

Morphological phylogenetics of the sea spiders (Arthropoda: Pycnogonida)

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Abstract

Pycnogonids or sea spiders are a group of marine arthropods whose relations to the chelicerates have been an issue of controversy. Higher-level phylogenetic relationships among the lineages of sea spiders are investigated using 36 morphological characters from 37 species from all extant families and a Devonian pycnogonid fossil. This is one of the first attempts to analyze the higher-level relationships of the Pycnogonida using cladistic techniques. Character homoplasy (implied weights) is taken into account to construct a polytomous, most-parsimonious tree in which two major clades within Pycnogonida are obtained. Clade A includes Ammotheidae paraphyletic with Colossendeidae, Austrodecidae and Rhynchothoracidae, and clade B is formed by Nymphonidae, Callipallenidae (apparently paraphyletic), Pycnogonidae and Phoxichilidiidae. The analysis of equally weighted data is presented and helps to identify those characters less consistent. The reduction of the chelifores, palps and ovigers – shown independently within each of the clades as parallel evolution events – challenges the assumption of a gradual mode of reduction within the group, according to analysis of unordered vs ordered characters. Most of the phylogenetic affinities proposed here are compatible with traditional classifications. However, traditional taxonomic characters need to be complemented by sets of anatomical, molecular and developmental data, among others, to produce more robust phylogenetic hypotheses on the higher- and lower-level relationships of the sea spiders.

Key words: Pycnogonida, phylogeny, morphology, character evolution, gradual reduction, sea spiders

See also Electronic Supplement at <http://www.senckenberg.de/odes/02-05.htm>

Introduction

Pycnogonida or sea spiders are an extraordinary group of marine arthropods containing more than 1100 species living in tropical to polar seas, from the shoreline to abyssal depths (Arnaud & Bamber 1987, Munilla 1999). The subphylum Pycnogonida (Hedgpeth 1955) is defined by striking autapomorphies that strongly support monophyly, such as the prominent external proboscis, an extremely reduced abdomen, and the presence of a ventral pair of appendages (ovigers) on the cephalic segment (Boudreaux 1979). Pycnogonids have recently been proposed as the sister-taxon of all other extant arthropods (Giribet et al. 2001). Other studies have carried out a controversy about the status of pycnogonids as either relatives of the Chelicerata (Snodgrass 1938, Weygoldt 1986, Wheeler et al. 1993, Wheeler & Hayashi 1998,

Edgecombe et al. 2000) or basal arthropods (Zrzavy et al. 1997, Giribet & Ribera 2000, Edgecombe et al. 2000, Giribet et al. 2001). The scarcity of fossil records has not helped to solve uncertainties about the evolutionary history of pycnogonids. Nonetheless, the Devonian fossil *Palaeoisopus problematicus* Broili has been used to infer ancestral conditions of the group (Stock 1994). Recently, findings of pycnogonid larvae from the Upper Cambrian 'Orsten' have been interpreted as new evidence to relate pycnogonids to chelicerates (Walossek & Müller 1997, Walossek & Dunlop in press).

The uncertain position of the sea spiders is also reflected in the lack of understanding of the phylogenetic relationships within the group. Few studies have addressed the phylogenetic relationships of the pycnogonids at family level, and none of them used explicit cladistic analysis (but see Lovely 1999). Hedgpeth (1955) proposed a clas-

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sification of eight families of pycnogonids based on the presence and complexity of the chelifores, palps and ovigers. However, he stated that it would be almost impossible to draw a phylogenetic family tree, referring to the failed attempts in separating some of the families due to the occurrence of 'transitional' genera such as *Pallenopsis* (Hedgpeth 1947). Hedgpeth also suggested a direction in the evolution of the group based on a gradual reductive trend. He regarded the Nymphonidae as the most generalized lineage. Its members have functional chelae, 10-segmented ovigers, and long palps. Pycnogonidae are considered the most derived due to the absence of all the head appendages (Hedgpeth 1947). Fry (1978) examined the morphological similarities among pycnogonids to re-classify the group using a numerical taxonomy approach. However, the unclear outcome of the analysis and the creation of about 20 new families did not receive much support (Arnaud & Bamber 1987, Munilla 1999). More recently, Stock (1994) expressed his 'personal philosophy' about the phylogenetic relationships of pycnogonids based on a comparison of the extant Pycnogonida with the fossil *Palaeoisopus problematicus*, based on the assumption that 10-segmented appendages were the plesiomorphic state (Fig. 1A). Stock reiterated the ideas of a gradual reduction stated by Hedgpeth (1947), but he proposed Ammotheidae as the basal lineage of the pycnogonids with *Eurycyde* as the possible most primitive extant form (Stock 1994). Munilla (1999) also concluded that pycnogonid phylogeny could be derived from the assumption of 'regressive evolution', or the gradual loss of structures over evolutionary time (Fig. 1B).

So far, the hypothesis of an evolutionary trend of successive reductions in the number of segments of the appendages has not been examined under current cladistic techniques, and the validity of the families as monophyletic groups, mainly Ammotheidae and Callipallenidae, needs to be revised. Here, I test the assumption of a reductive trend in the Pycnogonida examining the phylogenetic affinities among the main lineages using quantitative cladistic analysis.

Ordered multistate characters can be useful when assuming trends of reduction series (Wilkinson 1992). For example, one can assume – by introducing unordered characters – that all possible changes in the number of segments of the appendages have an equal chance to occur. Alternatively, it can be assumed that the chelifores, palps and ovigers of pycnogonids went through a gradual reduction from the maximum number of segments to a complete loss; then, ordered characters are used. The most parsimonious outcome is chosen after comparing the results of the 'ordered' with those of the 'unordered' analysis.

When dealing with high-level phylogenies, taxon sampling can affect resulting topologies (Bininda-Emonds et al. 1998). The Pycnogonida are characterized by great morphological plasticity, and there are excep-

tions for almost every morphological character state within families and genera (Thompson 1904). In this analysis, the exemplar method approach (Yeates 1995) was attempted. Although not exhaustive because of the extremely wide variation in some taxa and unavailability of material, in most cases genera polymorphic for the characters coded are represented by more than one species and not by a single hypothetical supraspecific terminal taxon (see discussion in Prendini 2001).

In this paper, I present a cladistic study of the major lineages of the subphylum Pycnogonida, based entirely on morphological characteristics of adults. The main goals of this study are: 1) to examine phylogenetic affinities among extant sea spiders under different coding strategies, 2) to quantitatively test the hypothesis of a gradual reduction trend of the chelifores, palps and ovigers, 3) to identify taxa of doubtful monophyly and thus in need of taxonomic revision, and 4) to discuss the compatibility of the presented cladistic results with existing classifications of Pycnogonida.

Additional information on this study is available from the Organisms Diversity and Evolution Electronic Supplement 02–05, Parts 1–3, on the internet at <http://www.senckenberg.de/odes/>.

Materials and methods

Taxon sampling

A total of 38 species belonging to 21 genera of extant Pycnogonida and one fossil species, *Palaeoisopus problematicus*, were included in the analysis (Appendix 1; Table 1; Electr. Suppl., Pt 3). For a matter of convenience, the traditional family assignments of pycnogonid genera have been used throughout the study while their validity is being tested. Ammotheidae and Callipallenidae, the most diverse families in terms of morphology and number of genera, are represented here by eight and five genera, respectively (*Pallenopsis* Wilson is included in the Callipallenidae following Child 1979). Colossendeidae is represented by a species of the type genus *Colossendeis* Jarzinsky and by a species of *Rhopalorhynchus* Wood-Mason. The monogeneric Rhynchothoracidae and Pycnogonidae, which are remarkably uniform, are represented here by single species. Nymphonidae, a cosmopolitan family with a large number of closely related species belonging to the type genus *Nymphon* Fabricius, is represented by three species.

Several factors influenced the selection of species for the analysis. Firstly, most of the taxa included in the analysis are part of the collections made by the author, mostly in North Queensland but also in the Colombian Caribbean (Arango 2000). Additional material was kindly provided by collaborators in Australia and overseas, but for a few species descriptions from the literature had to be used (all material sources listed in Electr. Suppl., Pt 1). Type genera and those whose members are abundant and/or of widest distribution were selected from each of the families. The genera for which more

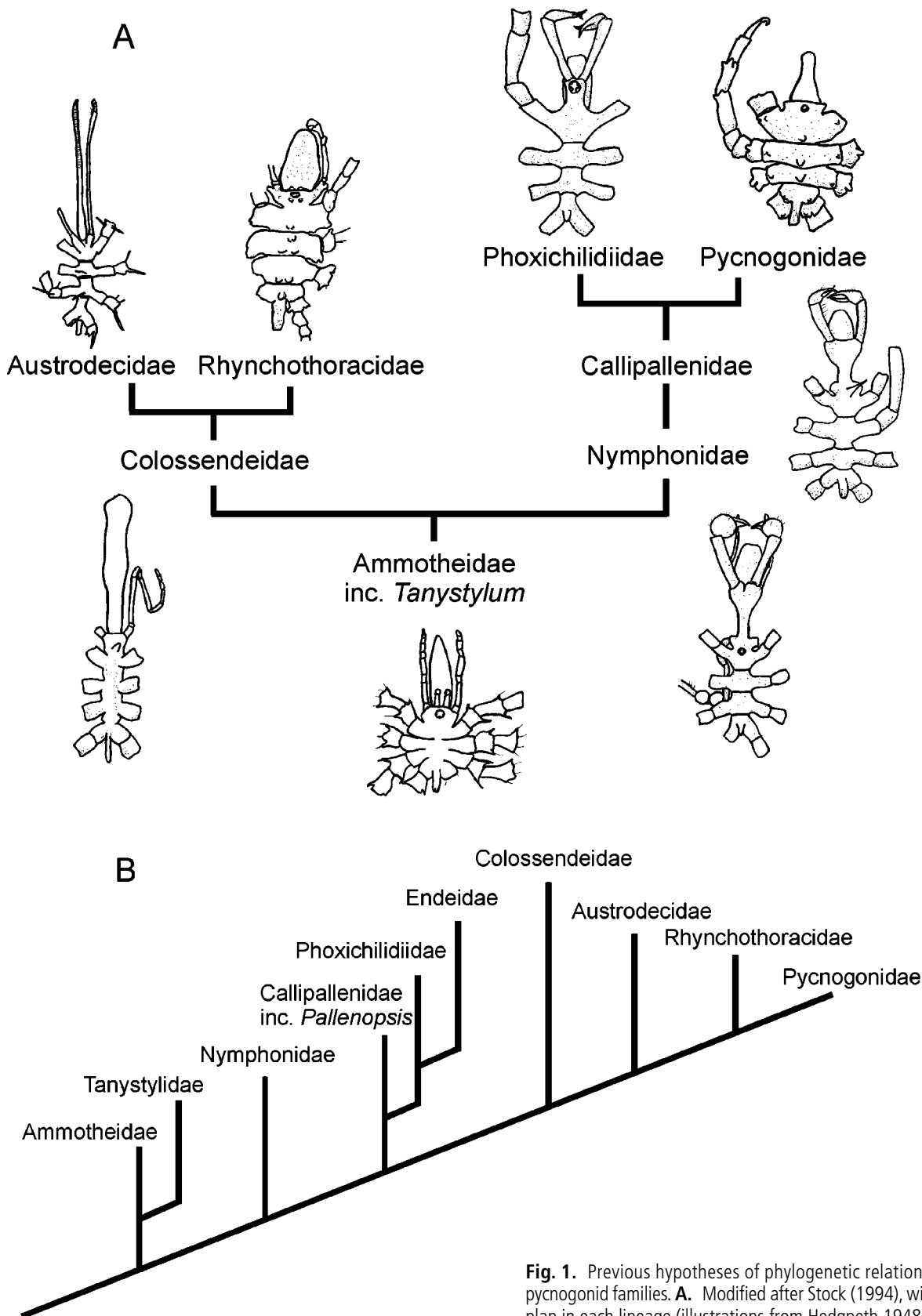


Fig. 1. Previous hypotheses of phylogenetic relationships among pycnogonid families. **A.** Modified after Stock (1994), with basic body plan in each lineage (illustrations from Hedgpeth 1948; Stock 1989, 1991). **B.** From Munilla (1999).

than one species were included due to intrageneric 'polymorphism' were: *Achelia* Hodge, *Ammothella* Verrill, *Anoplodactylus* Wilson, *Ascorhynchus* Sars, *Austrodecus* Hodgson, *Callipallene* Flynn, *Cilunculus* Loman, *Nymphon*, and *Tanystylum* Miers. 'Transitional' or problematic taxa such as *Pallenopsis*, *Tanystylum* and *Endeis* Philippi, whose taxonomic status had been a matter of debate, have been included to provide a test for such taxonomic hypotheses.

Most of the phylogenetically informative characters within the Pycnogonida are derived from structures absent in any other arthropod taxa (e.g. characters of ovigers and proboscis). For this reason it is very difficult to take outgroup relationships into account. *Palaeoisopus problematicus*, a well-known fossil pycnogonid from the Devonian, was introduced to the analysis in hope of a root for the cladograms. Another two species of fossil sea spiders from the Lower Devonian are known, but very few specimens have been examined and their

morphology is not well understood (Bergström et al. 1980). Characters of *P. problematicus* were coded according to published descriptions of the fossil specimens based on radiographs (Bergström et al. 1980). In the absence of algorithms that distinguish between inapplicable characters and missing data (Lee & Bryant 1999), the use of fossil taxa offers some difficulties when coding detailed morphological characters (Kitching et al. 1998). However, it remains the best option to provide a sister group for the extant Pycnogonida.

Characters

Thirty-six morphological characters of adult sea spiders were scored across the 38 species (Appendix 1, Table 1). Of the total number of characters, 20 are binary and 16 were coded as multistate. For those multistate characters that refer to the number of segments of the appendages, the actual number of

Table 1. Species and coding of morphological characters.

	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5												
<i>Palaeoisopus problematicus</i>	0	0	0	0	0	1	0	1	0	0	0	0	?	?	?	?	?	?	?	1	0	?	?	1	0	?	?	?	?	0	1	0	0	2	0	0	2	0
<i>Eurycyde raphiaster</i>	0	1	1	1	0	1	0	1	1	0	1	1	0	0	0	1	1	2	0	0	2	0	1	1	2	0	0	1	-	-	1	1	1	1	2	0		
<i>Achelia assimilis</i>	0	2	1	0	0	2	0	2	1	0	1	0	1	0	0	1	0	0	2	1	2	0	0	0	2	0	0	0	-	-	1	1	1	1	0	0		
<i>Achelia australiensis</i>	0	2	1	0	0	2	0	2	1	0	1	0	1	0	0	1	0	0	2	1	2	0	0	0	2	0	0	0	-	-	1	1	0	1	0	0		
<i>Ammothella n.sp.</i>	0	1	1	1	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	2	0	0	0	1	0	0	0	-	-	1	1	0	1	0	0	0		
<i>Ammothella biunguiculata</i>	0	1	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	2	0	0	0	2	0	0	0	0	-	-	1	1	0	1	0	0		
<i>Ammothea hilgendorfi</i>	0	2	1	0	0	1	0	1	1	0	1	0	1	0	0	0	0	0	1	0	2	0	0	1	4	0	0	0	-	-	1	2	0	2	1	0	0	
<i>Tanystylum haswelli</i>	0	2	1	0	0	5	0	5	1	0	1	0	1	0	0	1	0	0	2	1	2	0	0	0	2	0	1	0	-	-	0	3	0	1	0	0	0	
<i>Tanystylum rehderi</i>	0	2	1	0	0	3	0	3	1	0	1	0	1	0	0	1	0	0	2	1	2	0	0	0	1	0	1	0	-	-	1	3	0	1	0	0	0	
<i>Nymphopsis acinacispinata</i>	0	1	1	1	0	1	0	1	1	0	2	0	1	0	0	1	0	0	1	2	2	0	0	0	2	0	1	0	-	-	1	2	1	1	0	0	0	
<i>Ascorhynchus glaberrimus</i>	0	2	1	0	0	1	0	1	1	0	1	1	0	0	1	0	0	3	0	0	2	0	1	1	2	0	0	1	-	-	0	1	0	1	2	0	0	
<i>Ascorhynchus ramipes</i>	0	2	1	0	0	1	0	1	1	0	1	1	0	0	1	0	0	3	0	0	3	0	1	1	2	0	0	1	-	-	0	1	0	2	2	0	0	
<i>Ascorhynchus tenuirostris</i>	0	2	1	0	0	0	0	1	0	1	1	0	0	0	2	1	0	0	2	0	1	0	2	0	1	2	0	0	1	-	-	0	1	0	1	2	0	0
<i>Cilunculus armatus</i>	0	2	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	1	1	0	2	0	0	0	4	0	0	0	-	-	0	2	1	1	2	1	0	0
<i>Cilunculus sekiguchi</i>	0	2	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	1	1	0	2	0	0	0	2	0	0	0	-	-	0	2	0	1	2	1	0	0
<i>Nymphon micronesicum</i>	0	2	0	0	0	4	0	4	1	0	1	1	1	0	1	0	2	3	0	0	2	0	1	2	1	0	0	0	0	1	0	0	1	0	0	1	0	0
<i>Nymphon molleri</i>	0	2	0	0	0	4	0	4	1	0	1	1	1	0	1	0	2	3	0	0	2	0	1	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Nymphon surinamense</i>	0	2	0	0	0	4	0	4	1	0	1	1	0	0	1	0	2	3	0	0	1	0	1	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Colossendeis megalonyx</i>	1	-	-	-	0	0	0	0	1	0	1	1	0	1	-	-	-	-	0	2	0	0	1	0	0	0	0	1	-	-	0	0	0	2	0	0	0	
<i>Rhopalorhynchus tenuissimum</i>	1	-	-	-	0	0	0	0	1	0	1	1	0	1	-	-	-	-	0	0	0	1	0	0	0	0	1	-	-	2	0	0	2	0	0	0	0	
<i>Austrodecus glaciale</i>	1	-	-	-	0	3	0	3	4	0	3	0	1	0	0	1	2	1	1	0	3	0	1	0	0	0	1	0	-	-	0	3	0	2	0	0	0	
<i>Austrodecus gordonae</i>	1	-	-	-	0	3	0	3	5	0	4	0	0	0	1	2	1	1	0	0	0	1	0	2	0	1	0	-	-	0	3	0	2	0	0	0	0	0
<i>Rhynchothorax australis</i>	1	-	-	-	0	3	0	5	1	0	1	1	1	0	0	1	0	0	2	1	3	1	1	0	3	0	0	0	-	-	0	1	0	1	0	0	0	0
<i>Callipallene novaezealandiae</i>	0	2	0	0	1	-	1	-	1	0	1	0	1	0	0	0	2	1	0	1	2	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Callipallene brevisrostris</i>	0	2	0	0	1	-	1	-	1	0	1	0	1	0	1	0	2	1	0	0	2	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Parapallene famelica</i>	0	2	0	1	1	-	1	-	1	0	1	1	0	0	1	0	2	1	0	0	-	0	0	2	3	0	1	0	1	0	1	2	0	0	0	0	0	0
<i>Propallene saengeri</i>	0	2	0	0	0	6	1	-	1	0	1	0	0	0	1	1	2	3	0	0	2	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Pseudopallene ambigua</i>	0	2	0	0	1	-	1	-	1	0	1	1	0	1	-	-	-	-	2	0	2	0	0	0	1	0	0	0	1	1	0	1	0	0	0	2	0	
<i>Pallenopsis schmitti</i>	0	2	0	0	0	7	0	6	1	0	1	0	1	0	0	1	2	1	0	0	2	0	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0
<i>Anoplodactylus n.sp.</i>	0	2	0	0	1	-	1	-	4	1	-	0	1	0	0	1	0	1	0	1	0	2	0	0	0	3	1	-	-	1	1	0	1	1	0	0	0	0
<i>Anoplodactylus batangensis</i>	0	2	0	0	1	-	1	-	4	1	-	0	0	0	0	1	0	1	1	0	2	0	0	0	3	1	-	-	1	1	1	3	1	1	0	0	0	
<i>Anoplodactylus tenuicarpus</i>	0	2	0	0	1	-	1	-	4	1	-	0	0	0	1	0	0	1	0	0	2	0	0	0	3	1	-	-	0	1	1	0	1	0	0	0	0	
<i>Anoplodactylus glandulifer</i>	0	2	0	0	1	-	1	-	4	1	-	0	1	0	1	1	0	1	1	1	2	0	0	0	3	1	-	-	0	1	1	0	1	0	0	0	0	
<i>Anoplodactylus insignis</i>	0	2	0	0	1	-	1	-	4	1	-	0	0	0	0	0	1	0	0	2	0	0	0	3	1	-	-	1	1	1	0	1	0	0	0	0	0	
<i>Anoplodactylus n. sp.</i>	0	2	0	0	1	-	1	-	4	1	-	0	0	0	0	1	0	1	2	1	2	0	0	0	3	1	-	-	1	1	1	0	1	1	0	0	0	
<i>Anoplodactylus longiceps</i>	0	2	0	0	1	-	1	-	4	1	-	0	1	0	1	0	0	1	0	0	2	0	0	0	3	1	-	-	1	1	1	0	0	1	0	0	0	
<i>Endeis mollis</i>	1	-	-	-	1	-	1	-	3	1	-	0	1	0	1	0	1	3	0	0	1	0	0	1	4	1	-	-	-	-	1	0	0	1	1	0	0	0
<i>Pycnogonum litorale</i>	1	-	-	-	1	-	1	-	2	1	-	1	0	1	-	-	-	-	2	1	3	1	1	0	1	1	-	-	-	-	0	1	0	0	0	0	0	

segments present was entered as a character state instead of creating class intervals to reduce the number of states.

Different alternatives exist for the coding of inapplicable character states when a certain character is absent in some of the taxa. In the pycnogonids, this is a major problem when coding external morphological features, specifically those of the cephalic appendages and the cement glands because these are absent in several taxa. I use the reductive coding (Strong & Lipscomb 1999), denoting inapplicable characters (e.g. number of palp segments in those taxa having no palps) with “-”. There are problems associated with this reductive or separate coding, such as redundancy of absence states and their partial independence relative to the presence (Lee & Bryant 1999). However, when analyzed under unambiguous optimization settings – that is, not allowing semi-strict trees (default in Nona and PeeWee packages; Goloboff 1993b, 1997) – reductive coding is the option that best reflects the information content of the data collected when inapplicable values need to be introduced to the matrix (Strong & Lipscomb 1999).

All sixteen multistate characters were initially treated as unordered. Even if the ‘unordered’ analysis is justified because character transformations should not be assumed but tested by cladistic analysis (Hauser & Presch 1991), different assumptions of character transformations (e.g. ‘unordered’ and ‘ordered’) should be compared (Wilkinson 1992). Disagreement between the alternative treatments implies that phylogenetic inferences are sensitive to assumptions of character evolution (Wilkinson 1992), and a choice then has to be made. Those multistate characters for which hypotheses of transformation series could be assumed (e.g. 10-segmented to 9-segmented to 7-segmented to 6-segmented, etc.) were coded both as unordered and ordered (characters 1, 5, 7, 8, 10 in Appendix 1), and the results of both analyses were compared.

Character evaluation

The analyses were carried out without assigning a priori polarity to characters. Outgroup relationships and polarization of characters are issues difficult to approach in pycnogonids due to the lack of reliable sister taxa. *P. problematicus* has not been constrained as an outgroup to polarize the characters. It was expected to provide a root for the tree but is not meant to be used for a strict outgroup comparison. Assumptions can be made regarding the ancestral state of some characters, but there is no reason to assume that because *P. problematicus* is extinct and probably older than the extant forms, all its characters are therefore plesiomorphic.

In the following sections a discussion of the most relevant characters is presented, including assumptions about their evolution, as well as a brief overview of important features of the different lineages of pycnogonids.

Chelifores

The term ‘chelifores’ makes reference to the first pair of appendages frontally on the cephalon. They are believed to be homologues to the chelicerae in Arachnida. The presence of chelifores in adult pycnogonids (character 0, see Appendix 1) is considered a plesiomorphic state based on outgroup comparison (comparing them to the chelicerae) and on the ontogenetic criterion, since all pycnogonid larvae and juvenile forms have cheli-

fores. Chelifores are completely functional in adults of all members of the families Nymphonidae, Phoxichilidiidae (excluding *Endeis* after Child 1992), and most species of Callipallenidae. When present, chelifores of extant taxa have one or two basal segments forming the scape. The fossil specimens have three segments (character 1). Among modern species, and following the idea of a reductive trend, a two-segmented scape has been assumed as a primitive condition (Stock 1994, Munilla 1999). Different degrees of reduction of chelifores are found within the Ammotheidae, ranging from a few fully chelate species to species having no chelifores but just a single short segment on the front of the cephalon. The apomorphic state of complete absence of these appendages is characteristic of Pycnogonidae, Austrodecidae, Rhynchothoracidae, and the genus *Endeis*. The presence of spines on the chelifores (character 3) and teeth on the chelae (character 28) might have phylogenetic importance and these characters have been coded among those forms bearing chelifores. The former is an apparently stable feature within some genera of Ammotheidae (*Nymphopsis* Haswell, *Ammothella*, *Achelia*), the latter is useful for genera and species of Callipallenidae and Nymphonidae, respectively. The fossil *P. problematicus* has neither spines on the chelifores nor teeth on the chelae, and it is possible that their occurrence might be a derived feature of certain extant taxa. However, I refrain from accepting the absence of spines and teeth on chelifores as a plesiomorphic state based on knowledge of a single fossil species. Families of pycnogonids have been classified based on the presence-absence of chelifores, but it is unlikely, or at least not yet proven, that all the forms whose adults lack chelifores are phylogenetically related.

Palps

Considered homologues to pedipalps in arachnids. As with the chelifores, the absence of palps is a feature used to classify genera into families. When present, their pattern of segmentation shows a wide variation, from more than ten segments to a single segment (characters 5 and 7). Longer and more segmented palps have been assumed as the plesiomorphic condition (Stock 1994, Munilla 1999). The palps of the known fossils cannot be evaluated with complete certainty, but apparently they have nine segments (Bergström et al. 1980). Ten-segmented palps are coded for some Ammotheidae (e.g. *Eurycyde* Schiodte and *Ascorhynchus*) and Colossendeidae, although discrepancies exist in the literature regarding the counting of the basal portion of the palps of Colossendeidae as a segment. Palps of nine, eight or six segments are found in Ammotheidae, Austrodecidae, Rhynchothoracidae and some callipallenids. The number of palpal segments is polymorphic within some of the ammotheid genera coded (e.g. *Tanystylum*, *Ascorhynchus*). Although males and females are mostly similar in regard to the palps, presence/absence and number of segments have been coded separately for each sex to include differences observed in the callipallenid *Propallene* Schimkewitsch (characters 4–7). A transformation series of the palps towards reduction is tested coding the characters of number of segments as ordered.

Ovigers

Unlike the chelifores and the palps, there is no counterpart or structure homologous with the ovigers of pycnogonids in any

other arthropod group. They can be present or absent, and show different degrees of reduction and patterns of segmentation. These characters vary between males and females. Eleven-segmented ovigers are assumed here to occur in males and females of *P. problematicus*, since sex cannot be distinguished in the radiographs of the fossils. Presence of ovigers and number of segments were coded separately for males and females because they are sexually dimorphic. Females of Pycnogonidae, Phoxichilidiidae and *Endeis* lack ovigers completely. The terminal claw (character 11) of nymphonids and some ammotheids and callipallenids could be derived from the eleventh segment of the oviger observed in the fossil. However, an ancestral condition within the group could be the presence of a terminal claw as a remnant of the main claw of the propodus, retained during the modification of the oviger, if the latter is assumed to be a modified leg (Arnaud & Bamber 1987). Thus, the loss of the terminal claw could be seen as an apomorphic condition. Different types of spines can be present on the terminal segments of the ovigers (character 25), or spines can be completely absent, as in members of Phoxichilidiidae and Pycnogonidae, which is seen as a reversal (Stock 1994). Nymphonids, callipallenids, colossendeids and members of Ammotheidae share compound or denticulate spines. Denticulate spines are generally arranged in a single row on the last four segments. However, multiple rows of spines are present in colossendeids and two ammotheid genera, *Ascorhynchus* and *Eurycyde* (character 27). It is not possible to define either a plesiomorphic or an apomorphic condition for this character.

Legs

Characteristics of the propodus are useful to segregate genera and species of pycnogonids. The presence of auxiliary claws (character 12), or 'ungues' when compared to the pretarsal structure of spiders in Snodgrass (1952), has been an important character to recognize genera of ammotheid and callipallenid affinities, and species within Nymphonidae and Phoxichilidiidae. They are not evident in the fossil, and homology has not been established with similar chelicerate structures such as tridactyl claws believed to be ancestral (e.g. in *Nothrus* sp. (Acari); van der Hammen, 1986). The presence of heel spines is included to examine its phylogenetic informativeness (character 22). The absence of heel spines in divergent taxa appears as a parallel event of secondary loss.

Cement gland openings on the femora of males (in *Propalene* occurring on tibiae as well) are present in most pycnogonid taxa (character 13), suggesting this as the plesiomorphic state despite the character being uncodeable for the fossil *P. problematicus*. Absence of these structures in the unrelated taxa Colossendeidae, Pycnogonidae and *Pseudopallene* Wilson can be assumed to be due to loss. Cement glands are present as single or multiple openings, the former appearing as characteristic of more basal taxa. A clear pattern of the distribution of the type of cement glands cannot be distinguished among the families. Both pores and conspicuous tubes occur within Ammotheidae, Callipallenidae and Phoxichilidiidae (character 15). The situation is similar regarding cement gland position with respect to the femora, but a mid-dorsal position seems to be the general state (character 16 and 17).

Genital pores or gonopores are located ventrally on the second coxae of one, two, three, or all pairs of legs (characters 20 and 21). Multiple openings of the gonads are assumed to be a plesiomorphic condition when compared to chelicerates and euarthropods in general (Boudreaux 1979). Most of the female sea spiders have gonopores on all leg pairs, but females in *Rhynchothorax* Costa and *Pycnogonum* Brunnich, for instance, possess a single pair of gonopores. Within the Pycnogonida this state might be assumed as a secondary loss occurring independently in different lineages. In some members of Ammotheidae and Phoxichilidiidae, the genital pores of males are present on prominent ventral spurs of the coxae (character 32), that appear as an independent specialization of the reproductive outlets.

Trunk

The shape of the body (character 18) is estimated by the distance between the lateral processes of each of the segments. Elongate, slender forms appear to be more common in the group; they are characteristic of Nymphonidae, *Endeis*, and most Colossendeidae. Many tenuous forms are also found in *Anoplodactylus* (Phoxichilidiidae), and some Ammotheidae which also include discoid-shaped forms (e.g. *Achelia* and *Tanystylum*). A small and compact body characterizes Rhynchothoracidae and Pycnogonidae. The general appearance of the body of sea spiders has been related to factors of the physical environment, more elongate forms being common on deeper soft-bottom substrata, and medium and compact forms generally found in shallow waters exposed to strong wave action (Arnaud & Bamber 1987). However, this has not been found to be a reliable rule, and any form can occur in any type of habitat. The segmentation of the trunk can be clearly distinguished by marked dorsal lines, which is the general state and presumably ancestral, but many species show partial or complete absence of segmentation lines. Lack of segmentation is more common in the compact forms although *Colossendeis* species, many with well-separated lateral processes, show no signs of trunk segmentation.

The position of the ocular tubercle with respect to the cephalic segment is explored as a phylogenetic character (character 23). Its posterior position is a synapomorphy of Nymphonidae and Callipallenidae, believed to be a specialized state with no biological implications discovered so far. The shape of the tubercle, although diverse within the group, is not useful as a phylogenetic character due to frequent intraspecific variation (examples shown in King 1973). An anterior cephalic hood in which the proboscis is embedded occurs in the ammotheid *Cilunculus* and has been coded as an autapomorphy for the genus.

The position of the abdomen of pycnogonids is rather consistent within genera. A horizontal position is assumed to be the plesiomorphic form when compared to other arthropod groups, it is also the state observed in the fossil *P. problematicus*. The ancestral condition is present in some taxa, but the significance of an erect abdomen has yet to be explained. Different degrees of abdominal inclination have been observed but are all coded as erect if not colinear with the trunk.

Proboscis

The proboscis of pycnogonids has been considered a homologue of the proboscis in polychaetes (Henry 1953 cited in

Hedgpeth 1954, Sharov 1966), provoking suggested placement of the sea spiders closer to the basal arthropodan stock than to chelicerates (Sharov 1966). So far, there is no evidence that the proboscis is anything other than the elongated acron (Boudreaux 1979), a unique specialization within arthropods. Fry & Hedgpeth (1969) tried to code the different shapes of the proboscis using a system of geometrical shapes and coordinates. The coding presented in this study is based on the five main types of proboscis shapes these authors proposed (character 31), using the geometrical criteria but not the system of coordinates (see Electr. Suppl., Pt 2). The particular shape and length of the proboscis can usually define families and genera. In Colossendeidae and Austrodecidae, the proboscis is longer than the trunk (character 33). This is not expected to be a synapomorphy for these two lineages, but probably an independently attained specialization. A ventral position of the proboscis is described in the fossil species (Bergström et al. 1980). This position resembles that observed in the basal ammotheid genera (e.g. *Eurycyde*, *Ascorhynchus* and *Cilunculus*) but also in the callipallenid form *Pseudopallene*. Fry (1965) pointed out the possible phylogenetic relevance of characteristics of the musculature and internal structure of the proboscis. There is information on only six species from five distinct genera, thus this character could not be defined in the present analysis. Morphological adaptations to preferred prey, as shown for *Austrodecus*, *Rhynchothorax* and *Pycnogonum* (Fry 1965), could also be further investigated for evolutionary implications.

Cladistic analysis

A parsimony analysis under an 'a posteriori weighting' approach, using the implied weights of the package PeeWee (Goloboff 1993b), was carried out to produce a phylogeny of the Pycnogonida based on morphological characters. As a preliminary exploration and for the aim of comparison with the implied weights analysis, similar analyses were done in Nona and PAUP* version 4.0b.4a (Swofford 2000), entering the characters as equally weighted or 'unweighted'. These days it is commonly considered that an equally weighted analysis is a preliminary estimate of the relative value of the data (Kitching et al. 1998). Based on Farris' ideas on character weighting (Farris 1969), Goloboff proposed a non-iterative method that uses evidence on homoplasy to estimate character reliability (Goloboff 1993a). It does not depend on initial estimations of weights, and produces trees of maximum fit $F = \sum f_i$, which implies the characters to be maximally reliable (Goloboff 1993a, 1995). The fit of the character i is measured with $f_i = k / (k + es)$, where k is a constant that changes the concavity of the fitting function to allow homoplastic characters to have more or less influence, and es is the number of extra steps. No theoretical justification exists for selecting a particular k value (Turner & Zandee 1995; Prendini 2000). However, extreme values of k are not recommended since very mild concavity functions (lowest value of k) do not differ much from analysis with equal weights, and very strong functions cannot be justified (Goloboff 1993b). The concavity or k value in this analysis was set to 5, weighting less strongly against characters with homoplasy (Goloboff 1993a). When k values of 4 and below were introduced, the analysis resulted in 21 most-parsimonious trees and a decrease of 6–23% in total fitness compared to the results with $k = 5$. When the ex-

treme value of $k = 6$ was used, a slight increase in the total fit occurred (1%), but the topologies remained the same as with $k = 5$. Prior fits or weights of the characters were scaled to 10 as recommended by Goloboff (1993a, b).

Heuristic searches were run in PeeWee using the commands "hold500; hold/20; mult*50", i.e. hold 500 trees in memory; keep 20 starting trees in each replication; perform Tree-Bisection-Reconnection (TBR) swapping on 50 random addition replicates. The command "jump" was used for additional swapping among multiple 'islands' of trees (Goloboff 1995). This same analysis can be run in PAUP* 4.0 by selecting unambiguous optimization under the parsimony settings and entering the "Goloboff", "GPeeWee" and "GK" options. The analysis with equally weighted characters was run in Nona (under the same commands as PeeWee) and PAUP*. Heuristic searches employed 20 iterations of random stepwise addition of the taxa, tree space was sampled using 100 random addition sequence replicates with three trees sampled per iteration ($nchuck = 3$, $chuckscore = 1$) in PAUP*. The resulting trees were branch-swapped using TBR checking for shorter resolutions and to fill out tree space (as in Edgcombe et al. 2000). This procedure is computationally less demanding than the default options for search in PAUP*. Under a strict or unambiguous optimization, only those trees are shown on which all minimum branch lengths are greater than zero. This is the default procedure in Nona and PeeWee, but the same conditions of analysis can also be implemented in PAUP* ($pset collapse = minbrlen$) (G. F. Wilson pers. comm.).

The synapomorphies present in all the dichotomous trees yielded by PeeWee (total set of trees found using the command "poly-") are found using the command "apo" in PeeWee (Goloboff 1997). Relative degree of support for each node was also examined by means of the branch support indices (Bremer 1994). Bremer support values up to ten extra steps were calculated in PeeWee using the command "hold 1000; bsupport10", i.e. hold a maximum of 1000 trees and branch support indices up to 10 extra steps.

The results of the implied weights analysis, obtained using unordered characters, were compared to those obtained with five multistate characters coded as ordered (characters 1, 5, 7, 8, 10 in Appendix 1) and representing the number of segments of chelifores, palps and ovigers in males and females. Hypotheses of pycnogonid evolution were investigated by constraining clades proposed by other authors and comparing them with the present results using the commands "ref", "swap", "mv", and "cmp" in PeeWee (as in Szumik 1996, Prendini 2000).

Results

A single most-parsimonious polytomous tree of maximum fit ($F = 2391.9$ [53%], $L = 180$) was found using unordered characters and implied weights (Fig. 2). The two polytomies – one for *Achelia* species, the other for *Anoplodactylus* species – when uncollapsed produce nine equally most-parsimonious dichotomous trees. The strict consensus of the nine trees with these two nodes collapsed is the basis for the discussion of the pycnogonid relationships presented (Fig. 2).

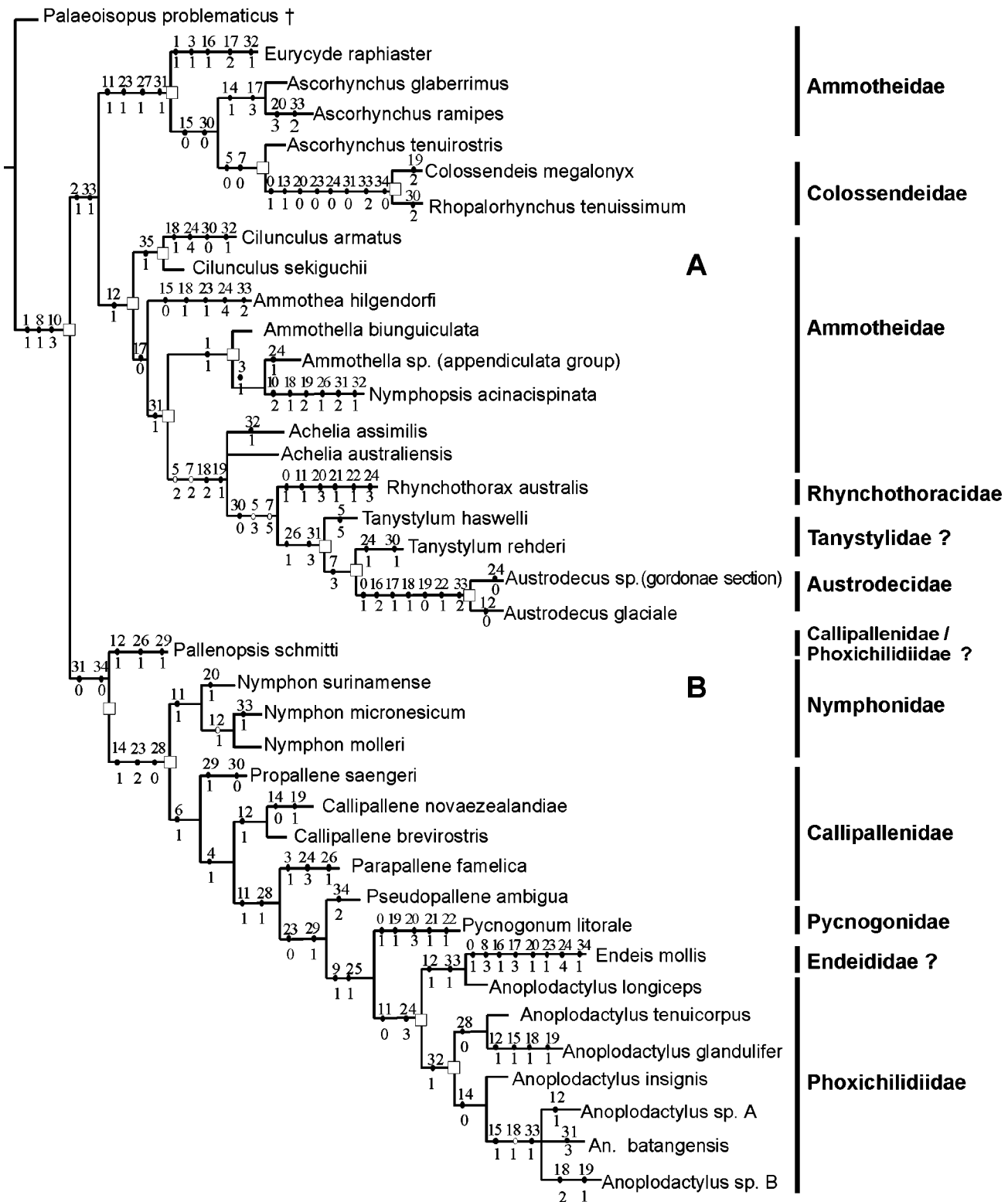


Fig. 2. Single most-parsimonious polytymous tree ($F = 2391.9$ [53%]; length = 180; $ci = 39$; $ri = 67$) obtained from the analysis of unordered characters and implied weights and presented as the preferred hypothesis of the pycnogonid phylogeny. This is the strict consensus tree of nine dichotomous resolutions. Solid circles represent synapomorphies present in all nine trees. Numbers above circles identify characters, numbers below circles the character states (Appendix 1). Nodes indicated by solid squares show a maximum Bremer support value of 3.

Two major lineages of pycnogonids are obtained: ammotheids grouped with Colossendeidae, Austrodecidae and Rhynchothoracidae (clade A in Fig. 2), and nymphonids grouped with callipallenids, Pycnogonidae, Phoxichilidiidae (including *Endeis*), with *Pallenopsis* as a sister-taxon (clade B). Clade A is supported by two synapomorphies: absence of chelae in the adults (character 2), and size of the proboscis relative to the trunk (character 33). However, character 33 changes twice from state 1 to state 2 in *Ascorhynchus ramipes* and *Austrodecus*. Clade B is supported by the shape and position of the proboscis (characters 31 and 34), although these characters are homoplastic, changing in *Anoplodactylus batangensis* and *Endeis mollis*, respectively. The support for the monophyly of living pycnogonids, A+B, is given mainly by the presence of fewer segments in chelifores and ovigers (characters 1, 8 and 10) in the living taxa than in the fossil *Palaeoisopus*.

The 'unweighted' analysis resulted in a single most-parsimonious tree ($L = 174$) (Fig. 3). This tree differs from the 'weighted' tree (Fig. 2) in the basal positions of *Cilunculus* and *Ammothea*, the derived position of Nymphonidae, and by *Pycnogonum* and *Pseudopallene* being placed within one clade.

After converting a subset of five multistates from unordered to ordered (characters 1, 5, 7, 8, 10 in Appendix 1), another set of searches in PeeWee yielded 100 most-parsimonious dichotomous trees. These are summarized in the strict consensus tree (Fig. 4) showing five collapsed nodes ($F = 2361.2$ [49%], $L = 195$). Fit decreased for eleven characters and increased for three when ordered characters were introduced (Table 2), resulting in a decrease in total fit of 4%.

Although most of the shallow clades were rather similar between the unordered and ordered analyses, the deep divergence of the two main clades is not obtained in the latter. Instead, a chain-like cladogram joins both major groupings (Fig. 4). A decreased resolution when the five multistates are coded as ordered (excepting character 10, which is uninformative as unordered) makes the alternative treatment of an 'unordered analysis' a better choice to represent their possible evolution. Characters 1, 5, 7, 8 and 10 are traced onto the initial proposed phylogeny (Fig. 5) to visualize the possible evolution of these characters according to the resulting topology in Fig. 2.

Discussion

Goloboff's method for estimating character weights during tree search (implied weights) is used here to construct a high-level phylogeny of Pycnogonida. Since the reliability of the characters is a logical implication of the trees being examined, this approach supports the notion

that there is no necessity to estimate weights prior to the analysis in a parsimony analysis. On the other hand, a parsimony analysis of equally weighted or 'unweighted' data can be regarded as a preliminary estimate of phylogeny and could only be defended with a claim that all the characters provide equally strong evidence. It is widely known that such a claim is usually rejected, in cladistic analyses some characters show a lot of homoplasy while others are perfectly hierarchical (Goloboff 1993a). Topological differences between the weighted and 'unweighted' analyses presented here are due to less weight given to the homoplastic characters by the implied weights method. A comparison of the consistency and retention indexes for each character between the implied weighted (iw) and the equally weighted (ew) reflects this action (Table 2).

The cladogram yielded by the implied weights analysis of unordered characters (Fig. 2) is the basis for the pycnogonid phylogeny discussed below. The internal relationships within each of the two major clades are described according to the conventional families of pycnogonids, which are used throughout the study while their validity is being tested.

Ammotheidae+Colossendeidae+Rhynchothoracidae+Austrodecidae

Two main groupings are found in clade A indicating the Ammotheidae as paraphyletic. The ammotheids *Eurycyde* and *Ascorhynchus* are grouped basally with Colossendeidae, supported by the presence of a terminal claw (character 11), multiple rows of spines on the ovigers (character 27), the mid-position of the ocular tubercle (character 23), and the shape of the proboscis (character 31), the latter two being reversals. Munilla (1999) showed the presence of multiple rows of spines on the ovigers as an autapomorphy of Colossendeidae, also noting – without further discussion – the curious presence of the multiple rows of spines in the ovigers of *Ascorhynchus*. Colossendeidae is a highly specialized family as indicated by the eight synapomorphies grouping its two most conspicuous genera, *Colossendeis* and *Rhopalorhynchus*. It is worth noting that the clade for ammotheids and colossendeids is characterized by species known from deeper waters, with the exception of a few *Eurycyde* and *Rhopalorhynchus* species. *Eurycyde* and *Cilunculus* have been considered primitive forms among extant pycnogonids, based on sutures on the proboscis and abdomen believed to be remains of an ancestral segmentation pattern (Stock 1994). *Ammothea* is presented here as sister taxon of ((*Ammothella*+*Nymphopsis*) (*Achelia*+*Rhynchothorax* + (*Tanystylum* +*Austrodecus*))), in contrast to the unresolved position of the genus presented by Lovely (1999).

At least 40 genera have been placed within Ammotheidae at one time or another. Of these, about 30 are general-

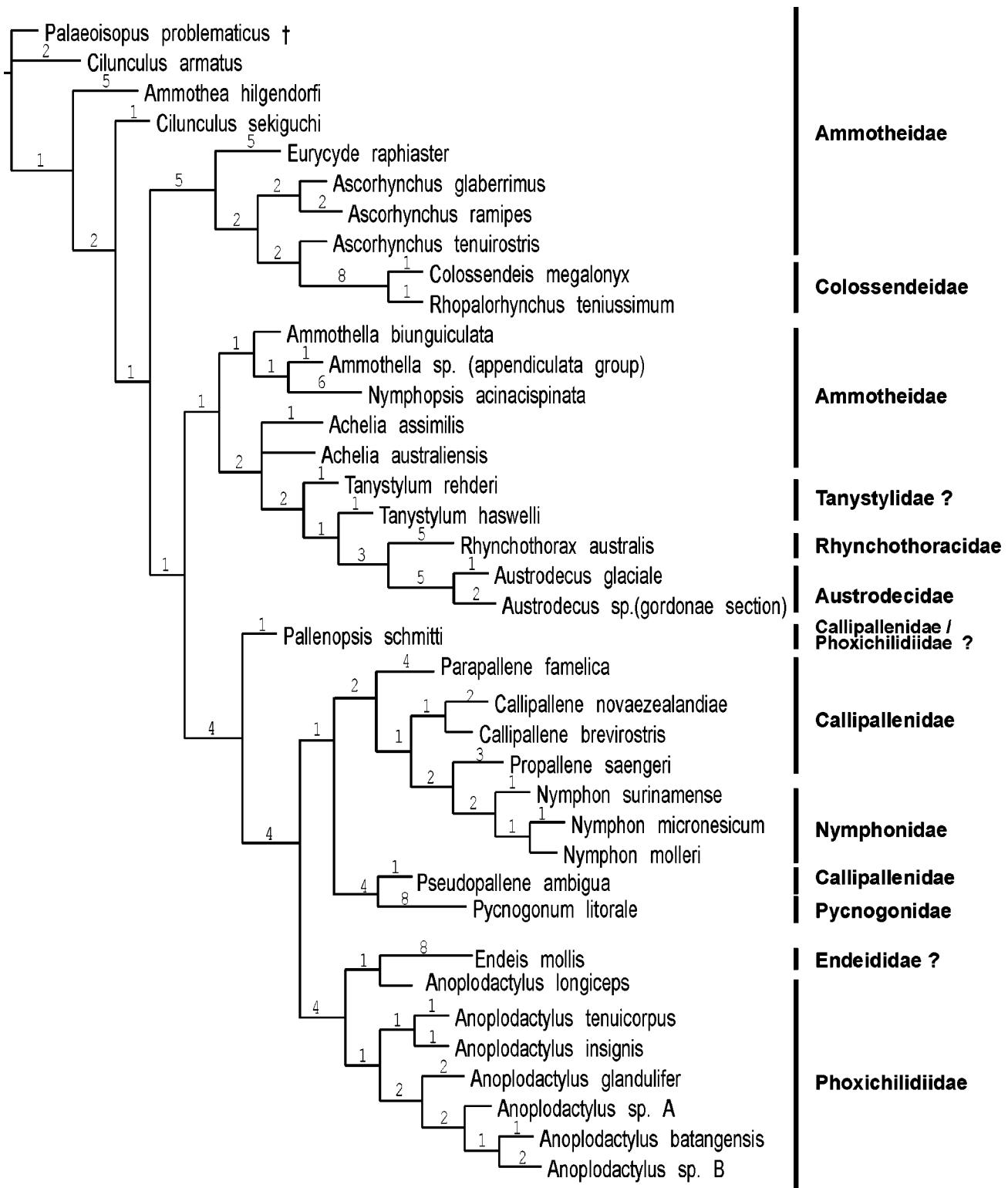


Fig. 3. Single most-parsimonious tree (length = 174; ci = 40; ri = 69) obtained with unordered and equally weighted characters. Branch lengths are shown.

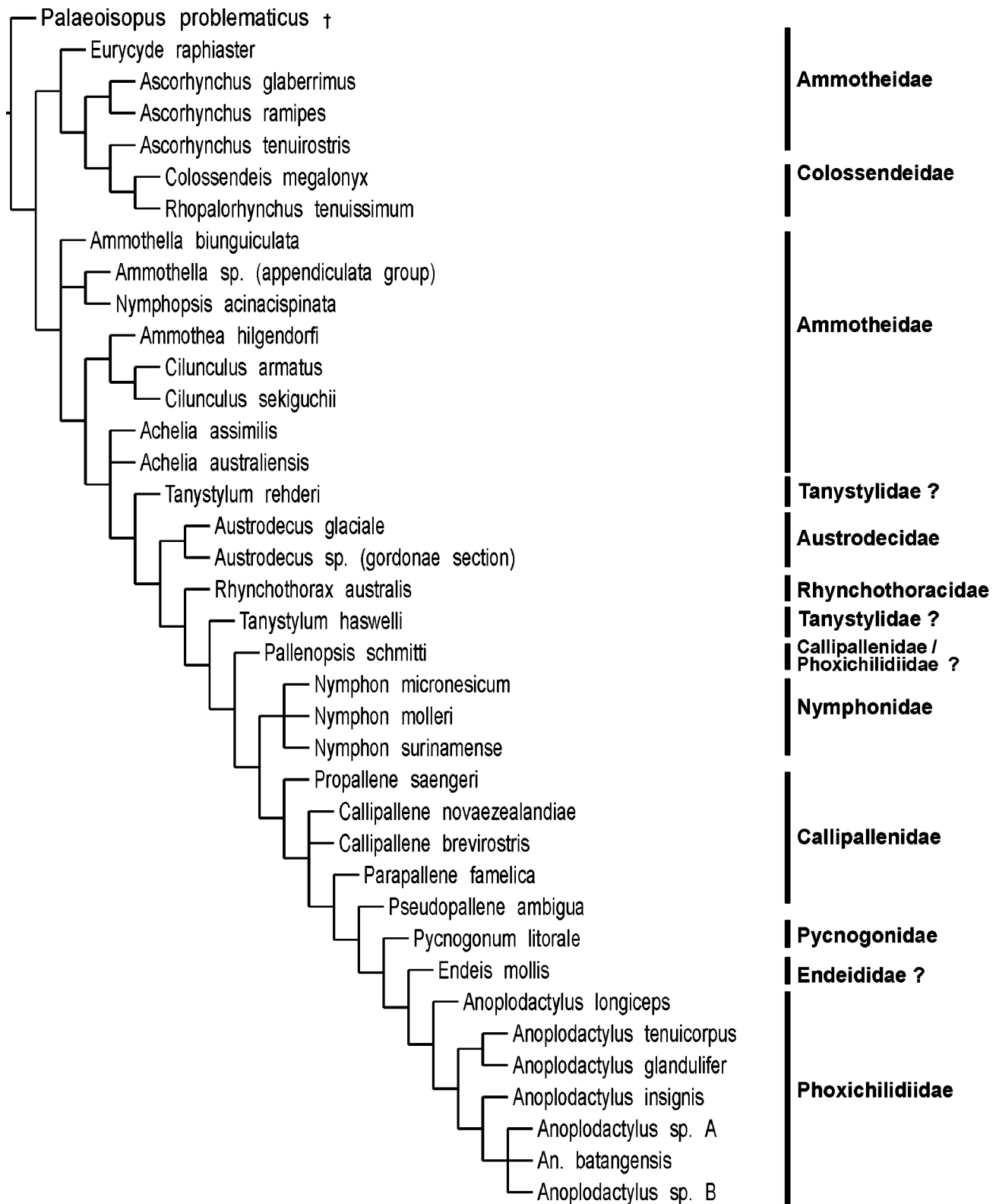


Fig. 4. Single most-parsimonious polytomous tree ($F = 2361.2$ [49%]; length = 195; $ci = 36$; $ri = 69$) obtained with five multistate ordered characters (1, 5, 7, 8, 10) under implied weights. This is the strict consensus tree of 100 most-parsimonious dichotomous resolutions.

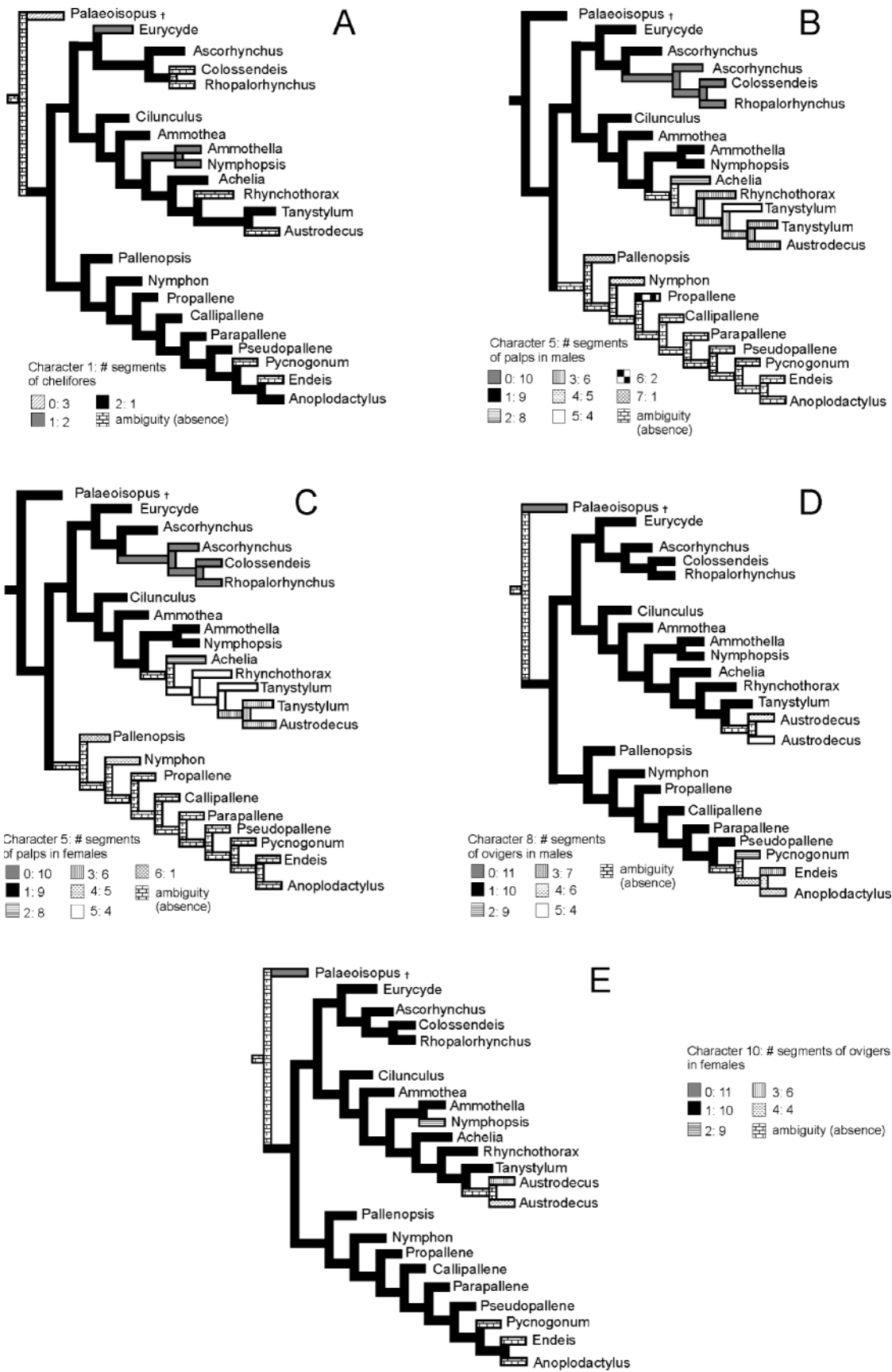


Table 2. Character statistics for 'unordered' and 'ordered' analyses. Characters coded as ordered in bold. Consistency index (ci) and retention index (ri) are given for the analyses with implied weights (iw) and with equal weights (ew). Prior fits or weights of all characters scaled to 10. Fit values were obtained from the 'implied weights' analysis.

Char	Unordered analysis					Ordered analysis				
	fit	ci (iw)	ri (iw)	ci (ew)	ri (ew)	fit	ci (iw)	ri (iw)	ci (ew)	ri (ew)
0	55.5	20	33	25	50	55.5	20	33	25	50
1	83.3	66	66	66	66	83.3	66	75	50	50
2	100.0	100	100	50	92	83.3	50	92	50	92
3	71.4	33	33	33	33	71.4	33	33	33	33
4	100.0	100	100	50	91	100.0	100	100	50	91
5	100.0	100	100	100	100	71.4	77	93	77	93
6	100.0	100	100	50	92	100.0	100	100	50	92
7	100.0	100	100	85	87	83.3	85	96	66	86
8	83.3	83	85	83	85	62.5	62	88	55	85
9	100.0	100	100	50	87	100.0	100	100	50	87
10			Uninformative			83.3	50	66	80	66
11	55.5	20	66	20	66	55.5	20	66	20	66
12	41.6	12	56	12	56	38.4	11	50	12	56
13	71.4	33	33	50	66	71.4	33	33	50	66
14	62.5	25	72	20	63	62.5	25	72	20	63
15	50.0	16	61	20	69	50.0	16	61	20	69
16	55.5	33	63	33	63	55.5	33	63	33	63
17	55.5	42	71	42	71	45.4	33	57	37	64
18	35.7	18	40	28	66	33.3	16	33	25	60
19	45.4	25	33	25	33	41.6	22	22	22	22
20	50.0	37	16	42	33	50.0	37	16	42	33
21	83.3	50	0	50	0	83.3	50	0	50	0
22	50.0	16	61	20	69	55.5	20	69	20	69
23	55.5	33	63	40	72	55.5	33	63	40	72
24	41.6	36	68	40	72	41.6	36	68	40	72
25	100.0	100	100	50	87	100.0	100	100	50	87
26	62.5	25	50	20	33	55.5	20	33	20	33
27	100.0	100	100	100	100	100.0	100	100	100	100
28	71.4	33	71	33	71	71.4	33	71	33	71
29	71.4	33	66	33	66	71.4	33	66	33	66
30	41.6	22	41	33	66	41.6	22	41	28	58
31	41.6	30	63	33	68	38.4	27	57	33	68
32	55.5	20	55	20	55	55.5	20	55	20	55
33	45.4	25	64	22	58	38.4	20	52	20	52
34	55.5	33	50	40	62	55.5	33	50	40	62
35	100.0	100	100	50	0	100.0	100	100	50	0

ly accepted as valid taxa (Fry & Hedgpeth 1969, Child 1998). The great morphological diversity within the family has led taxonomists to propose some genera as separate families, e.g. in the case of the Tanystylidae (Schimke-witsch 1913). In the present study, *Tanystylum* appears as an ammotheid genus closely related to *Achelia*, *Rhynchothorax* and *Austrodecus*. Some authors fail to recognize a common origin for *Achelia* and *Tanystylum*, arguing that there is a lower number of palp segments in *Tanystylum* (Hedgpeth 1954, Clark 1977, Munilla 1999). The results of the present study differ from that position and agree with Stock's classification in showing *Achelia*

and *Tanystylum* as closely related taxa (Stock 1954). However, no synapomorphies were found to hold *T. haswelli* Child and *T. rehderi* Child in a single clade. A similar case is presented by the *Ammothella* species: *Ammothella* sp. *appendiculata*-group is grouped together with *Nymphopsis*, solely by the presence of spines in the chelifores. A single character grouping the two species together is not considered to be strong evidence to modify the status of *Ammothella* and *Nymphopsis*.

The affiliations of *Rhynchothorax* have long been uncertain. The genus was associated to ammotheid forms (e.g. Bouvier 1923), considered a genus of the family

Tanystylidae (Hedgpeth 1955), or to belong to the Colossendeidae (see Arnaud & Krapp 1990). More recently, Stock (1994) suggested its affinity to *Austrodecus*. Thompson (1904) had given it family status, creating Rhynchothoracidae which has been widely accepted and redefined more recently (Arnaud & Bamber 1987, Arnaud & Krapp 1990). According to the present results, *Rhynchothorax* appears closely related to the ammotheids *Achelia* and *Tanystylum*. Characteristics of the trunk (characters 18 and 19), number of palp segments (characters 5 and 7), and the position of the abdomen (character 30) bring *Rhynchothorax* closer to the small ammotheid forms. Munilla (1999) had suggested Rhynchothoracidae to be the sister taxon of Pycnogonidae, based on the presence of a single genital pore in the females of both taxa, assuming it to be a product of 'regressive' evolution. That pattern of relationship is not obtained in this analysis.

Austrodecidae is a compact and homogeneous family with highly specialized characteristics, the most remarkable being the pipette-like proboscis and the extreme reduction of the ovigers. *Austrodecus* species used to be considered members of Tanystylidae (Hedgpeth 1947), before Stock created the family Austrodecidae (Stock 1954). In this study the Tanystylidae are rendered paraphyletic by the Austrodecidae. This close relationship is based on the trend observed in *Tanystylum* towards a proboscis tapered downward (character 31), and on the sharing of simple spines on the ovigers of both taxa (character 26). The presence of six-segmented palps in the females of *T. rehderi* and the species of *Austrodecus* shows these taxa to be closely related (character 7), segregating them from *T. haswelli* and *Rhynchothorax* in which females possess four-segmented palps.

A major revision of the relationships of the genera in Ammotheidae is long overdue (Fry & Hedgpeth 1969). A revision of the family is a difficult task, mainly because of the large number of species to be examined and the scarcity of type material available for rare genera. The relationships presented here are a first impression of what might have been the course of evolution in this lineage.

(Nymphonidae+Callipallenidae+Pycnogonidae+Phoxichilidiidae)+Pallenopsis

The shape and the frontal position of the proboscis (characters 31 and 34) are synapomorphies that segregate clade B from the rest of the Pycnogonida. Nymphonidae appears as a monophyletic group based on the presence of a terminal claw on the ovigers (character 11) in the three *Nymphon* species. This family has been considered a relatively homogeneous lineage of numerous (ca. 240) closely related species. *Nymphon surinamense* exhibits a different position of the male genital pores (character 20) and was selected as representative of the species complex with no auxiliary claws (character 12), contrasting with *N. molleri* and *N. micronesicum*.

The position of the ocular tubercle (character 23) and the presence of teeth on the chelae (character 28) relate Nymphonidae and callipallenid genera. According to Stock (1994), these two lineages are closely related. However, an electrophoretic study had shown Nymphonidae as a basal clade of the Pycnogonida distant from the Callipallenidae (Munilla & De Haro 1981). This plesiomorphic condition has also been supported by the idea that Nymphonidae species show a generalized plan of the Pycnogonida closer to that of an arachnid (Hedgpeth 1947). From the information currently available, it is not possible to ascertain the ancestral conditions of the extant Pycnogonida, until more fossil evidence becomes available. Nevertheless, in this study Nymphonidae appears as a fairly basal group of pycnogonids at the same time related to the callipallenids.

Males from both Nymphonidae and Callipallenidae generally possess one cement gland (character 14), except for some species of *Callipallene* such as *C. brevirostris* with more than one, and the genus *Pseudopallene* that shares the absence of cement glands with *Pycnogonum* and *Colossendeis*. The Callipallenidae appear as a paraphyletic group, containing the Phoxichilidiidae, Endeidae and Pycnogonidae, all related by the absence of palps in the females (character 6). *Pseudopallene* links the callipallenids to (*Pycnogonum*+*Endeis*+*Anoplodactylus*) based on the anterior position of the ocular tubercle and the ventral orientation of the chelae (characters 23 and 29, respectively). The absence of ovigers in the females (character 9) and the glabrous condition of the ovigers in males bring together the Pycnogonidae and Phoxichilidiidae. The position of Pycnogonidae as derived from a callipallenid ancestor reflects the same relationships proposed by Stock (1994) in his diagram (Fig. 1A).

The genus *Endeis* has been considered by some specialists as a distinct entity that needs to be placed as a separate family, Endeidae (Hedgpeth 1947, King 1973) or Endeididae (Child 1992), but Stock (1965) preferred to include it in the Phoxichilidiidae. The present cladogram shows *Endeis* as closely related to the Phoxichilidiidae based on the absence of a terminal claw on the ovigers (character 11). *E. mollis* appears as sister to *Anoplodactylus longiceps* which could be taken as representative of *Anoplodactylus* species with auxiliary claws (character 12) and a relatively long proboscis (character 33). Although *E. mollis* is grouped with *A. longiceps* by these two characters, *Endeis* has clear autapomorphies (Fig. 2) that support it as a taxon separate from *Anoplodactylus*. The inclusion of *Phoxichilidium*, the most probable sister taxon to *Anoplodactylus*, in future analyses might be of help to test the proximity of *Anoplodactylus* and *Endeis*.

The paraphyly of *Anoplodactylus* might be explained by the enormous variability within the genus in the characteristics of the cement glands, shape and segmentation

of the trunk, size and shape of the proboscis, and a number of other characters. The absence of palps, of ovigers in females, and the number of segments of the male ovigers are quite stable among the species, but the internal relationships of the diverse genus *Anoplodactylus* are worthy of further detailed studies.

Pallenopsis has been considered a transitional genus between Callipallenidae and Phoxichilidiidae (Hedgpeth 1947), classified as a callipallenid (see Hedgpeth 1948, Child 1979) and as a phoxichilidiid (Stock 1978). In the present analysis *Pallenopsis* is the basal taxon of clade B, which node is supported by the presence of a single cement gland (character 14), the middle position of the ocular tubercle (character 23), and the absence of teeth on the chelae (character 28). However, *Pallenopsis* shares these characters with taxa from one or the other family, and a certain variation is also known within the genus (e.g. toothed chelae in *P. mascula* Bamber). Child (1992) has suggested *Pallenopsis* might deserve familial rank, this particular data set is agnostic and does not give indication whether to relate *Pallenopsis* to Callipallenidae or Phoxichilidiidae.

Character evolution

Presence/absence and features of the head appendages are the most commonly used characters relating families and genera of pycnogonids. Since it has been argued that a gradual reduction of the appendages might have occurred within the group, the assumption of an order in the evolution from highest to lowest number of appendage segments should show the most-parsimonious resolution of pycnogonid phylogeny. The present results do not show support for this argument. The most parsimonious trees were obtained when the number of segments of chelifores, palps and ovigers were coded as unordered. This suggests that there has not been a strict gradual reduction of the appendages throughout the evolution of the group. The mapping of the characters onto the proposed phylogeny (Fig. 5 A–E) shows that a trend of reduction and loss of the appendages occurs independently in each of the two major clades.

Regarding the evolution of the chelifores, according to the cladogram proposed here, a complete loss of the chelifores in adults has independently occurred on five occasions (Fig. 5A). Chelate larval stages and juveniles are known for most of the taxa in which chelifores are lost in the adult stage. The functional importance of the chelae in larvae and juveniles is believed to be related to their parasitic habits (King 1973, Staples & Watson 1987), but the absence of chelae in adults has not been discussed in functional or ecological terms.

The absence of palps (characters 4 and 5) appears as an apomorphic condition relating *Pycnogonum*, Phoxichilidiidae and callipallenid taxa, although males of a

few genera of Callipallenidae (e.g. *Propallene*) have 1- or 2-segmented palps. It could be argued that it was within the callipallenids that sea spiders lacking palps began to diversify. A reduction in the number of palp segments is also evident within the ammotheid clade (*Achelia*+*Rhynchothorax*+*Tanystylum*), but complete loss does not occur (Fig. 5B, C). The reduction or absence of palps cannot yet be explained in terms of their functional or ecological significance. The relevance of the palps as sexually dimorphic features, in some callipallenid taxa such as *Propallene*, remains to be studied. Setae and glands observed on the palps of *Nymphon* and ammotheid species are believed to be sensory structures used for the recognition of prey (in King 1973, Arnaud & Bamber 1987). However, for those taxa in which palps are completely absent alternative sensory structures are yet to be recognized. Detailed developmental and physiological studies might help to form hypotheses on the significance of the loss of palps, which seems to have occurred first in the females of callipallenid forms, suggesting also that sexual dimorphism might be involved.

A reduced number of oviger segments in males is common to Pycnogonidae and phoxichilidiids; in Austrodecidae not only the numbers of segments are lower (Fig. 5D), but ovigers are also extremely reduced in overall size (Stock 1957). A total absence of ovigers has been observed in species of *Pycnogonum* (Stock 1968, Child 1998), as well as in a few *Austrodecus* (Stock 1991). However, the process of reduction seems to be different since the size of the ovigers of *Pycnogonum* males is not as extreme as in *Austrodecus*. The number of oviger segments in females appears as an uninformative character in the analysis (character 10, Fig. 5E). Although it is not possible to identify a common origin for the reduction of the ovigers in the females, their complete absence (character 9) clearly defines the clade for Pycnogonidae and Phoxichilidiidae, including *Endeis* (Fig. 2). Ecological or functional differences between species with conspicuous, long ovigers and those with reduced or absent ovigers are not well studied. However, males of species lacking ovigers have been observed carrying the eggs cemented to the ventral side of the trunk (Child 1998). Again, as well as for the palps, the reduction of the ovigers especially in the males is shown as a parallel event in the two major clades of the Pycnogonida.

Previous classifications

The fit and length of the trees obtained in this study were evaluated against previous classifications. Constraints were forced onto the tree according to relationships previously suggested by Stock (1994) and by Munilla (1999), both summarizing the most accepted traditional classification of the Pycnogonida.

Table 3. Clades enforced according to previous classifications, and their fit compared to the phylogeny proposed in this study. Prior fits or weights of all characters scaled to 1.

Tree	Fit difference	Characters with better fit	Characters with worse fit
Colossendeidae+Rhynchothoracidae+ Austrodecidae (Stock, 1994)	-4.6	0(2/1.6); 20(1/0.5); 22(2/1.2); 30(1/0.4)	5(1/1.7); 7(2/2.9); 12(1/0.3); 15(1/0.5); 18(1/0.2); 26(1/0.7); 27(1/1.7); 31(1/0.3)
Rhynchothoracidae+Austrodecidae (Stock, 1994)	-1.0	0(1/0.7); 20(1/0.5); 22(1/0.5)	7(1/1.7); 26(1/0.7); 31(1/0.3)
<i>Pallenopsis</i> as a phoxichilidiid (Stock, 1978)	-6.3	22(1/0.5); 29(1/1.2)	4(1/1.7); 6(1/1.7); 9(1/1.7); 14(1/0.7); 15(1/0.5); 25(1/1.7)
<i>Pallenopsis</i> as a callipallenid (Hedgpeth, 1948; Child, 1979)	-2.3	22(1/0.5)	14(1/0.7); 23(1/0.5); 28(1/0.9)
Pycnogonidae+Rhynchothoracidae (Munilla, 1999)	-8.5	0(1/0.7); 11(1/0.7); 20(1/0.5); 21(1/1.7); 22(1/0.5)	4(1/1.7); 5(1/1.7); 6(1/1.7); 7(1/1.7); 9(1/1.7); 12(1/0.3); 14(1/0.7); 15(1/0.5); 17(1/0.5); 25(1/1.7); 33(1/0.4)
<i>Endeis</i> +Phoxichilidiidae (Stock, 1994)	-0.7	No better fit for any character	12(1/0.3); 33(1/0.4)

According to his diagram (Fig. 1A), Stock seemingly proposed a clade Colossendeidae+Rhynchothoracidae+Austrodecidae (Stock 1994). When this group was enforced, an overall decrease in total fit of 4.6 was observed, eight characters decreasing and four characters increasing in fit (Table 3). A clade formed by these three taxa does not explain the data set well. However, when a clade for *Rhynchothorax* and *Austrodecus* was constrained (according to Stock's diagram), the total fit of the proposed phylogeny was just slightly affected (Table 3). The absence of chelifores (character 0) is the main character with a better fit, but the type of spines in the ovigers (character 26) and the shape of the proboscis (character 31) are not synapomorphies of *Rhynchothorax*+*Austrodecus* (Table 3).

When *Pallenopsis* was constrained to Phoxichilidiidae, as proposed by Stock (1978), there was a decrease in total fit of 3% with nine characters having a worse fit. Then, enforcing *Pallenopsis* as a callipallenid taxon, the fitness was just two units lower than the unconstrained cladogram, but only one character performed better (Table 3). The basal position of *Pallenopsis* in clade B suggests it could be proposed as a higher taxon, but it might also indicate lack of sufficient informative characters to attach the genus to any of the known taxa.

When the clade Rhynchothoracidae+Pycnogonidae proposed by Munilla (1999) was enforced, the overall fitness decreased consistently, with 11 characters showing a worse fit under this constraint. These results show that the grouping of the two families is far from being the most explanatory for the current data set. The con-

straint of *Endeis* as a sister taxon of the Phoxichilidiidae revealed a very slight decrease in total fit, but none of the characters showed a better fit (Table 3). The close relationship between *Anoplodactylus* and *Endeis* has been shown here, and I propose that *Endeis* be left within the Phoxichilidiidae until further evidence becomes available to decide whether the two genera are within a single family or whether the two families should be grouped within a higher taxon.

A clade for callipallenids, phoxichilidiids (including *Endeis*) and Pycnogonidae, as proposed by both Stock (1994) and Munilla (1999), is supported in this analysis. However, the monophyly of Callipallenidae and the status of *Pallenopsis* and *Endeis* need to be clarified.

Conclusions

The phylogeny proposed shows two main lineages of extant sea spiders. Ammotheidae is shown as a paraphyletic group including *Rhynchothorax*, *Austrodecus* and Colossendeidae. These are segregated from a clade combining Callipallenidae, also a paraphyletic group, with Nymphonidae, Pycnogonidae and Phoxichilidiidae. The absence of chelae in adults is a main feature supporting the divergence of these two main clades. Unordered, weighted characters provided the most parsimonious and consistent resolution of a pycnogonid phylogeny on the morphological data set used. A strict gradual reduction of the appendages in a manner of ladder-like evolution is not supported. Instead, a trend of reduc-

tion of the palps and ovigers has occurred independently in each of the two major clades.

In general terms, the outcome of this study agrees with previous classifications, especially the one proposed by Stock (1994), except for his ideas of *Pallenopsis* as a phoxichilidiid genus, and of a clade consisting of Colossendeidae, Austrodecidae and Rhynchothoracidae. Pycnogonid phylogeny is yet not clear. Little work has been done based on a set of traditional taxonomic characters, and there is a strong need for additional characters from different sources to either contrast the clades proposed or give a more robust support. The lack of appropriate outgroups for comparison and polarization remains a major problem in attempts to relate pycnogonids to other arthropod taxa, and to recognize ancestral character states.

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Appendix 1

Morphological characters, states, and scores for phylogenetic analysis

Characters referred to in the list as “*Ordered*” were considered as such only when indicated in the analysis.

0. Chelifores: present (0); absent (1).
1. (Where applicable.) Number of segments of the chelifore scape: 3-segmented (0); 2-segmented (1); 1-segmented (2). *Ordered*.
2. Chelae: present (0); absent (1).
3. (Where applicable.) Chelifores, dorsal spines on scape: absent (0); present (1).
4. Palps, in males: present (0); absent (1).
5. Palps, number of segments in males: 10-seg. (0); 9-seg. (1); 8-seg. (2); 6-seg. (3); 5-seg. (4); 4-seg. (5); 2-seg. (6); 1-seg. (7). *Ordered*.
6. Palps, in females: present (0); absent (1).
7. Palps, number of segments in females: 10-seg. (0); 9-seg. (1); 8-seg. (2); 6-seg. (3); 5-seg. (4); 4-seg. (5); 1-seg. (6). *Ordered*.
8. Ovigiers, number of segments in males: 11-seg. (0); 10-seg. (1); 9-seg. (2); 7-seg. (3); 6-seg. (4); 4-seg. (5). *Ordered*.
9. Ovigiers, in females: present (0); absent (1).
10. Ovigiers, number of segments in females: 11-seg. (0); 10-seg. (1); 9-seg. (2); 6-seg. (3); 4-seg. (4). *Ordered*.
11. Ovigiers, terminal claw: absent (0); present (1).
12. Propodi, auxiliary claws: absent (0); present (1).
13. Cement gland(s): present on femora or other leg segments (0); completely absent (1).
14. Cement gland(s), on femora: one on each femur (0); multiple on each femur (1).
15. Cement gland shape: pore(s) or slit on the cuticle (0); tube(s) or protuberances (1).
16. Cement gland position on legs: dorsal (0); lateral (1); ventral (2).
17. Cement gland(s), on femora: located distally (0); at mid-point (1); proximal (2); distributed all along femora (3).
18. Trunk: elongate shape, crurigers or lateral processes separated by distance at least equal to their own diameter (0); intermediate shape: crurigers separated by less than their own diameter, but never touching (1); compact shape: crurigers touching (2).
19. Trunk: distinctly segmented, the three lines of segmentation dorsally visible (0); partially segmented, only one or two lines visible (1); lines of segmentation not distinct (2).
20. Genital pores in males: present on all four pairs of legs (0); present on second, third and fourth pairs of legs (1); present on third and fourth pairs of legs (2); present on fourth pair of legs only (3).
21. Genital pores in females: present on all four pairs of legs (0); present on fourth pair of legs only (1).
22. Propodi, heel spines: present (0); absent (1).
23. Ocular tubercle: anterior on cephalon (0); equidistant to anterior and posterior margins (1); posterior on cephalon (2).
24. Ovigiers, largest segment: sixth (0); fifth (1); fourth (2); third (3); second (4).
25. Ovigiers, spines on last segments: present (strigilis) (0); absent (1).
26. Ovigiers, spines: compound or denticulate (0); simple (1).
27. Ovigiers, spine arrangement: in single row (0); in multiple rows (1).
28. Chelae, teeth: present (0); absent (1).
29. Chelae, orientation: opposing each other (0); pointing downwards, in front of tip of proboscis (1).
30. Abdomen: horizontal in same direction as trunk (0); erect diagonally or pointing upwards (1).
31. Proboscis, shape (see Electr. Suppl., Pt 2): A = straight (0); B = inflated proximally, acute distally (1); C = inflated distally (2); D = tapering or pipette-like (3).
32. Second coxae of last pairs of legs in males, ventral spurs: absent (0); present (1).
33. Proboscis, length: less than half length of trunk (0); equal (\pm 1mm) to half length of trunk (1); at least equal to trunk length (2).
34. Proboscis, position: frontal and fixed (0); in angle and movable (1); ventral and highly movable (2).
35. Cephalic hood: present (0); absent (1).