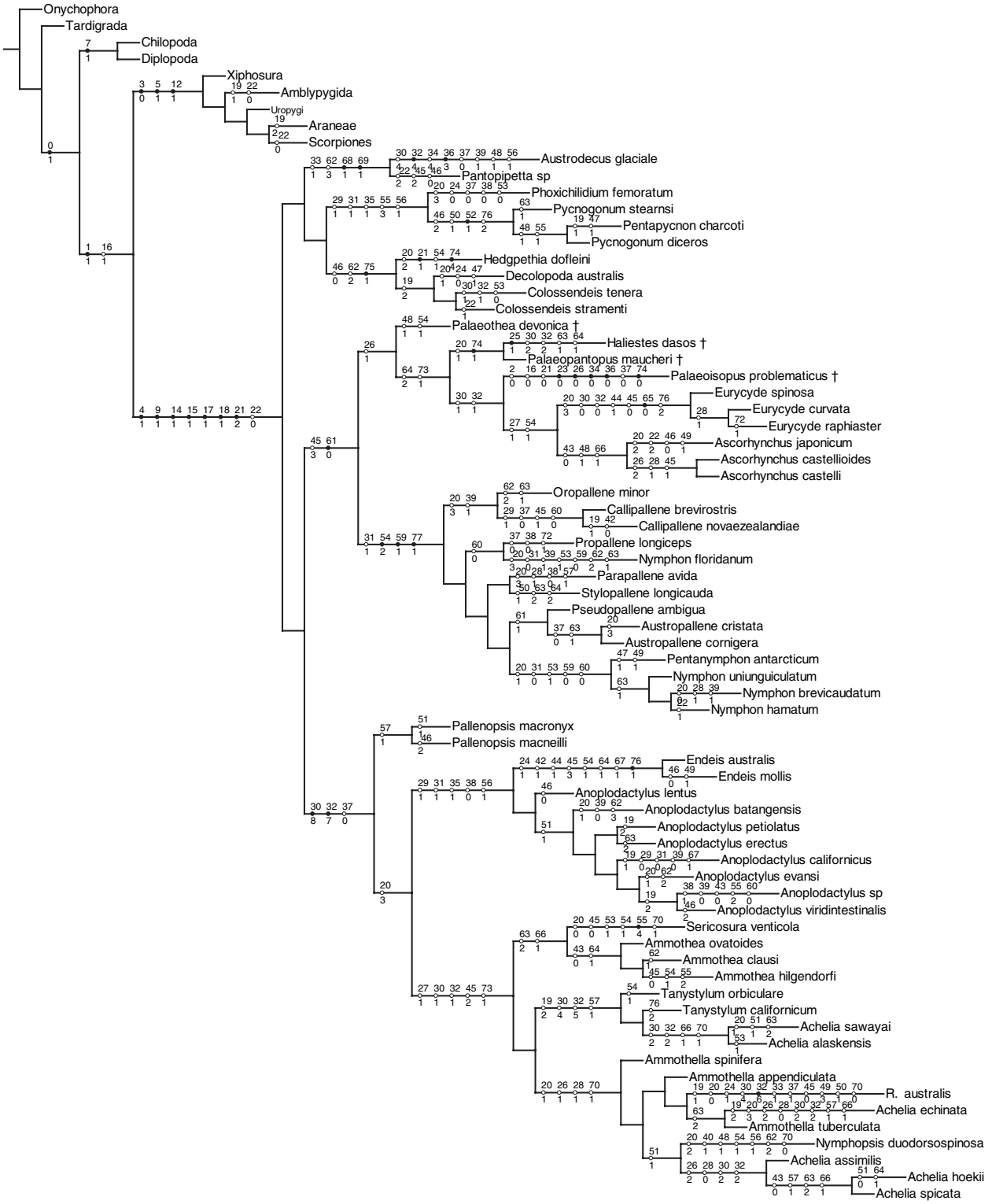

Fig. 8. Morphological characters (Appendix 2) traced onto the optimal topology found (Fig. 3) in an attempt to signal the nodes requiring a stronger test, that are those for which additional evidence is needed from non-genotypic sources, and how the present hypothesis of relationships is supported by morphological information available. The length of this tree with only morphological characters active is 406 steps, 68 longer than the resulting MPTs of the separate analysis of morphological characters in Fig. 4. The mapping of characters on to this tree is complemented by the list of molecular synapomorphies presented in the Appendix 3.

hand, Rhynchothoracidae, another group characterized by the absence of chelifores is tentatively related to ammotheids, not unexpectedly repeating the pattern obtained with morphology alone in Arango (2002), given the sparse molecular data available (see Methods) for this rare lineage. Affinities between Rhynchothoracidae, Austrodecidae and Pycnogonidae based on body shape, segmentation of palps and ovigers and number of female gonopores are not evident here (see discussion in Arango, 2002).

The strong grouping of ammotheids and phoxichilidiids including *Endeis*, could be in agreement with the concurrence of similar embryological traits observed in species of these families such as small eggs with complete and symmetrical cleavage (Sanchez, 1959), although similar embryology is described in *Pycnogonum litorale* (Sanchez, 1959; King, 1973). A peculiar spiral type of cleavage observed in a *Callipallene* species (Sanchez, 1959; King, 1973) and a different type of total cleavage in *Nymphon* (Sanchez, 1959; King, 1973) remain to be compared with embryological features in other species. Observations of embryology and larval development are known for species from a few genera, but they would definitely be useful for expanding the range of potentially informative sources.

The deeper nodes of the pycnogonid phylogeny, although strongly supported in a total evidence analysis, are not strongly defended by known morphological synapomorphies. Potentially informative structural characters with known variation within Pycnogonida, such as sperm morphology (van Deurs, 1974a,b) and eye structure (Heß et al., 1996), are to be scored across a much broader range of terminals. The task is to investigate different sources (e.g., internal anatomy, development, gene order, etc.) that could provide additional evidence to test phylogenetic hypotheses in Pycnogonida.

The absence or reduction of appendages in many taxa causes proliferation of missing or inapplicable data as well as homoplasy. In Arango (2002) the use of implied weights allowed the exploration of how homoplastic characters can have more or less influence in the phylogeny of the group, but nodes were not strongly supported. The need for more characters led to a subsequent preliminary molecular approach and combined analysis of nine major taxa, morphology and $\approx$1.2 kb of nuclear ribosomal DNA. In this work we largely increased the data set available for Pycnogonida, and provided a strong test of the phylogeny using a total evidence approach under iterative pass (IP) optimization, which contributed substantially to the improvement in tree length compared with the initial RAS, TBR and tree fusing rounds (not shown). Similar results and outcomes have been reported before with the use of IP optimization (Giannini and Simmons, 2003; Wheeler, 2003b; Sorhannus, 2004). The analysis presented here constitutes a more consistent approach and more aggressive search for most parsimonious topologies, which should provide a strong, more reliable higher-level phylogeny testing for monophyly and interfamilial affinities. The progress in non-supraspecific taxon sampling largely depends upon exhaustive fieldwork and wide collaboration with research teams sampling a variety of habitats, in particular those from deeper or remote areas. Although a complete test for monophyly for each of the genera is not attempted here, several species groups representing diversity in morphology can provide more reliable results at a higher level. In future investigations, additional terminals and the inclusion of more non-genotypic characters are needed to test the stability of the clades. Regarding the molecular data, further analyses will focus on the dependence and sensitivity of these results to variation in analytical assumptions (e.g., indel and gap extension costs). Finally, at the level of arthropod evolution studies, the great variation observed among the pycnogonid lineages, and the phylogenetic position of pycnogonid taxa sampled, need to be carefully considered, as they can potentially have large impacts in their results and interpretations.

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Appendix 1

Distribution of 78 morphological characters among 72 taxa, 63 are exemplar Pycnogonida species. Character states scored 0–8, ? = unknown; – = inapplicable. Polymorphic states: A = 0/1; B = 4/5/6.

	012345678901234567890123456789012345678901234567890123456789012345678901234567
Euperipatoides/Rubbergia/ Peripatus	00-10000100-000?0000-01----------
Macrobiotus/Milnesium/ Hypsibius	00-10010101-00000000-01----------
Lithobius/Cormocephalus	10--0031000-00000000-011----------
Polyxenus/Orthoporus/ Unixenus/Narceus	10--0031000-00000000-011----------
Limulus polyphemus	11000120001112001000011----------
Typopeltis kasnakowi	11000120001112000000011----------
Centruroides/Pandinus	11000120001112000000001----------
Atrax/Aphonopelma/Nesticus/ Zorocrates	11000120001012001002011----------
Damon annulatipes	11000120101012001001000 1----------
Haliestes dasos†	11111?201110????11?01?01001000 2?20101110?????000?????0?????120111??000?011???
Palaeoisopus problematicus†	11011?201110????01?000000000?0101000000?0-01103100??0?10?????102221??0??0010???
Palaeothea devonica†	11111?20?110????11?0??01?01??0?0?0?0??????????101?????1?????--0001??0???00????
Palaeopantopus maucheri†	11111?20?110????11??1201?0???03030101?????????200?????01??????221??0???0?1???
Achelia assimilis	11111020111001111111120100210020201010110001022002010002000 0--1101000010012000
Achelia sawayai	11111020111001111112120100210020201010110001022002010001010 0--1201100010012000
Achelia alaskensis	11111020111001111112320100210020201010110001022002000101010 0--1101100010012000
Achelia hoekii	11111020111001111111320100210020201010110000022002000010100--1211100010012000
Achelia spicata	11111020111001111111A020100210020201010110000021002010001010 0--1201100010012000
Achelia echinata	11111020111001111112320100210020201010110001022002000001010 0--1201100010012000
Ammothea clausi	11111020111001111110320100210010101010110000021 0-20000010000--1211100000012000
Ammothea hilgendorfi	11111020111001111110320100210010101010110000001002000012000 0--2211100000012000
Ammothea ovatoides	11111020111001111110320100210010101010110000021002000001000 0--221110000001 20?0
Ammothella appendiculata	11111020111001111110120100111010101010110001020002000102000 0--1101000010012000
Ammothella spinifera	11111020111001111110120100111010101010110001021002000001000 0--1101000010012000
Ammothella tuberculata	11111020111001111110120100111010101010110001022012000001000 0--1201000010012000
Anoplodactylus batangensis	11111020111001111110120100200 1-1--41-000-0010110020100031--0113101000000-02001
Anoplodactylus erectus	11111020111001111110320100200 1-1--41-0011001011002010003 1--0110201000000-02001
Anoplodactylus californicus	11111020111001111111320100200080704 1-000-0010110020100031--0110101010001102001
Anoplodactylus sp.	11111020111001111112320100200 1-1--41-010-0000110020100021--0010101000000-02001
Anoplodactylus viridintestinalis	11111020111001111112320100200 1-1--41-0011001012002010003 1--0110101000000-02001
Anoplodactylus petiolatus	11111020111001111112320100200 1-1--41-0011001011002010003 1--0110101000000-02001
Anoplodactylus evansi	11111020111001111110120100200 1-1--41-0011001011002010003 1--0112101000001-02001
Anoplodactylus lentus	11111020111001111110320100200 1-1--41-0011000010002000003 1--0110101000000-020?1
Ascorhynchus castellioides	11111020111001111110020100211010101011 10-0000110120001120010--1221100010012000
Ascorhynchus castelli	11111020111001111110020100211010101011 10-0000110120001120-10--1221100010012000
Ascorhynchus japonicum	11111020111001111110222100110010101011 10-0100300110001120010--122110001001 20?0
Austrodecus glaciale	11111020111001111110020110---0404140301100012110132001001--0--32010011000-2???
Austropallene cornigera	11111020111001111110020100200 1-1--101010-0102320020000210001113111010 0?0-020?2
Austropallene cristata	11111020111001111110320100200 1-1--101000-1112320020000210001113111 0?00?0-02002
Callipallene brevirostris	11111020111001111110320100200 1-1--1010110010210002000021000100000100000 0-02002
*Callipallene novaezealandi*ae	11111020111001111111320100200 1-1--1010110000210002000021000100000100000 0-02002
Colossendeis stramenti	11111020111001111112021110---00000101110-1----00020001-00010--22010000000-21-?

Appendix 1
Continued

	0123456789012345678901234567890123456789012345678901234567890123456789012345678901234567
Colossendeis tenera	11111020111001111112020110---01010101110-1----000--000000010--22010000000-21-?
Decolopoda australis	11111020111001111112120101100000000101110-1----010000?10000101122010000000021-?
Endeis mollis	11111020111001111110320110---1-1--31-001-0101300010000121--0--0111010000--2011
Endeis australis	11111020111001111110320110---1-1--31-001-0101310020000121--0--0111010000--2011
Eurycyde raphiaster	11111020111001111110320100111000000101110-0011010020001120010--1220000001011202?
Eurycyde spinosa	11111020111001111110320100110000000101110-0011010020001120010--1200000001001202?
Eurycyde curvata	11111020111001111110320100111000000101110-0????100?0001120010--1200000001001202?
Hedgpethia dofleini	11111020111001111110210110---00000101110-1----0003?0?1100010--22010000000-31-?
Nymphon brevicaudatum	11111020111001111111002010020105050101111001121200200012100000000101000000000200?
Nymphon floridanum	11111020111001111110320100200050501011111-0102300020001210000002101000000002002
Nymphon hamatum	11111020111001111110121100200050501011110-?????100????1-1000?000101000000020??
Nymphon uniunguiculatum	11111020111001111110120100200050501011110-0102300020001210000000101000000020??
Nymphopsis duodorsospinosa	11111020111001111112220100111010101010111001021012010012 1--0--2101000000012000
Pallenopsis macneilli	11111020111001111110020100200080701020110001212002000001010011010100000001020??
Pallenopsis macronyx	11111020111001111110020100200080701 0?010-0012110020100010100110001000000 1020??
Pantopipetta sp	11111020111001111110022110---03031101110-00122000??0?10?0000--32010011000-2???
Parapallene avida	11111020111001111110320100201 1-1--101100-1----000200002101011020010000 00-020?2
Oropallene minor	11111020111001111110320100200061--1011110011231002000021000110210100000 0-020??
Pentanymphon antarcticum	11111020111001111111012010020005050101110-0102301010001210000000001000000002???
Pentapycnon charcoti	11111020111001111111020110---1-1--21-110-1----21131011011--0--1001000000--2???
Phoxichilidium femoratum	11111020111001111110320100200 1-1--41-000001001100200000 31--0110001000000-02001
Propallene longiceps	11111020111001111110020100200061-0101000-0112310020000210001000001000000102002
Pseudopallene ambigua	11111020111001111110020100200 1-1--101100-1----200200002100011110210000 00-02002
Pycnogonum diceros	11111020111001111110020110---1-1--21-110-1----20131011111--0--2201000000--2?20
Pycnogonum stearnsi	11111020111001111110020110---1-1--21-110-1----20031011131--0--0101000000--2020
Rhynchothorax australis	11111020111001111111020110---0406110111100010020131001030000--11010000000-2???
Sericosura venticola	11111020111001111110020100210010101010110001001002000114 0000--2201100010012000
Stylopallene longicauda	11111020111001111110020100 2001-1--101110-1----200210002100011012210000?0-02002
Tanystylum californicum	11111020111001111112320100210040501010110001022002000001 0100--1101000000012020
Tanystylum orbiculare	11111020111001111112320100210 0B0B0101011000102200200001 10100--2101000-00012000

Appendix 2

List of 78 morphological characters used in this study. Description and earlier references of previously used characters are signaled in bold with initial and number of character in that study. A# in Arango (2002); E# in Edgecombe (2004); WH# in Wheeler and Hayashi (1998).

0 Sclerotization of cuticle into hard, articulated exoskeleton: (0) absent; (1) present (**E17**).

1 Tagmosis into prosoma-opisthosoma: (0) absent; (1) present **(E190)**.

2 Opisthosoma greatly reduced forming a slender tube emerging from the last pair of legs: (0) absent; (1) present **(E193)**.

3 Body carapace or shield: (0) present; (1) absent.

4 Body extending in lateral processes: (0) absent; (1) present.

5 Endosternum (ventral tendons fused into prosomal endosternum): (0) absent; (1) present. **(E207).**

6 Appendage of deutocerebral segment: (0) jaw; (1) walking leg; (2) Chelicera/Chelifore; (3) antenna. (**E103**). This is a crucial character to resolve pycnogonids position in arthropod data sets. Segmental correspondence recently discussed in Edgecombe (2004), Maxmen et al. (2005), Manuel et al. (2006), Jager et al. (2006), and others cited therein. In this particular data set we are favoring the hypothesis that chelicerae of chelicerates and chelifores of pycnogonids are homologous (see Discussion in Vilpoux and Waloszek, 2003; Manuel et al., 2006; Jager et al., 2006), in opposition to the argument by Maxmen et al. (2005) suggesting they are convergent structures innervated from different segmental neuromeres. If pycnogonids are at the base of the arthropod tree and not related to euchelicerates as suggested by Maxmen et al. then chelifores/chelicerae are a plesiomorphy of Arthropoda or a product of convergence. In this study, the chelifore = chelicerae hypothesis follows the scoring from previous arthropod data sets based on classical embryological studies (e.g., Sanchez, 1959) and now on novel hox gene expression data.

7 Mandible: (0) absent; (1) present **(E119)**.

8 Triradiate pharyngeal lumen: (0) absent; (1) present. **(E99)**

9 Enlarged external fixed proboscis with terminal triangular mouth (internally observed in Austrodecidae): (0) absent; (1) present **(WH4)**.

10 Post oral antenna: (0) present; (1) absent **(WH1)**.

11 Pedipalps chelate: (0) absent; (1) present.

12 Ganglia of post oral appendages fused into a single nerve mass: (0) absent; (1) present. **(E51).**

13 Number of median eyes: (0) none; (1) four; (2) two. (**E77**).

14 Orientation of retinula cells: (0) eversely oriented; (1) inversely oriented. Supraspecific Pycnogonida have been coded with retinula cells inversely oriented according to results in Heß et al. (1996) **(E79)**.

15 Eyes *Tapetum lucidum*: (0) absent; (1) present. The presence of *Tapetum lucidum* is coded as an apomorphy of the supraspecific terminal Pycnogonida according to Heß et al. (1996).

16 Telson: (0) present; (1) absent **(E261)**.

17 Ovigers: (0) absent; (1) present. **(E141)**

18 Genital pores location: (0) on body segment; (1) on coxae of legs **(E275, 276)**.

19 Trunk segmentation: (0) fully segmented; (1) partially segmented; (2) trunk not segmented **(A19)**.

20 Abdomen position: (0) horizontal; (1) diagonal to body; (2) bent or pointing ventrally; (3) erect **(A30)**. One state has been added to code for the bending of the abdomen pointing downwards in *Ascorhynchus* and *Nymphopsis*, or the extreme ventral position observed in *Hedgpethia*.

21 Anus position: (0) ventral on last segment of abdomen; (1) ventral on reduced abdomen; (2) apically on abdomen. Generally all pycnogonid taxa have the anus at the tip of the abdomen and can be seen dorsally. *Hedgpethia*, is the only genus included here in which the anus is positioned ventrally on a extremely reduced abdomen. Another Colossendeid genus, *Rhopalorhynchus*, shares this state (illustration in Arango, 2003b). No description of the anus is available for the fossils *H. dasos* and *P. devonica*.

22 Ocular tubercle: (0) present; (1) absent; (2) reduced (**E85**).

23 Leg shape: (0) flattened, laterally compressed; (1) tubular. This character codes for the unique flattened shape of the legs described in *P. problematicus* (Bergström et al., 1980).

24 Loss of chelifores when adulthood reached in pycnogonids: (0) absent; (1) present. (see Fig. 5). The loss of chelifores with the last molt when they convert to adulthood in many genera of sea spiders is coded as an apomorphic event. This is the same as character 0 in Arango (2002) just re-stated so the process of losing the chelifores is the character used **(A0)**.

25 Chelae with finger reversal: (0) absent; (1) present. In most known pycnogonid genera the movable finger of each chela is placed ventrally to the immovable finger and articulates downward. In *Decolopoda* and *Dodecolopoda* (not included here) two of the polymerous taxa of Colossendeidae the movable finger is placed dorsally and articulated upward (see in Child, 1995a).

26 Chelifore scape number of segments: (0) three; (1) two; (2) one. When chelifores are absent coded as inapplicable (**A1** but unordered).

27 Loss of chelae when adulthood reached: (0) absent; (1) present (see Fig. 5E,F,G) **(A2)**.

28 Chelifores dorsal spines on scape: (0) absent; (1) present **(A3)**.

29 Palps males: (0) present; (1) absent **(A4)**.

30 Palps number of segments in male pycnogonids: (0) 10; (1) nine; (2) eight; (3) seven; (4) six; (5) five; (6) four; (7) two; (8) one **(A5)**.

31 Palps female: (0) present; (1) absent **(A6)**.

32 Palps number of segments in female pycnogonids: (0) 10; (1) nine; (2) eight; (3) seven; (4) six; (5) five; (6) four; (7) one **(A7)**.

33 Segmentation line at base of palp: (0) present; (1) absent. In most pycnogonids the palps are visibly segmented from the body. In Austrodecidae and Rhnchothoracidae the first segment of the palp does not articulate (no segmentation line visible) but appears as a lateral process.

34 Ovigers number of segments in male: (0) 11; (1) 10; (2) nine; (3) seven; (4) six; (5) five; (6) four **(A8)**.

35 Oviger female: (0) present; (1) absent **(A9)**.

36 Oviger female number of segments: (0) 11; (1) 10; (2) nine; (3) six **(A10)**.

37 Ovigers terminal claw (in both sexes when present in females otherwise in males, same applies to characters 55–58): (0) absent; (1) present **(A11)** (Fig. 6A,B).

38 Propodus heel: (0) present; (1) absent. The heel is especially conspicuous in Phoxichilidiidae and some Callipallenids.

39 Auxiliary claws: (0) absent; (1) present **(A12)** (Fig. 7D,E).

40 Auxiliary claws position: (0) ectal dorsally from main claw; (1) lateral to main claw. The tiny auxiliary claws in *Anoplodactylus* when present, are found laterally, implanted on the main claw, not arising dorsally from the base of the main claw as in other sea spiders (Fig. 7F).

41 Cement glands on femora and/or other leg segments: (0) present; (1) absent **(A13)**.

42 Cement glands number: (0) one; (1) multiple **(A14)**.

43 Cement glands type: (0) pore or slit; (1) tube (Fig. 7G) **(A15)**.

44 Cement gland position on a transverse axis on femora: (0) dorsal (Fig. 7G); (1) lateral; (2) ventral **(A16)**.

45 Cement gland position on a longitudinal axis on femora: (0) proximal; (1) at or near midpoint (Fig. 7G); (2) distal; (3) distributed or scattered **(A17)**.

46 Trunk shape: (0) elongate; (1) medium; (2) compact **(A18)** (Fig. 5).

47 Trunk segments number: (0) four; (1) five. The significance of the polymery in some families of pycnogonids has not been investigated. *Pentanymphon antarcticum*, *Pentapycnon charcoti* and *Decolopoda australis* (fig. 3e in Dunlop and Arango, 2005) show five-segmented body, instead of the usual four. Six-segmented trunk species such as

Dodecolopoda mawsoni or *Sexanymphon mirabilis* were not available for this study.

48 Dorsomedian trunk tubercles: (0) absent; (1) present. Conspicuous tubercles dorsally are coded as apomorphic, e.g., *Nymphopsis* (Fig. 4D,E).

49 Genital pores position in males: (0) all pairs of legs; (1) second, third and fourth pairs of legs; (2) third and fourth pair of legs; (3) fourth pair of legs **(A20)**. Detail of a male genital pore in Fig. 7(I).

50 Pair of legs having genital pores in female: (0) all pairs of legs; (1) fourth pair of legs; (2) first, second and third pair of legs **(A21)**.

51 Genital spurs ventrally on second coxae of males: (0) absent; (1) present. Mature males of *Anoplodactylus* and some ammotheids show prominent spurs ventrally on second coxae of the third and fourth pair of legs. These spurs bear a small genital pore at the tip.

52 Genital pores position in females: (0) Ventrally on second coxae (Fig. 7H); (1) Dorsally on second coxae. Dorsal genital pores in females appear as an autapomorphy of Pycnogonidae. The location of female gonopore could not be observed nor was available from material or descriptions for the extinct taxa, *Decolopoda australis*, *Hedgpethia dofleini Nymphon hamatum* and *Pantopipetta* sp.

53 Heel spines: (0) present; (1) absent **(A22)**.

54 Ocular tubercle position: (0) anterior; (1) at or near midpoint; (2) posterior (as in *Nymphon*, Fig. 5C) **(A23)**.

55 Oviger longest segment: (0) sixth; (1) fifth; (2) fourth; (3) third; (4) one **(A24)**.

56 Oviger spines (developed strigilis): (0) present; (1) absent **(A25)** (Fig. 6E,F).

57 Oviger spines type: (0) compound; (1) simple **(A26)**.

58 Oviger number of rows of spines: (0) one; (1) multiple **(A27)**.

59 Oviger apophysis in males: (0) absent; (1) present. Only present in species of Callipallenidae and Nymphonidae, in which mature males show a distal apophysis on the fifth segment of the ovigers (Fig. 7C).

60 Chelae teeth: (0) present; (1) absent **(A28)**.

61 Chelae orientation: (0) opposing each other; (1) pointing down; (2) pointing upwards. A third state is included to code the state described from reconstructions of the Silurian *H. dasos* showing the chelae pointing upwards (see supplementary information in Siveter et al., 2004) **(A29)**.

62 Proboscis shape: (0) A; (1) B; (2) C; (3) D (Fry and Hedgpeth, 1969; Arango, 2002) **(A31)**.

63 Proboscis relative length: (0) less than half the trunk length; (1) half or near half the trunk length (45–55%); (2) more than half the trunk length (larger than 55% of trunk length). The description of states (1) and (2) has been modified as in Siveter et al. (2004) **(A33)**.

64 Proboscis position: (0) frontal; (1) diagonal with respect to body axis; (2) ventral **(A34)**.

65 Proboscis segmentation: (0) present; (1) absent. A unique basal segment in form of pedestal is observed in *Eurycyde* species (Fig. 6D). It is considered to be a primitive character by Stock (1994). The plesiomorphic state cannot be defended until there is a clear hypothesis of homology regarding the anterior segmentation of pycnogonids and other arthropods.

66 Proboscis articulated by basal unsclerotized arthrodial membrane for mobility (Arnaud and Bamber, 1987): (0) absent, not as evident; (1) present. A flexible articulation at the base of the proboscis gives vertical and some lateral movement to the proboscis observed in ammotheid taxa. In species with a short, apparently fixed proboscis like the nymphonids or callipallenids, this articulation is not observed.

67 Mouth surrounded by sensory setae: (0) absent; (1) present.

68 Proboscis annulation: (0) absent; (1) present. A pipette-like, thin, curved proboscis as autapomorphic of the Austrodecidae (Fig. 6C).

69 Mouth configuration in pycnogonids: (0) Trilobate, terminally opening at front of proboscis (Fig. 6B); (1) Bilobate, ventrodistally on proboscis (Fig. 6A). It distinguishes the specialized bilobate mouth configuration of Austrodecidae; however, the triradiate structure of the proboscis is observed internally (Fahrenbach and Arango, in press).

70 Proboscis wall incisures shaping the mouth: (0) absent; (1) present (Figs 6B.D). The evolutionary or functional significance of these deep incisures are unknown but can be related to food specialization in the ammotheid taxa.

71 Proboscis ventral protuberances in females: (0) absent; (1) present. Conspicuous ventral bumps occur in mature females of *Anoplodactylus californicus*, *A. evansi* and 12 other species not included in this study. This dimorphism seems to have some intraspecific variation in *A. californicus* and has been related to gynandromorphy (Child, 1978). Its variation with age or any other factors, or the functional significance of these outgrowths is yet unknown (Arango and Maxmen, 2006).

72 Palps size proportional to proboscis: (0) longer, distal segments can touch the mouth; (1) shorter, not in contact with mouth.

73 Chelifores size proportional to proboscis: (0) as long or longer than proboscis, in contact with mouth; (1) shorter, not in contact with mouth (Fig. 6E,G).

74 Abdominal segmentation in pycnogonids: (0) five-segmented; (1) three-segmented; (2) one segment peg-shaped; (3) reduced, not visible dorsally. This refers only to the part of pycnogonid body referred to the abdomen, and is coded inapplicable in the other arthropods.

75 Eggs found attached to ovigers of males: (0) present (see Fig. 6D); (1) absent. Eggs attached to male ovigers have not been found in any Colossendeidae or Austrodecidae species and is highly unlikely it could due to sampling effort considering the large numbers of individuals collected of these taxa in old and recent expeditions. It is possible these two families have a different mode of reproduction involving hosts as has been suggested before (for Austrodecidae see Stock, 1957; Arnaud and Bamber, 1987; Child, 1995a).

76 Egg masses configuration attached to ovigers: (0) Separate balls or bracelets on each oviger (Fig. 6D); (1) layers or sheaths held by the two ovigers; (2) single mass attached to both ovigers. The form of masses of eggs attached to ovigers seems to represent a consistent character at genus level. The separate balls or bracelets is the most general form, sheaths or layers observed in *Endeis* and a single mass held by both ovigers seen in *Eurycyde* but also in *Pycnogonum* (in King, 1973).

77 Larval type; (0) typical protonymphon; (1) almost square body, flattened dorsoventrally, thin, long, lash like third segments of second and third appendages, ''free swimming larva'' (as in Bogomolova and Malakhov, 2003) [named ''encysted larva'' in the summary by Bain (2003)]; (2) no protonymphon stage after hatching, earliest larva with rudiments of first pair of walking legs. In most cases, the coding based on supraspecific terminals according to literature (Bogomolova and Malakhov, 2003 and earlier literature cited therein).

Appendix 3

Synapomorphies involving DNA sequences diagnosing major clades of Pycnogonida. Only non-ambiguous transformations included. This list complements the morphological evidence presented in Fig. 8.

Pycnogonida

28S

Char. 164: – → A
Char. 165: T → C
Char. 175: – → G
Char. 180: – → C
Char. 186: – → G
Char. 187: – → G
Char. 189: – → T
Char. 228: G → A
Char. 232: C → G
Char. 236: T → G

Char. 243: T → –
Char. 264: G → C
Char. 267: C → T
Char. 269: A → G
Char. 275: C → T
Char. 289: T → –
Char. 293: – → T
Char. 311: A → T
Char. 322: G → T
Char. 338: C → A
Char. 387: G → T
Char. 419: G → T
Char. 429: A → G
Char. 447: A → G
Char. 546: T → C
Char. 605: T → C
Char. 766: – → A
Char. 786: – → A
Char. 891: – → T
Char. 909: – → G
Char. 915: – → C
Char. 916: – → A
Char. 946: AG → C
Char. 977: C → G
Char. 987: T → G
Char. 992: – → T
Char. 997: A → G
Char. 1001: A → T
Char. 1014: G → –
Char. 1021: G → C
Char. 1032: T → –
Char. 1048: T → –
Char. 1116: A → T
Char. 1122: G → T
Char. 1146: C → G
Char. 1150: G → C
Char. 1153: G → A
Char. 1179: T → C
Char. 1189: – → G
Char. 1196: – → A
Char. 1231: G → C
Char. 1291: – → G
Char. 1318: – → T
Char. 1343: – → G
Char. 1348: – → G
Char. 1357: G → T
Char. 1372: G → T
Char. 1427: T → –
Char. 1449: T → –
Char. 1457: G → T
Char. 1488: C → –
Char. 1521: T → A
Char. 1571: G → T
Char. 1593: G → C
Char. 1603: – → G
Char. 1684: C → –
Char. 1714: G → C
Char. 1749: A → T
Char. 1760: C → A
Char. 1763: G → A
Char. 1773: C → –
Char. 1785: G → T
Char. 1901: – → G
Char. 1938: C → T
Char. 2019: – → C
Char. 2091: A → G
Char. 2106: T → C
Char. 2113: G → C
Char. 2146: A → T
Char. 2155: – → G
Char. 2167: G → A
Char. 2170: A → G
Char. 2184: A → T
Char. 2239: – → T
Char. 2397: G → C
Char. 2399: C → T
Char. 2404: T → C
Char. 2413: C → A
Char. 2426: C → T
Char. 2436: – → T
Char. 2453: G → A
Char. 2457: A → T
Char. 2473: T → C
Char. 2490: A → G
Char. 2631: T → C
Char. 2648: T → C
Char. 2685: – → T
Char. 2701: – → C
Char. 2928: T → –
Char. 2954: C → T
Char. 3011: G → A
Char. 3068: T → C
Char. 3118: T → C
Char. 3195: C → T
Char. 3199: C → T
Char. 3211: G → A
Char. 3235: – → G
Char. 3244: – → C
Char. 3248: – → G
Char. 3346: – → C
Char. 3363: – → C
Char. 3376: A → G
Char. 3422: T → C
Char. 3426: T → G
Char. 3428: C → T
Char. 3443: A → G
Char. 3464: T → C
Char. 3483: T → C
Char. 3556: A → G
Char. 3664: A → G
Char. 3755: G → T
Char. 3843: G → T
Char. 3916: T → C
Char. 3950: T → C
Char. 3958: G → A
Char. 4003: T → C
Char. 4004: A → G
Char. 4013: G → C
Char. 4014: A → G
Char. 4034: G → A
Char. 4066: T → C
Char. 4081: G → T
Char. 4102: C → T
Char. 4120: – → G
Char. 4121: – → A
Char. 4123: – → G
Char. 4127: – → T
Char. 4134: G → C
Char. 4138: T → A
Char. 4187: G → A
Char. 4190: T → C
Char. 4193: C → T
Char. 4219: – → C
Char. 4225: G → –

Char. 4227: A → C
Char. 4234: – → C
Char. 4362: C → A
Char. 4380: G → A
Char. 4385: G → A
Char. 4421: C → A
Char. 4432: T → C
Char. 4433: G → C
Char. 4476: G → A
Char. 4541: C → –
Char. 4545: T → –
Char. 4594: G → C
Char. 4599: A → G
Char. 4624: G → T
Char. 4670: – → C
Char. 4744: C → A
Char. 4792: – → A
Char. 4805: C → T
Char. 4810: A → C
Char. 4841: T → C
Char. 4849: G → A
Char. 4904: T → C
Char. 4906: C → A
Char. 4919: T → G
Char. 4935: G → T
Char. 4937: A → G
Char. 5103: T → C
Char. 5196: C → T

18S
Char. 5259: C → G
Char. 5279: A → T
Char. 5337: – → A
Char. 5352: T → C
Char. 5398: G → C
Char. 5400: C → G
Char. 5429: – → C
Char. 5488: – → C
Char. 5506: – → A
Char. 5608: – → G
Char. 5695: – → C
Char. 5731: C → T
Char. 5733: A → G
Char. 6116: G → A
Char. 6138: T → G
Char. 6143: C → T
Char. 6145: G → A
Char. 6169: C → T
Char. 6178: – → T
Char. 6183: – → T
Char. 6189: – → C
Char. 6192: – → T
Char. 6215: – → T
Char. 6250: C → G
Char. 6265: T → G
Char. 6266: T → C
Char. 6271: T → A
Char. 6280: C → A
Char. 6353: C → –
Char. 6377: A → G
Char. 6417: G → C
Char. 6674: G → C
Char. 7050: C → T
Char. 7069: A → –
Char. 7122: T → C
Char. 7144: T → C
Char. 7226: C → T
Char. 7232: G → C
Char. 7415: A → G
Char. 7601: G → T
Char. 7617: C → –
Char. 7642: – → T
Char. 7647: G → A
Char. 7655: C → A
Char. 7673: G → T
Char. 7679: C → G

COI
Char. 7826: C → T
Char. 7831: C → A
Char. 7832: C → A
Char. 7833: A → C
Char. 7884: G → T
Char. 7946: C → T
Char. 7966: T → A
Char. 7968: A → T
Char. 8035: C → T
Char. 8052: – → T
Char. 8190: G → A
Char. 8267: T → A
Char. 8296: T → A
Char. 8297: C → G
Char. 8302: A → T
Char. 8303: A → T
Char. 8394: A → T
Char. 8460: G → T
Char. 8468: T → A
Char. 8528: C → T
Char. 8550: C → A
Char. 8600: C → T
Char. 8658: A → T
Char. 8667: C → T
Char. 8730: C → A
Char. 8734: C → T
Char. 8749: – → A
Char. 8755: T → A
Char. 8756: C → A
Char. 8775: C → A
Char. 8789: C → AT
Char. 8790: C → A
Char. 8850: C → T
Char. 8908: – → T

12S
Char. 8997: A → T
Char. 9096: C → T
Char. 9261: T → A
Char. 9264: T → A
Char. 9300: A → G
Char. 9500: – → A
Char. 9505: A → T
Char. 9539: G → A

16S
Char. 9605: A → G
Char. 9653: – → T
Char. 9774: T → C
Char. 9788: A → T
Char. 9909: T → C
Char. 9926: T → –
Char. 10000: T → –
Char. 10005: A → –
Char. 10137: A → T

Char. 10148: A → T
Char. 10398: T → A
Char. 10436: G → A
Char. 10439: A → T
Char. 10444: A → T

H3
Char. 10635: G → C
Char. 10739: G → A
Char. 10765: G → A
Char. 10825: G → A

Pycnogonidae

18S
Char. 7362: G → T

COI
Char. 7647: A → G

H3
Char. 10656: G → A
Char. 10712: C → T
Char. 10714: T → G
Char. 10739: A → G
Char. 10744: T → G
Char. 10778: C → A
Char. 10782: – → T
Char. 10786: G → –
Char. 10789: A → C
Char. 10791: A → T
Char. 10818: T → C
Char. 10825: A → G
Char. 10835: C → T

Nymphonidae (exc. N. floridanum*)*

28S
Char. 190: C → T
Char. 255: T → G
Char. 910: C → T
Char. 944: T → C
Char. 946: C → T
Char. 1094: C → T
Char. 1151: C → T
Char. 1153: A → T
Char. 1162: A → T
Char. 1170: C → T
Char. 1207: T → –
Char. 1220: T → C
Char. 1226: T → C
Char. 1231: C → G
Char. 1247: – → T
Char. 1348: G → A
Char. 1370: C → T
Char. 1395: T → C
Char. 1431: G → A
Char. 1538: T → A
Char. 1554: T → A
Char. 1639: T → –
Char. 1666: C → T
Char. 1676: – → C
Char. 1679: – → G
Char. 1821: C → G
Char. 1831: T → A
Char. 1846: G → T
Char. 2135: G → T
Char. 2285: – → A
Char. 2288: G → A
Char. 2342: – → T
Char. 3253: G → T
Char. 3307: G → –
Char. 3363: C → G
Char. 3417: A → T
Char. 3770: G → –
Char. 3858: A → –
Char. 4773: G → T

18S
Char. 5539: A → G

COI
Char. 7877: A → T
Char. 7954: T → C
Char. 8049: A → T
Char. 8186: A → T
Char. 8198: A → T
Char. 8256: A → T
Char. 8283: A → T

12S
Char. 8953: T → A
Char. 9091: T → C
Char. 9116: – → A
Char. 9117: – → A
Char. 9133: A → G
Char. 9153: T → A
Char. 9258: T → A
Char. 9290: A → –
Char. 9361: – → C
Char. 9434: T → A
Char. 9436: T → A
Char. 9525: A → –

16S
Char. 9657: A → T
Char. 9696: T → G
Char. 9710: A → C
Char. 9778: A → T
Char. 9787: T → A
Char. 9984: T → A
Char. 10121: – → C
Char. 10170: T → A
Char. 10219: – → A
Char. 10250: T → –
Char. 10444: T → A
Char. 10452: T → C

H3
Char. 10675: T → C
Char. 10711: G → A
Char. 10818: T → C

Austrodecidae

28S
Char. 115: A → T
Char. 173: C → T

Char. 199: AGT → C
Char. 230: A → G
Char. 232: G → A
Char. 254: T → C
Char. 259: C → T
Char. 260: G → C
Char. 335: T → –
Char. 339: – → T
Char. 367: T → C
Char. 374: G → C
Char. 402: G → A
Char. 416: T → C
Char. 550: G → T
Char. 561: G → C
Char. 577: – → A
Char. 582: T → C
Char. 611: A → G
Char. 613: C → T
Char. 633: – → C
Char. 638: T → G
Char. 660: G → T
Char. 694: G → T
Char. 700: – → G
Char. 806: C → –
Char. 826: C → T
Char. 832: G → C
Char. 874: C → –
Char. 899: – → C
Char. 910: G → A
Char. 911: G → A
Char. 974: C → –
Char. 1017: G → A
Char. 1019: T → C
Char. 1128: – → G
Char. 1133: – → A
Char. 1134: – → C
Char. 1135: – → G
Char. 1184: G → –
Char. 1186: C → –
Char. 1207: T → –
Char. 1226: T → –
Char. 1283: – → A
Char. 1284: – → A
Char. 1301: C → T
Char. 1349: – → T
Char. 1359: C → A
Char. 1365: T → C
Char. 1370: T → G
Char. 1423: A → T
Char. 1452: C → T
Char. 1457: T → –
Char. 1572: C → T
Char. 1602: C → G
Char. 1620: C → G
Char. 1655: C → T
Char. 1668: C → T
Char. 1674: A → C
Char. 1709: – → T
Char. 1730: – → A
Char. 1731: T → A
Char. 1736: – → A
Char. 1763: A → T
Char. 1791: – → G
Char. 1796: – → T
Char. 1813: – → C
Char. 1831: T → G
Char. 1841: – → C
Char. 1900: – → A
Char. 1901: G → A
Char. 1970: G → T
Char. 2020: – → T
Char. 2027: G → A
Char. 2038: C → A
Char. 2048: C → G
Char. 2195: – → A
Char. 2224: C → T
Char. 2350: G → T
Char. 2407: – → T
Char. 2410: – → G
Char. 2413: A → T
Char. 2421: C → T
Char. 2427: A → T
Char. 2430: C → T
Char. 2457: T → C
Char. 2705: A → –
Char. 2707: G → T
Char. 2708: A → T
Char. 2711: A → –
Char. 2712: A → T
Char. 2840: C → T
Char. 2866: – → T
Char. 2867: C → T
Char. 3056: C → T
Char. 3061: T → G
Char. 3062: G → A
Char. 3232: A → C
Char. 3244: C → T
Char. 3307: G → –
Char. 3363: C → A
Char. 3369: G → C
Char. 3399: G → T
Char. 3406: A → C
Char. 3417: G → T
Char. 3709: – → T
Char. 3723: G → A
Char. 3755: T → A
Char. 3800: C → G
Char. 3840: T → A
Char. 3966: – → A
Char. 3981: – → T
Char. 3993: T → A
Char. 3997: T → A
Char. 4227: C → G

18S

Char. 4249: G → A
Char. 5284: A → C
Char. 5332: T → C
Char. 5355: T → G
Char. 5398: C → A
Char. 5402: A → T
Char. 5416: C → G
Char. 5512: C → A
Char. 5570: T– → A
Char. 5580: – → T
Char. 5641: T → C
Char. 5701: C → A
Char. 5729: G → A
Char. 5856: A → T
Char. 6098: T → C
Char. 6099: C → T
Char. 6125: C → T
Char. 6154: A → G
Char. 6189: C → A

Char. 6240: G → A
Char. 6314: A → G
Char. 6439: G → A
Char. 6610: C → G
Char. 6629: A → T
Char. 6708: A → G
Char. 6713: A → G

16S
Char. 9599: A → T
Char. 9603: A → G
Char. 9608: G → T
Char. 9741: T → G
Char. 9754: A → G
Char. 9757: A → G
Char. 9759: T → –
Char. 9821: T → A
Char. 9870: A → T
Char. 9959: T → G
Char. 9970: T → –
Char. 10014: T → –
Char. 10017: T → G
Char. 10142: A → T
Char. 10170: T → A
Char. 10198: A → –
Char. 10228: – → G
Char. 10369: T → C
Char. 10393: A → G
Char. 10449: C → G
Char. 10499: T → –
Char. 10507: A → T
Char. 10513: – → G

H3
Char. 10564: A → T
Char. 10648: C → A
Char. 10650: T → A
Char. 10724: A → G
Char. 10747: G → C
Char. 10759: C → T

Austrodecidae + *Colossendeidae* + (P. femoratum + *Pycnogonidae)*

28S
Char. 349: G → C
Char. 450: T → C
Char. 696: G → CT
Char. 747: – → A
Char. 796: – → G
Char. 797: – → G
Char. 855: G → T
Char. 973: C → A
Char. 1210: C → –
Char. 1235: G → C
Char. 1241: C → T
Char. 1309: C → G
Char. 1486: G → –
Char. 1504: G → –
Char. 1512: G → –
Char. 1556: C → –
Char. 1557: G → T
Char. 1650: T → C
Char. 1663: – → T
Char. 1728: – → C
Char. 1757: G → –
Char. 1809: – → C
Char. 1839: T → G
Char. 1922: T → C
Char. 2002: C → T
Char. 2005: T → A
Char. 2205: C → T
Char. 2216: G → –
Char. 2296: C → T
Char. 2333: – → T
Char. 3217: A → G
Char. 3264: – → A
Char. 3364: – → T
Char. 3660: G → T
Char. 3790: A → G
Char. 4760: – → C
Char. 4776: G → C
Char. 5225: G → A

18S
Char. 7068: C → –
Char. 7097: A → C
Char. 7634: G → –
Char. 7771: A → G
Char. 7797: G → T
Char. 7800: T → A
Char. 8177: A → T
Char. 8314: A → G
Char. 8765: C → A
Char. 8780: T → C

12S
Char. 9104: C → T
Char. 9108: T → –
Char. 9142: T → A
Char. 9156: A → T
Char. 9158: – → A
Char. 9271: G → A
Char. 9330: A → T
Char. 9342: A → T
Char. 9476: T → A
Char. 9509: A → T

16S
Char. 9624: – → A
Char. 9683: A → T
Char. 9769: C → T
Char. 9787: T → A
Char. 9811: T → A
Char. 9885: A → T
Char. 10158: T → –
Char. 10220: – → G
Char. 10305: – → T
Char. 10482: A → T
Char. 10487: A → T

H3
Char. 10849: G → C
Char. 10862: T → C

Phoxichilidiidae (Inc. Endeis, exc. P. femoratum*)*

28S
Char. 165: C → T
Char. 648: A → T

Char. 686: T → A
Char. 823: C → T
Char. 880: – → C
Char. 1094: C → A
Char. 1122: T → C
Char. 1164: T → G
Char. 1248: – → T
Char. 1263: T → G
Char. 1303: – → G
Char. 1350: – → C
Char. 1355: – → A
Char. 1512: G → –
Char. 1523: C → T
Char. 1524: C → G
Char. 1615: T → G
Char. 1810: C → –
Char. 1891: – → T
Char. 1938: T → –
Char. 1960: C → G
Char. 1970: G → C
Char. 1982: T → G
Char. 2414: – → T
Char. 2418: T → G
Char. 3240: – → T
Char. 3307: C → T
Char. 3346: C → G
Char. 3363: C → A
Char. 3422: C → T
Char. 3475: C → T
Char. 3493: C → T
Char. 3708: A → C
Char. 3800: G → –
Char. 4689: – → G
Char. 4699: – → C
Char. 4766: – → G
Char. 5243: – → G
Char. 5247: A → C

18S
Char. 5441: C → T
Char. 6203: A → C
Char. 6208: A → T

COI
Char. 7829: G → A
Char. 8198: A → T
Char. 8275: C → A
Char. 8528: T → A

12S
Char. 8937: A → G
Char. 8962: T → A
Char. 8977: T → A
Char. 8992: T → A
Char. 9104: C → T
Char. 9295: T → A
Char. 9312: A → T
Char. 9314: A → T
Char. 9362: A → –
Char. 9364: A → –
Char. 9500: A → T
Char. 9542: T → –

16S
Char. 9599: A → T
Char. 9683: A → T
Char. 9741: T → A
Char. 9759: T → G
Char. 9796: A → T
Char. 9823: A → T
Char. 9935: A → T
Char. 10046: A → –
Char. 10061: T → –
Char. 10127: – → T
Char. 10128: A → T
Char. 10325: T → –
Char. 10333: G → T
Char. 10470: T → A
Char. 10490: A → T

H3
Char. 10871: C → T

Colossendeidae

28S
Char. 180: C → T
Char. 206: C → A
Char. 253: – → T
Char. 403: G → A
Char. 545: C → T
Char. 598: A → G
Char. 864: – → C
Char. 868: G → A
Char. 873: – → T
Char. 886: G → A
Char. 1164: C → G
Char. 1165: G → C
Char. 1231: C → G
Char. 1296: T → C
Char. 1310: – → T
Char. 1352: C → T
Char. 1431: AC → T
Char. 1513: C → T
Char. 1516: G → T
Char. 1544: T → G
Char. 1590: C → –
Char. 1601: G → A
Char. 1603: G → –
Char. 1644: C → –
Char. 1691: – → C
Char. 1695: G → C
Char. 1714: C → –
Char. 1725: T → –
Char. 1753: – → A
Char. 1765: C → G
Char. 1781: G → A
Char. 1785: T → C
Char. 1787: T → G
Char. 1814: C → A
Char. 1914: G → –
Char. 1930: C → G
Char. 1966: G → C
Char. 2040: G → A
Char. 2288: G → A
Char. 2331: A → C
Char. 2368: C → –
Char. 2469: C → T
Char. 2647: C → T
Char. 2708: A → G
Char. 2902: T → C
Char. 3216: – → A

Char. 3221: – → A
Char. 3417: G → C
Char. 3444: G → A
Char. 3493: T → C
Char. 3548: C → T
Char. 3750: C → G
Char. 3826: G → T
Char. 4199: G → T
Char. 4466: G → A
Char. 4504: T → –
Char. 4623: A → G
Char. 4706: C → G
Char. 5232: A → G
Char. 5235: G → –

18S
Char. 5554: – → G
Char. 5666: G → T
Char. 5940: T → A
Char. 6210: – → A
Char. 6212: – → C
Char. 6213: – → C
Char. 7081: – → C
Char. 7639: T → C
Char. 7640: C → T

12S
Char. 8903: A → T
Char. 8913: T → A
Char. 8922: T → A
Char. 8960: T → A
Char. 8962: T → A
Char. 9076: T → A
Char. 9129: – → A
Char. 9429: A → T
Char. 9433: – → A
Char. 9442: T → A
Char. 9470: – → T
Char. 9514: T → –
Char. 9537: – → A

16S
Char. 9659: T → A
Char. 9717: C → T
Char. 9767: G → T
Char. 9769: T → A
Char. 9774: C → T
Char. 9777: T → A
Char. 9805: G → A
Char. 9808: T → A
Char. 9906: T → C
Char. 9928: – → T
Char. 9942: – → A
Char. 10004: T → –
Char. 10037: – → T
Char. 10249: A → T
Char. 10275: G → –
Char. 10325: T → A
Char. 10333: G → –
Char. 10350: A → G
Char. 10354: A → G
Char. 10436: A → T
Char. 10447: A → G
Char. 10470: T → A
Char. 10511: A → T
Char. 10532: T → A
Char. 10540: T → A

H3
Char. 10662: A → T

Eurycyde + Ascorhynchus + (Callipallenid – Nymphonids) group

28S
Char. 180: C → G
Char. 660: G → T
Char. 686: T → A
Char. 856: – → T
Char. 903: T → C
Char. 1263: T → G
Char. 1319: C → T
Char. 1510: A → G
Char. 1590: C → –
Char. 1605: C → –
Char. 1714: C → –
Char. 1737: C → T
Char. 1804: – → C
Char. 1834: C → –
Char. 1867: – → T
Char. 1880: C → G
Char. 2180: G → A
Char. 2290: – → A
Char. 2356: – → T
Char. 2371: C → A
Char. 3039: G → A
Char. 3375: C → A
Char. 3806: A → G
Char. 3826: G → A
Char. 4797: C → –

18S
Char. 5450: T → C
Char. 7074: C → A
Char. 7602: A → C

COI
Char. 8076: T → A
Char. 8243: A → –
Char. 8275: C → A
Char. 8489: C → T
Char. 8528: T → A
Char. 8550: A → G
Char. 8559: A → T
Char. 8850: T → A

12S
Char. 9076: T → –
Char. 9225: – → T
Char. 9350: T → A
Char. 9380: A → T
Char. 9405: A → T

16S
Char. 9585: A → T
Char. 9639: T → –
Char. 9781: T → A
Char. 9826: G → A
Char. 9889: A → G
Char. 10106: T → A

Char. 10128: A → T
Char. 10314: A → T

H3
Char. 10714: T → G
Char. 10821: T → G

*Pycnogonida except Austrodecidae + Colossendeidae + (*P. femoratum *+ Pycnogonidae)*

28S
Char. 246: G → C
Char. 248: T → G
Char. 718: A → C
Char. 761: C → A
Char. 823: G → C
Char. 852: – → C
Char. 862: – → C
Char. 868: G → T
Char. 871: A → T
Char. 965: – → A
Char. 969: – → T
Char. 1091: – → T
Char. 1168: A → –
Char. 1282: – → G
Char. 1460: T → A
Char. 1484: – → A
Char. 1493: T → A
Char. 1511: – → T
Char. 1551: – → C
Char. 1619: – → T
Char. 1674: A → –
Char. 1695: G → C
Char. 1751: G → –
Char. 1765: C → A
Char. 1787: T → G
Char. 1821: – → C
Char. 1914: G → T
Char. 1928: C → T
Char. 1952: – → C
Char. 2218: A → T
Char. 2336: C → G
Char. 2357: – → C
Char. 2417: T → C
Char. 2418: G → T
Char. 2630: A → T
Char. 2686: – → C
Char. 2705: A → G
Char. 3192: T → C
Char. 3204: C → T
Char. 3313: – → C
Char. 3323: – → T
Char. 3359: A → G
Char. 3403: – → C
Char. 3424: – → G
Char. 3432: G → A
Char. 3493: T → C
Char. 3548: C → T
Char. 3743: C → T
Char. 3750: C → T
Char. 3858: – → A
Char. 3865: – → A
Char. 4532: C → G
Char. 4547: C → T
Char. 4731: – → G
Char. 4738: – → C
Char. 4740: – → A
Char. 4741: T → A
Char. 4773: C → G

18S
Char. 5497: – → G
Char. 5539: G → A
Char. 5553: – → G
Char. 5594: T → C
Char. 5666: G → C
Char. 5696: C → T
Char. 6194: – → A
Char. 6203: – → A
Char. 6205: – → C
Char. 6208: – → A
Char. 7635: G → T
Char. 7639: T → C

CO1
Char. 7987: T → A
Char. 8021: – → A
Char. 8049: C → A
Char. 8135: A → T
Char. 8234: T → A
Char. 8322: C → T
Char. 8324: T → A
Char. 8345: T → A
Char. 8551: C → T
Char. 8562: C → T
Char. 8769: A → G
Char. 8771: C → T
Char. 8795: T → A
Char. 8836: A → G
Char. 8853: T → A

12S
Char. 8897: T → A
Char. 8910: – → A
Char. 8936: A → G
Char. 9009: A → T
Char. 9012: T → C
Char. 9214: – → A
Char. 9347: A → T
Char. 9403: T → A

16S
Char. 10010: A → T
Char. 10057: G → A
Char. 10227: T → A
Char. 10229: A → T
Char. 10265: A → T

H3
Char. 10604: T → C
Char. 10605: C → A
Char. 10608: T → A
Char. 10629: A → T

Callipallenids + Nymphonids group

28S
Char. 254: T → C
Char. 255: C → T
Char. 343: G → T

Char. 359: G → T
Char. 369: T → C
Char. 605: C → A
Char. 611: A → G
Char. 614: C → T
Char. 617: C → T
Char. 648: A → C
Char. 779: T → C
Char. 868: T → A
Char. 891: T → G
Char. 909: G → T
Char. 910: G → C
Char. 912: C → T
Char. 971: C → –
Char. 1155: C → T
Char. 1162: G → A
Char. 1229: – → T
Char. 1235: G → C
Char. 1336: G → C
Char. 1359: C → T
Char. 1360: C → T
Char. 1365: T → –
Char. 1370: T → C
Char. 1460: A → G
Char. 1538: – → T
Char. 1565: T → G
Char. 1618: C → AG
Char. 1620: C → –
Char. 1639: G → T
Char. 1666: T → C
Char. 1710: T → –
Char. 1731: T → C
Char. 1732: C → T
Char. 1754: C → –
Char. 1768: A → C
Char. 1777: – → A
Char. 1786: – → G
Char. 1873: T → C
Char. 1947: T → C
Char. 2135: T → G
Char. 2422: C → A
Char. 2427: A → C
Char. 2439: G → T
Char. 2592: A → G
Char. 2709: G → C
Char. 2853: C → T
Char. 2930: T → A
Char. 3050: C → A
Char. 3056: C → T
Char. 3177: G → C
Char. 3417: G → A
Char. 3712: G → A
Char. 3737: – → A
Char. 3738: – → A
Char. 3811: G → A
Char. 3840: T → C
Char. 3870: C → T
Char. 4014: G → T
Char. 4019: C → G
Char. 4133: T → A
Char. 4134: C → A
Char. 4139: – → T
Char. 4215: G → C
Char. 4219: C → A
Char. 4480: C → T
Char. 4487: G → T
Char. 4504: T → –
Char. 4509: T → G
Char. 4532: G → A
Char. 4693: – → G
Char. 4696: T → G
Char. 4759: T → C
Char. 4774: – → T
Char. 4775: – → C
Char. 4793: T → –
Char. 4807: T → A
Char. 5196: T → C

18S

Char. 5277: T → –
Char. 5420: – → C
Char. 5432: – → T
Char. 5463: – → G
Char. 5489: – → A
Char. 5490: – → A
Char. 5501: – → A
Char. 5549: – → G
Char. 5570: T → C
Char. 5594: C → –
Char. 5600: C → –
Char. 5608: G → T
Char. 5641: T → G
Char. 5704: C → T
Char. 6125: C → A
Char. 6126: T → A
Char. 6165: C → G
Char. 6193: – → C
Char. 6194: A → G
Char. 6215: T → G
Char. 6223: T → A
Char. 6250: G → T
Char. 6331: – → T
Char. 6335: G → T
Char. 6351: A → G
Char. 7077: T → C

COI

Char. 7794: C → T
Char. 7797: G → A
Char. 7800: T → C
Char. 7811: T → A
Char. 7813: A → T
Char. 7844: A → T
Char. 7847: A → T
Char. 7854: G → A
Char. 7877: T → A
Char. 7923: A → G
Char. 7931: T → A
Char. 7954: G → T
Char. 7997: A → T
Char. 8006: A → T
Char. 8019: T → A
Char. 8062: C → T
Char. 8072: T → C
Char. 8124: T → A
Char. 8125: C → G
Char. 8149: C → T
Char. 8163: A → G
Char. 8181: T → A
Char. 8183: A → T
Char. 8208: G → T
Char. 8211: A → T
Char. 8222: T → A

Char. 8223: A → G
Char. 8231: T → A
Char. 8233: T → A
Char. 8250: A → C
Char. 8258: G → T
Char. 8277: A → T
Char. 8290: G → A
Char. 8291: T → C
Char. 8314: A → T
Char. 8325: C → G
Char. 8331: C → G
Char. 8332: T → C
Char. 8367: C → T
Char. 8374: A → T
Char. 8375: C → T
Char. 8427: A → T
Char. 8516: A → G
Char. 8531: C → T
Char. 8536: G → C
Char. 8538: G → A
Char. 8539: G → A
Char. 8578: G → T
Char. 8580: T → A
Char. 8599: T → A
Char. 8713: A → G
Char. 8744: T → G
Char. 8745: C → G
Char. 8747: C → A
Char. 8765: C → A
Char. 8775: A → C
Char. 8868: C → T

12S

Char. 8904: G → T
Char. 8951: T → A
Char. 8953: A → T
Char. 8967: A → G
Char. 8989: – → T
Char. 8997: T → A
Char. 9017: C → T
Char. 9098: G → T
Char. 9138: A → T
Char. 9140: A → T
Char. 9157: G → T
Char. 9175: A → T
Char. 9186: T → A
Char. 9189: T → A
Char. 9247: T → A
Char. 9274: C → A
Char. 9277: G → T
Char. 9278: G → A
Char. 9279: T → A
Char. 9300: G → A
Char. 9306: T → A
Char. 9309: G → AC
Char. 9340: A → T
Char. 9358: T → –
Char. 9455: T → C
Char. 9545: C → A
Char. 9546: T → A
Char. 9548: A → T

16S

Char. 9592: T → A
Char. 9607: C → A
Char. 9610: A → G
Char. 9629: A → –
Char. 9679: G → –
Char. 9681: A → –
Char. 9686: G → –
Char. 9689: C → –
Char. 9753: A → –
Char. 9789: – → A
Char. 9888: T → A
Char. 9909: C → A
Char. 9910: A → G
Char. 9967: – → T
Char. 10109: C → T
Char. 10120: G → A
Char. 10132: A → T
Char. 10140: G → T
Char. 10147: T → –
Char. 10148: T → A
Char. 10160: T → A
Char. 10172: T → A-
Char. 10229: T → –
Char. 10258: T → –
Char. 10347: T → C
Char. 10348: G → T
Char. 10367: T → A
Char. 10395: A → T
Char. 10511: A → T

H3

Char. 10558: T → A
Char. 1238: – → G
Char. 1309: T → G
Char. 1313: G → C
Char. 1520: C → T
Char. 1521: A → G
Char. 1718: G → –
Char. 1748: G → C
Char. 1922: T → A
Char. 2368: T → –
Char. 3204: T → C
Char. 3237: – → T
Char. 3346: A → G
Char. 3432: A → G
Char. 4779: C → G
Char. 5338: G → A
Char. 6194: T → C
Char. 7080: A → G
Char. 7083: C → T
Char. 7956: C → A
Char. 8240: A → G
Char. 8595: A → T
Char. 8700: A → T
Char. 8734: T → A
Char. 8753: T → A
Char. 8865: A → G
Char. 8963: T → C
Char. 9247: T → –
Char. 9413: – → T
Char. 9429: A → –
Char. 9459: A → G
Char. 9542: T → C
Char. 9564: T → A
Char. 9587: T → A
Char. 9603: A → G
Char. 9623: – → A
Char. 9653: A → G
Char. 9657: A → T
Char. 9774: C → T

Char. 9796: A → T
Char. 9991: T → –
Char. 10010: T → A
Char. 10071: T → –
Char. 10198: A → –
Char. 10320: T → A
Char. 10446: A → –
Char. 10555: A → G
Char. 10587: T → C
Char. 10730: G → T
Char. 10786: C → G

Ammotheids + Rhynchothoracidae group

28S
Char. 232: G → –
Char. 233: C → A
Char. 257: T → C
Char. 261: G → C
Char. 329: – → G
Char. 338: A → –
Char. 643: G → C
Char. 697: A → G
Char. 903: T → G
Char. 951: C → –
Char. 953: T → –
Char. 955: C → –
Char. 958: T → –
Char. 963: C → –
Char. 964: C → –
Char. 965: A → –
Char. 969: T → –
Char. 971: C → –
Char. 973: C → –
Char. 977: G → –
Char. 980: G → –
Char. 981: G → –
Char. 984: G → –
Char. 987: G → –
Char. 988: AG → –
Char. 989: G → –
Char. 992: T → –
Char. 993: T → –
Char. 1064: – → A
Char. 1155: C → T
Char. 1207: T → –
Char. 1228: G → –
Char. 1365: T → C
Char. 1423: A → C
Char. 1431: A → T
Char. 1457: C → A
Char. 1495: A → T
Char. 1502: C → G
Char. 1557: G → –
Char. 1659: G → T
Char. 1682: T → A
Char. 1708: – → T
Char. 1760: A → –
Char. 2214: C → A
Char. 3820: T → C
Char. 3843: T → A
Char. 3847: T → C
Char. 4445: C → T
Char. 4523: G → C
Char. 4584: G → C
Char. 4694: – → G
Char. 4796: T → C
Char. 4797: C → A
Char. 4807: T → A

18S
Char. 5644: G → A
Char. 6162: C → T
Char. 6175: – → T
Char. 6205: C → G
Char. 7080: G → T
Char. 7083: T → C

COI
Char. 7783: A → T
Char. 7844: A → T
Char. 7922: T → A
Char. 7971: G → A
Char. 7972: A → T
Char. 7996: A → T
Char. 8531: C → G
Char. 8695: G → A

12S
Char. 9049: – → A
Char. 9302: – → A
Char. 9340: A → T
Char. 9403: A → T
Char. 9449: A → G
Char. 9476: T → A
Char. 9509: A → T

16S
Char. 9635: – → T
Char. 9653: T → A
Char. 9826: G → T
Char. 9850: – → T
Char. 9852: C → T
Char. 9995: A → –
Char. 10030: – → C
Char. 10057: A → T
Char. 10094: – → A
Char. 10332: A → –
Char. 10387: G → T
Char. 10472: G → A

Phoxichilidiids + Ammotheids

28S
Char. 328: T → C
Char. 335: T → G
Char. 359: G → T
Char. 582: T → A
Char. 653: C → T
Char. 1280: – → G
Char. 1493: A → –
Char. 1656: – → G
Char. 1705: – → C
Char. 1821: C → G
Char. 1897: T → G
Char. 2144: C → A
Char. 3811: G → C
Char. 3840: T → –
Char. 4444: C → T
Char. 4779: – → C

18S
Char. 5638: T → –

COI
Char. 7749: A → T
Char. 8245: A → T
Char. 8637: A → T
Char. 8787: T → G

12S
Char. 8972: T → C
Char. 9251: – → A
Char. 9305: G → A
Char. 9322: A → G
Char. 9438: A → T
Char. 9495: T → A
Char. 9514: T → A

16S
Char. 9581: – → T
Char. 9600: A → T
Char. 10022: T → A
Char. 10132: A → T
Char. 10448: – → C

H3
Char. 10715: C → T
Char. 10727: A → G
Char. 10786: G → T
Char. 10821: T → C

Pallenopsidae + (Phoxichilidiids + Ammotheids)

28S
Char. 234: – → T
Char. 545: C → T
Char. 567: C → A
Char. 697: – → A
Char. 732: – → A
Char. 737: T → G
Char. 824: C → T
Char. 1076: – → T
Char. 1107: – → T
Char. 1109: – → T
Char. 1159: – → G
Char. 1164: C → T
Char. 1196: A → G
Char. 1220: G → C
Char. 1278: – → C
Char. 1291: G → C
Char. 1309: C → T
Char. 1318: T → C
Char. 1354: G → T
Char. 1370: T → A
Char. 1457: T → C
Char. 1604: – → C
Char. 1615: – → T
Char. 1644: C → G
Char. 1810: G → C
Char. 2283: G → C
Char. 2368: C → G
Char. 3232: A → G
Char. 3275: T → C
Char. 3307: G → C
Char. 3380: – → C
Char. 3708: – → A
Char. 3723: G → T
Char. 3795: A → T
Char. 3800: C → G
Char. 4445: A → C
Char. 4623: A → G
Char. 4714: – → G
Char. 5246: G → A

18S
Char. 5424: – → C
Char. 5494: – → G
Char. 5579: – → C
Char. 5629: – → C
Char. 5641: T → C
Char. 6196: – → C
Char. 7593: G → A

COI
Char. 7819: A → T
Char. 7822: A → T
Char. 7838: A → T
Char. 7841: A → T
Char. 7960: T → A
Char. 8253: A → C
Char. 8283: A → T
Char. 8331: C → T
Char. 8552: A → T
Char. 8558: A → T
Char. 8877: A → T

12S
Char. 8992: C → T
Char. 9004: G → A
Char. 9039: – → T
Char. 9067: – → AT
Char. 9309: G → –
Char. 9358: T → A
Char. 9442: T → A

16S
Char. 9595: T → A
Char. 9608: G → T
Char. 9666: T → A
Char. 9944: T → –
Char. 9951: T → A
Char. 10075: – → A
Char. 10107: A → T
Char. 10124: T → A
Char. 10198: A → T
Char. 10282: C → –
Char. 10291: A → T
Char. 10294: A → T
Char. 10321: G → A
Char. 10327: T → A
Char. 10354: A → G
Char. 10376: G → A
Char. 10467: T → A
Char. 10487: A → –
Char. 10508: – → G

H3
Char. 10561: C → T

Char. 10567: A → C
Char. 10668: C → A
Char. 10699: G → A
Char. 10852: C → T
Char. 10868: C → T

Colossendeidae + (P. femoratum + *Pycnogonidae*)

28S
Char. 179: A → C
Char. 718: A → T
Char. 744: – → C
Char. 805: – → C
Char. 912: C → T
Char. 977: G → A
Char. 1163: T → –
Char. 1394: T → A
Char. 1529: – → C
Char. 1565: T → C
Char. 1628: G → A
Char. 1733: C → T
Char. 1751: G → T
Char. 1857: – → G
Char. 1941: – → C
Char. 1943: – → A
Char. 2180: G → A
Char. 2203: G → A
Char. 2418: G → A
Char. 3811: G → C
Char. 3836: A → –
Char. 4120: G → T
Char. 4536: G → –
Char. 4742: A → C
Char. 4746: C → –

18S
Char. 5486: – → T
Char. 7363: C → T
Char. 7642: T → C

COI
Char. 7822: A → T
Char. 7854: G → A
Char. 8027: – → A
Char. 8062: C → T
Char. 8250: A → T
Char. 8313: A → T
Char. 8319: T → C
Char. 8357: T → A

12S
Char. 8937: A → G
Char. 9206: – → A
Char. 9434: T → A
Char. 9460: A → T
Char. 9472: T → A
Char. 9513: T → A
Char. 9525: A → –

16S
Char. 9655: T → A
Char. 9826: G → A
Char. 9941: – → AT
Char. 9962: – → A
Char. 10022: T → C
Char. 10101: – → T
Char. 10152: A → T
Char. 10227: T → –
Char. 10291: A → T

H3
Char. 10665: A → C
Char. 10804: T → A
Char. 10821: T → G
Char. 10839: G → A
Char. 10900: A → C