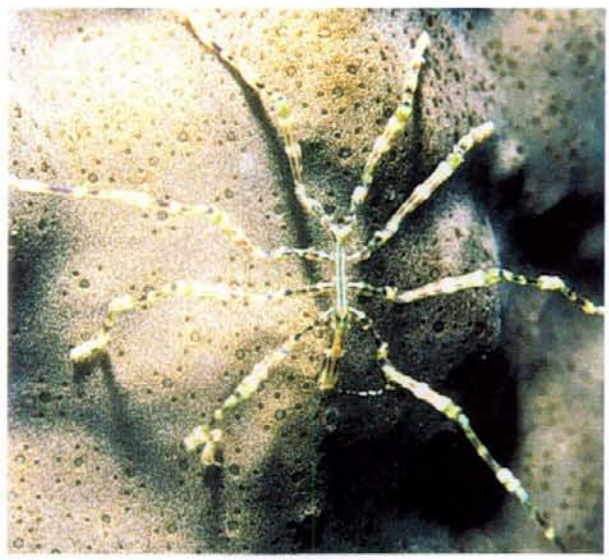
Morphological and Molecular Phylogenetic Analysis of the Sea Spiders (Arthropoda, Pycnogonida) and Taxonomic Study of Tropical Australian forms



Thesis submitted by
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in February 2002

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Claudia P. Arango

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ABSTRACT

Pycnogonida is a subphylum of marine arthropods showing unique characteristics. Their position within the Arthropoda is not yet clear, but strong evidence has suggested they may be the extant sister taxon to all other arthropods. The phylogenetic affinities among the extant families of pycnogonids: Ammotheidae, Colossendeidae, Callipallenidae, Nymphonidae, Phoxichilidiidae, Pycnogonidae, Austrodecidae, Rhynchothoracidae, and the position of problematic genera such as *Endeis*, *Pallenopsis* and *Tanystylum*, are uncertain. Traditionally, it has been assumed that an evolutionary trend of gradual reduction of numbers of segments of the appendages, mainly involving chelifores, palps and ovigers (head appendages) has taken place within the group. Modern cladistic techniques have not been applied to resolve phylogenetic conflicts of the sea spiders. I approached the problem of the uncertain higher-level phylogenetic affinities of pycnogonids to propose hypotheses of relationships based on cladistic analysis of morphological characters, thereby testing the hypothesis of a reduction trend. Additionally, I used a preliminary molecular approach to confront the morphological results. This is one of the first attempts to use molecular data in the study of systematics of pycnogonids. Phylogenetic relationships among the main lineages of extant sea spiders were studied using cladistic analysis of 36 morphological characters and 38 species from all the recognized families. A preliminary exemplar method was employed, and different assumptions of multistate character transformations were used to trace the evolution of the head appendages. Fragments of nuclear ribosomal DNA (18S and 28S) were sequenced to reconstruct the phylogenetic relationships among six higher taxa of sea spiders. Hypotheses of relationships were obtained from separate and combined analyses of these data sets under both maximum parsimony and maximum likelihood criteria. Trees derived from the molecular data set were compared with those from the set of 36 morphological characters previously analysed. Estimates of phylogeny were found to be significantly different between the molecular and the morphological data set and possible causes for incongruence, such as the coding of inapplicable characters in morphology and a very reduced set of taxa in the molecular analysis, are discussed. The position of Colossendeidae was a major cause of conflict, being supported as a relative of Ammotheidae by morphological characters but appearing closely related to Callipallenidae and Nymphonidae with DNA data. With the molecular characters, *Austrodecus* is identified as a basal taxon for the rest of the pycnogonids included, differing from its close relationship to ammotheids shown by morphology. Using morphological data, the family Ammotheidae appeared as paraphyletic as did Callipallenidae. *Pallenopsis* was related to *Anoplodactylus* according to DNA but not morphology. Although

no clear pattern of overall relationships among sea spiders is yet defined, several patterns useful for future systematic work have been noted. New sets of characters and compilation of data from all available sources will probably provide a better picture. Ontogenetic transformation could give some insights into character evolution, and knowledge of ecological traits is needed to complement morphological observations. A collection of fresh material of numerous species of sea spiders from the Great Barrier Reef and other localities of Queensland was useful for the phylogenetic analyses and also contributed to the knowledge of the marine fauna of Australia. Thirty-three species of tropical shallow-water sea spiders collected from the Queensland coast, the Great Barrier Reef and the Coral Sea are reported here. Among these were six undescribed species in the genera *Austrodecus*, *Anoplodactylus* and *Pycnogonum*, and other nine species, mostly of Indo-West pacific distribution not previously recorded for Australia.

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LIST OF PUBLICATIONS

Based on the work of this thesis, the following papers have been accepted or submitted to scientific journals for publication :

Arango CP. 2001. Sea spiders (Pycnogonida) from the Great Barrier Reef, Australia, feed on fire corals and zoanthids. Memoirs of the Queensland Museum 46:656.

Arango CP. 2000. Three species of sea spiders (Pycnogonida) from Santa Marta, Colombian Caribbean. Boletin de Investigaciones Marinas y Costeras 29:59-66.

Arango CP. In press. Morphological phylogenetics of sea spiders (Arthropoda, Pycnogonida). Organisms Diversity and Evolution.

Arango CP. In press. Sea spiders from the Great Barrier Reef area: New species, new records and ecological annotations. Journal of Natural History.

Arango CP. Molecular approach to the phylogenetics of Pycnogonida (Arthropoda) using nuclear ribosomal DNA and morphology. Submitted to Molecular Phylogenetics and Evolution.

Arango CP and Brodie GD. In press. Observations of predation on the tropical nudibranch *Okenia* sp. by the sea spider *Anoplodactylus longiceps* Williams (Pycnogonida, Arthropoda). The Veliger.

Lee A. C. and Arango CP. Two new species and other records of sea spiders (Pycnogonida, Arthropoda) from tropical North Queensland, Australia. Submitted to Memoirs of the Queensland Museum.

Statement on sources

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Claudia P. Arango

23rd May 2002

Date

CHAPTER ONE

General Introduction

1.1 Overview

The Pycnogonida (Gr. *pyknos* = crowded + *gony* = knee), commonly named sea spiders, are a distinct group of marine arthropods of uncertain affinities, frequently linked to the Chelicerata (Snodgrass, 1938; Firstman, 1973; Manton, 1977; Weygoldt, 1986; Wheeler et al., 1993; Zrzavý et al., 1997; Wheeler and Hayashi, 1998; Edgecombe et al., 2000; Giribet and Ribera, 2000; Regier and Shultz, 2001). The unique and very conspicuous characters of pycnogonids, including an external proboscis, an additional pair of appendages (called ovigers), and the reduction of the abdomen to a peg-shaped vestige, have caused controversy and made them difficult to relate to any other arthropod group (Boudreaux, 1979; Ax, 1987). DNA data have also shown discrepancies among different analyses with the Pycnogonida appearing as sister group of the chelicerates or basal to the main extant lineages of arthropods (Giribet and Ribera, 2000; Edgecombe et al., 2000). Recently, the most complete data set available in arthropod phylogeny studies has shown the latter option to be the most likely (Giribet et al., 2001). The special morphological characters, the unexpected relationships shown by DNA sequences, and also the scarcity of fossil records, are facts that create uncertainty in our own interpretations of pycnogonid affinities. There are ca. 1200 species of pycnogonids and it is believed there are many more species to be discovered mainly from the deep-sea. The known species are distributed in 80 genera and eight or nine families as they are: Ammotheidae, Austrodecidae, Callipallenidae, Colossendeidae, Nymphonidae, Phoxichilidiidae, Pycnogonidae, Rhynchothoracidae, and Endeididae a monogeneric family sometimes included in Phoxichilidiidae.

Ecologically, sea spiders are essentially marine benthic dwellers that occur from the shoreline to abyssal depths in all the seas around the world. They range in size from tiny midgets having leg spans of only 2 mm, to deep-sea giants with leg spans of up to 75 cm; the larger species are usually found at deeper habitats. Sea spiders are mostly epibenthic and carnivorous, some species have been described in parasitic associations with hydroids, molluscs and echinoderms (Arnaud and Bamber, 1987). Taxonomic descriptions of new species are still the most common type of publication on sea spiders. Monographs on pycnogonids started to appear in the late 1800s and have covered most of the regions of the world. Biological and ecological work related to feeding and reproductive traits has also been

carried out, however this has been done mainly on temperate or polar species (Fry, 1965; De Haro, 1978; Davenport et al., 1987; see review in Arnaud and Bamber, 1987).

1.2 Morphology of Pycnogonida

The body of the sea spiders is always very reduced and sometimes appears to be only a connector between each pair of legs; thus, the digestive and reproductive organs have migrated to the legs. Most species have four body segments, each of them bearing a pair of walking legs (Fig. 1). However, some deep-sea species can have five or six body segments and ten or twelve legs respectively ('polymerous forms' in Hedgpeth, 1947), which is a very unusual phenomenon in arthropods, and yet to be explained. The first segment or cephalon bears the ocular tubercle housing four simple eyes (typically pigmented), the proboscis, the first pair of walking legs and three other pairs of appendages: the chelifores above the proboscis, the palps laterally, and the ovigers ventrally. The most prominent external feature of pycnogonids is the proboscis. It is a moveable organ and shows wide variation in size and shape among families. The shape and internal structure has been related to specialised feeding habits, sometimes specific to a particular host among parasitic species (Fry, 1965; Staples and Watson, 1987). The ocular tubercle can be a tall, pointed protuberance or a low tubercle situated dorsally on the midline of the cephalon. Some species lack the ocular tubercle (especially abyssal and psammophilic species). The chelifores, believed to be homologues of the chelicerae in arachnids (Winter, 1980), consist of the scape or proximal part, and the chela, which has a fixed finger and a moveable finger articulated on the palm. In some species the fingers are robust and denticulate, in others they are very feeble. Chelifores are present in all the larval stages and juveniles known so far, but in some taxa, the chelifores disappear with the last moult before adulthood. The palps, presumed to be homologues of arachnid pedipalps, are multi-segmented and seem to have sensory, cleaning and feeding functions (see review in Arnaud and Bamber, 1987). However, there are three families in which palps are completely absent. Additionally, the ovigers are another pair of appendages joined to the cephalon on its ventral surface (Fig. 1.1). The males use these appendages to carry the eggs until hatching; in some species they also carry the larvae after hatching. Ovigers have a very particular configuration with a sickle-shaped terminal portion, with denticulate or simple spines. It is suggested the ovigers are important for grooming (Davenport et al., 1987), however, there might be some more important duties related to the mating and parenting behaviour, since it has been observed that legs can also clean the body, in both sea spiders with ovigers and those without. Females of some taxa lack ovigers, and these structures are completely absent in both sexes of some *Pycnogonum* species.

The legs of pycnogonids have generally eight segments, and a main distal claw, and many species have auxiliary claws placed dorsolaterally to the main claw. Males possess cement glands on the femora that secrete the substance used to wrap the eggs and attach them to the ovigers. The outlet can be a long duct, a short tube, a single pore, or a number of tiny pores located dorsally or ventrally on the femur. Males and females can be easily differentiated by the absence of ovigers in females of the families Phoxichilidiidae and most Pycnogonidae species, otherwise the presence of cement glands on the femora indicates a male individual [although hermaphrodite specimens are known for some species (see Miyazaki and Makioka, 1993)].

The fertilisation of the eggs is known to be external, the female releases the eggs and the male fertilises and attaches them to the ovigers in a single mass. The process has been observed in *Phoxichilidim femoratum*, *Endeis* species (see King, 1973), *Pycnogonum litorale* (Jarvis and King, 1972) (Tomaschko and Bückmann, 1997), and mating behaviour has also been recorded in *Propallene longiceps* (Nakamura and Sekiguchi, 1980). Regarding reproduction and development, the best-known species is *Pycnogonum litorale*, a common species of the north Atlantic that shows specific associations with a hydroid and a sea anemone (Jarvis and King, 1972; Behrens, 1984; Wilhelm et al., 1997; Tomaschko and Bückmann, 1997). Reproductive biology has also been studied in *Endeis laevis* (Jarvis and King, 1975), *Nymphon* species (King and Jarvis, 1970) and *Parapallene famelica* from the east coast of Australia (Hooper, 1981), among few others. Pycnogonids do not have a planktonic stage in their life cycle, and this is believed to have implications for the patterns of distribution and a possible high rate of speciation (Bamber, 1998b). During the post-embryonic period in some species of callipallenids and nymphonids the embryos stay on the parent's ovigers until they reach a well-developed stage (after the fourth instar) (Nakamura, 1981). However, most species hatch to a free-swimming protonymph. Pycnogonid protonymphs resemble a nauplius larva of the crustaceans, but they have a proboscis, bear chelifores with strong pincers, and have two other pairs of appendages with a single terminal claw. There is little information to date on morphological characters of larvae and juvenile stages.

1.3 Taxonomy and systematics of Pycnogonida

The taxonomy of Pycnogonida is principally based on the presence and the characteristics of the appendages on the cephalic segment. Characters of chelifores, palps and ovigers (presence, number of segments, configuration) define the artificial classification of families currently in use. Characters of the propodus and the cement glands are useful for identification at genus and species levels.

Some of the most relevant publications on the taxonomy of the Pycnogonida are early monographs (Hoek, 1881; Loman, 1908 and others) and more recently the series of works by J. Hedgpeth (1947, 1954), J. H. Stock (1975, 1994) and C. A. Child (between 1982 and 1998). The group has been reviewed by Helfer and Schlottke (1935), Fage (1949), King (1973) and Arnaud and Bamber (1987) with comprehensive reports on species from specific locations(Gordon, 1944; Hedgpeth, 1948; 1949; Stock, 1954; Fry and Hedgpeth, 1969 among others). Taxonomy and some aspects of general biology have been the concern of most of the published works, however novel information on the ecology of some species has been produced more recently (Mercier and Hamel, 1994; Sheerwood et al., 1998; Rogers et al. 2000; Arango, 2001).

Phylogeny of the Pycnogonida has been little studied. Hedgpeth (1947) established a system of classification based on traditional morphological distinctions. This classification is currently accepted and followed by most of the students of the group, with few changes made along the time. A hypothesis of a reduction series of the number of segments as an evolutionary trend towards the loss of appendages, has been behind this traditional classification (Hedgpeth, 1947; Stock, 1994) (Classification by Stock, 1994 is shown in Fig. 3.1). This has been a simple explanation accepted to describe the phylogeny of the group, but it is important to provide the tools to be able to test this taxonomic hypothesis. . The current methods available for the analysis of morphological and molecular data provide an opportunity to confront the hypothesis of a gradual reduction and present alternatives about the evolutionary history of the sea spiders.

1.4 This study

In this work I approach the problem of the uncertain higher-level phylogenetic affinities of pycnogonids with the aim of 1) proposing a phylogeny based on morphological characters to confront the hypothesis of a reduction trend. With the aid of cladistic techniques, a close account of the problems and gaps in the knowledge of the group is given and the taxa in need of detailed revision at lower levels are indicated. 2) Complementing and confronting the analysis of morphological characters with molecular data from two regions of nuclear DNA that are commonly used in studies of arthropod phylogeny. This is one of the first attempts to use molecular data in the systematics of pycnogonids, so it is also taken as an exploration of the usefulness of the genes selected for the study of higher-level phylogenetic relationships of sea spiders.

The analyses of morphological and molecular information were possible after the collection of sea spiders from different habitats in the shallow waters of North Queensland that gave me

access to more than 30 species representing seven out of eight families and included undescribed species and new records for Australia. However, limitations during the course of the project were related to the small number of specimens usually collected for most of the species, restricting the availability of material for microscopical and DNA analyses. The molecular analysis included only a limited subset of taxa that could be reliably sequenced for both genes.

Nonetheless, this study on systematics of the Pycnogonida exposes the problematic issues in the study of their phylogeny in the context of modern cladistic techniques and DNA analysis, and proposes affinities among main extant lineages that are worthy of further examination using additional sets of characters e.g. anatomical or ecological. In addition, the collection and description of numerous species of sea spiders from the Great Barrier Reef and other localities of Queensland is a contribution to the knowledge of the marine fauna of Australia. It also adds information that fills some gaps in the knowledge of the biogeographical and ecological patterns of distribution of the understudied tropical sea spiders. In the Chapter 2, I report the species collected during the study, followed by the cladistic analysis of morphological characters in the Chapter 3. The study of molecular phylogenetics of the Pycnogonida in Chapter 4 includes a simultaneous analysis of the two data sets (morphology and DNA) and a discussion of similarities and conflicts between the estimates of phylogeny from each of the data partitions. Finally, there is a general discussion and implications for future research of the main outcomes of this study. This is a pioneering work on molecular phylogenetics of pycnogonids and gives a preliminary framework for further studies. The evolution of morphological characters and traditional classification is analysed in the context of modern cladistic techniques for the first time; and scarcely studied taxonomy and ecology of tropical Australian sea spiders are revised suggesting new directions for research on pycnogonids.

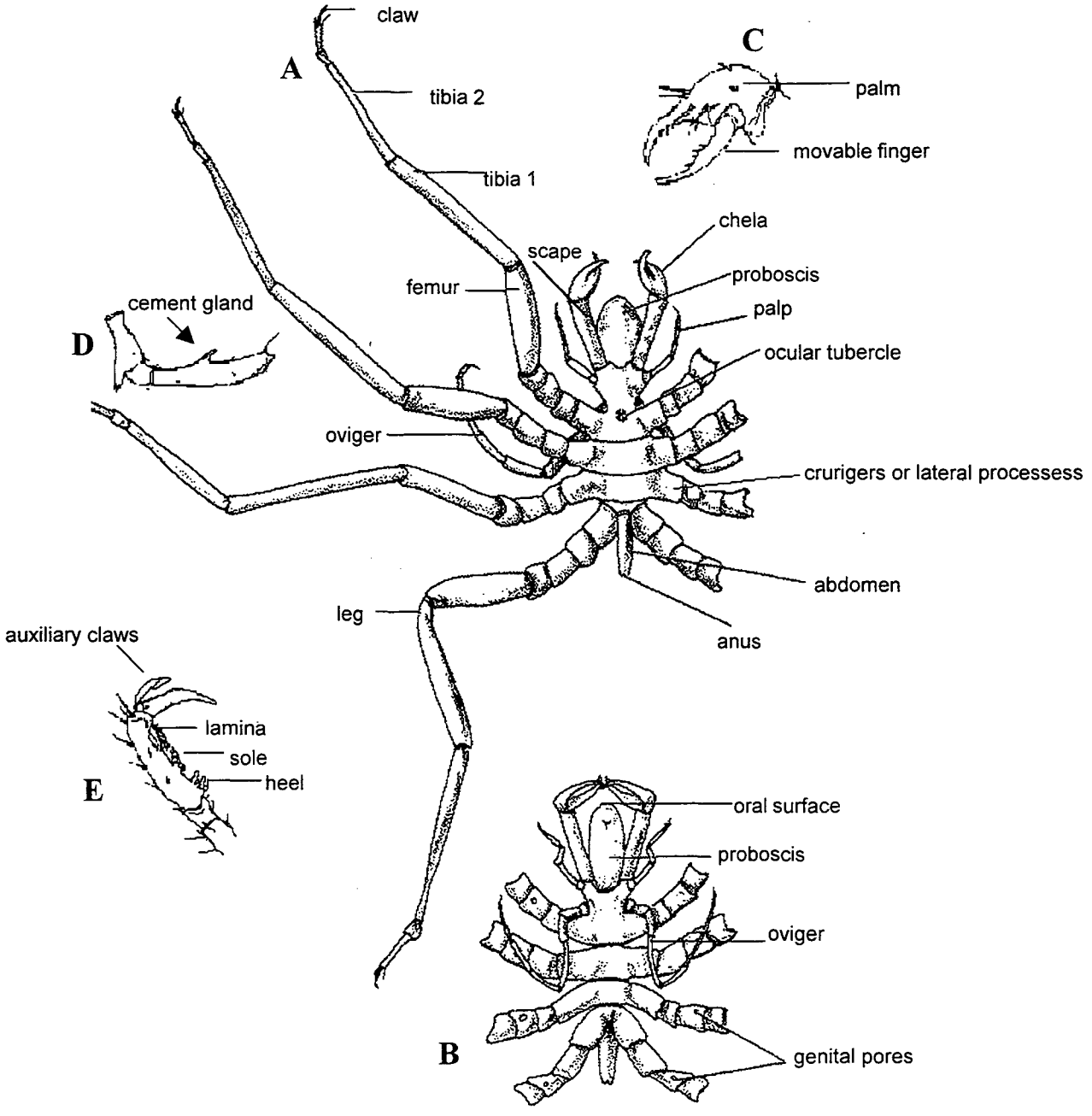

Figure 1.1. Diagram of a pycnogonid. A. Dorsal view of trunk and legs (drawn on the left side only). B. Ventral view. C. Detail of the chela. (palm + finger = chela, scape + chela=chelifore). D. Coxa 2, coxa 3 and femur with dorsal cement gland. E. Detail of propodus. Modified from Hickman (1973) and Child (1998).

CHAPTER TWO

Sea spiders (Pycnogonida, Arthropoda) from the Great Barrier Reef and Northeastern Australia: new species, new records and ecological annotations

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2.1 Summary

Thirty-three species of tropical shallow-water sea spiders are reported from the Queensland coast and coral reef microhabitats in the Great Barrier Reef and the Coral Sea. Undescribed species in the genera *Ammothella*, *Callipallene*, *Austrodecus*, *Anoplodactylus* and *Pycnogonum* are reported, as well as nine species, mostly of Indo-West Pacific distribution, not previously recorded for Australia. Rare species such as *Rhopalorhynchus tenuissimum* and *Nymphopsis acinacispinata* are reported, and the genus *Anoplodactylus* is shown to be highly diverse in the area. The intertidal green alga *Cladophora prolifera* was found to support a rich pycnogonid fauna, sheltering a total of fourteen species at two different sites. In coral reefs, coral rubble, macroalgae and zoanthids were found to support pycnogonid populations. This study highlights the diversity of pycnogonids in shallow water habitats and contributes to the knowledge of tropical faunae. It also illustrates the strong need for taxonomic and phylogenetic revision of some of the major taxa. Illustrations for most of the records and a brief discussion on zoogeographical and ecological aspects of tropical pycnogonid fauna are included.

2.2 Introduction

The first descriptions of Australian species of pycnogonids are those included in the extensive work by Hoek (1881) resulting from the Challenger Expedition. He accounted for six deep-sea species from southeastern Australian waters. Hoek was followed by Haswell (1884) who described eight new species from the eastern coast; then Carpenter (1892; 1893) reported new species from the northern Torres Strait region. Almost twenty years later Flynn (1918; 1919a; 1919b; 1929) redescribed the species reported by Haswell and added new species to the Australian list. Williams (1933; 1940; 1941) reported a new species from the Queensland coast, reviewed the fauna of Rottnest Island in Western Australia and revised the genus

Anoplodactylus. Stock (1954) described new species from the Great Barrier Reef (GBR) as part of a larger study of the fauna of the Indo-West Pacific, Australia and New Zealand collected by the Th. Mortensen expedition. Clark (1963) provided a comprehensive report on Australian pycnogonid fauna based on material deposited at the Australian Museum; of 42 species reported, 22 species were recognised as new to science, most of them from the New South Wales area.

Ten years later, an sporadic encounter with a Rhopalorhynchus was reported from the vicinity of Brisbane (Monod, 1971) and was followed by Stock (1973a; 1973b) who published two contributions to the knowledge of southeastern Australian sea spiders, and Child (1975) who aimed to continue filling the gap of knowledge around the Australian coasts with his report of pycnogonids from Western Australia. South and Western Australian waters have not been studied for sea spiders since the 1970s, and very few contributions have been made during the last three decades for any region. Staples (1979) described new species of *Propallene* and accounted for 28 species of pycnogonids known from Queensland at that time (Staples, 1982). Child's (1990) contribution to the pycnogonid fauna of the GBR is still the most recent published paper dealing specifically with Australian sea spiders. Child (1990) added fifteen species to the fauna of Queensland and reported six new to science, all from the GBR. Judging by the findings of new forms in his limited sampling from just two of the hundreds of islands, he suggested that the GBR could shelter a high diversity of pycnogonids. More recently, Stock (1994) published a report on Pycnogonida collected by major expeditions in the Indo-West Pacific and included two species from the Australian Northern Territory.

A total of 115 species reported from Australian waters were accounted for in the literature prior to this study. All recognised pycnogonid families are represented, and shallow water habitats hold a high diversity of Ammotheidae, Phoxichilidiidae and Callipallenidae, as noted by Clark (1963). Material deposited in museums around Australia more recently is still waiting for inclusion. A greater and more systematic effort is needed to clearly characterize the Australian pycnogonid fauna.

In the Indo-West Pacific there has been a greater effort in the study of the pycnogonid fauna. Reports have been compiled on pycnogonids collected during major expeditions from East Indian waters (Loman, 1908), Indian waters (Calman, 1923; Stock, 1953) and Japanese waters (Hedgpeth, 1949). Major contributions were derived from the collection deposited in the Zoologisk Museum Kobenhavn, including lots from Australia and New Zealand (Stock, 1954), and from the Galathea and Anton Bruun expeditions in the Indian and Pacific Oceans (Stock, 1968). More recently, a series of papers dealing with collections from the western Pacific Islands has increased the number of species known from tropical Pacific waters

(Bamber, 1997a; 1997b; 2000; Child, 1982b; 1983; 1988b; 1990; 1991; 1996; 1998a; Müller, 1990a; 1990b; 1990c; 1992a; 1992b). About 200 species are known so far from the shoreline down to 3500 m depth in Indo-West Pacific waters. *Anoplodactylus* (Phoxichilidiidae), and some Ammotheidae forms are the most diverse in shallow waters, while genera such *Colossendeis*, *Ascorhynchus* and *Pallenopsis* are common in bottoms deeper than 100 m, although shallower water representatives are also known.

The present study reports species of sea spiders from twenty-one sites in northeastern Australia, including four reefs in the Coral Sea (Table 1). The southern-most site is at the Swain Reefs in the Capricorn section of the GBR and the northern-most is Green Island, in the Cairns section. Thirty-three species are reported, of which six had not been described before, thirteen are new for Queensland and nine of them, mostly of Indo-Pacific distribution, are new records for Australia. Seven out of eight recognised families are represented in the collection, missing the rare Rhynchothoracidae, although *Rhynchothorax vallatus* was described from the GBR (Child, 1990). The intertidal green alga *Cladophora prolifera* (Roth) Kützing is reported as a pycnogonid-rich substrate, harbouring diverse pycnogonid communities in the Townsville area. In coral reefs, substrata such as zoanthids, hydrozoan corals and species of macroalgae are found to be important components in the understudied ecology of tropical sea spiders.

Identification keys for the families and genera reported here are included in Appendix 1. A key for the species of *Anoplodactylus* reported is in Appendix 2.

The species reported in this study are:

Ammotheidae Dorhn 1881

Achelia assimilis (Haswell, 1884)

Achelia nana Loman, 1908

Ammothella stauromata Child, 1982

Ammothella n. sp. *

Ascorhynchus tenuirostris Carpenter, 1892

Nymphopsis acinacispinata Williams, 1933

Tanystylum haswelli Child, 1990

Tanystylum rehderi Child, 1970

Austrodecidae Stock 1954

Austrodecus n. sp. ☼

Colossendeidae Hoek 1881

Rhopalorhynchus tenuissimum (Haswell, 1884)

Nymphonidae Wilson 1878

Nymphon molleri Clark, 1963

Nymphon micronesicum Child, 1982

Callipallenidae Hilton 1942

(?) Pallenopsis hoeki Miers, 1884

Callipallene n. sp. *

Callipallene novaezealandiae Stock, 1954

Parapallene famelica Flynn, 1929

Propallene saengeri Staples, 1979

Seguapallene cf. *micronesica* Child, 1983

Pigrogromitus timsanus Calman, 1927

Phoxichilidiidae Sars 1891

Anoplodactylus batangensis (Helfer, 1938)

Anoplodactylus n. sp. A ☼

Anoplodactylus digitatus Böhm, 1879

Anoplodactylus n. sp. B ☼

Anoplodactylus glandulifer Stock, 1954

Anoplodactylus longiceps Stock, 1951

Anoplodactylus tenuicorpus Child, 1991

Anoplodactylus tubiferus (Haswell, 1884)

Anoplodactylus versluysi Loman, 1908

Anoplodactylus pectinus Hedgpeth, 1948

Endeis biseriata Stock, 1968

Endeis flaccida Calman, 1923

Endeis mollis Carpenter, 1904

Pycnogonidae Wilson 1878

Pycnogonum n. sp. ☼

☼ This species is named and described in Arango (in press). * This species is named and described in Lee and Arango (submitted)

2.3 Materials and Methods

This study is based mostly upon material I collected with the aid of collaborators from James Cook University and other institutions in North Queensland (see acknowledgements). Collections were made during 1998-2001 in the area of Townsville, the Central section, the Cairns section, and the Capricorn sections of the Great Barrier Reef (Table 2.1). Most of the species were collected in shallow water habitats, including the intertidal zones of sandy

shores, reef flats, reef slopes, and a few species come from benthic samples down to 52m depth. Different collection techniques were used depending on the habitat sampled, bearing in mind that tropical shallow-water species are usually much smaller than their counterparts from temperate locations and deeper waters.

Table 2.1 Collection sites of pycnogonids in Queensland and the Coral Sea, Australia. The common name for the location mentioned in the table with details of the habitat are included under 'Material examined' of each of the species.

Locality	Latitude, Longitude
Townsville, North Queensland	
Cape Ferguson, Turtle Bay	19°15'S, 147°03'E
Rowes Bay	19°14'S, 146°47'E
Cleveland Bay	19° 07'S, 146°47'E
Townsville Marina	19°15'S, 146°50'E
Coral Sea	
Chilcott Island	16°56'S, 150°01'E
Flinders Reef	17°30'S, 149°10'E
Holmes Reef	16°29'S, 147°51'E
Willis Reef	16°18'S, 149°58'E
Great Barrier Reef, Central Section	
Pandora Reef	18°49'S, 146°26'E
Goold Island	18°10'S, 146°10'E
Great Palm Island, Cannon Bay	18°40'S, 146°35'E
Orpheus Island, Pioneer Bay	18°36'S, 146°29'E,
Rib Reef, reef slope	18°28'S, 146°52'E
Picnic Bay, Magnetic Island	19°10'S, 146°51'E
Geoffrey Bay, Magnetic Island	19°09'S, 146°52'E
GBR Cairns Section	
Green Island	16°10'S, 146°05'E
GBR, Capricorn Section	
Swain Reefs	21°36'S, 152°05'E
North Fitzroy Reef	23°35'S, 152°09'E
Other Localities in Queensland	
Lucinda jetty	18°35'S, 146°29'E
Cairns Marina, Trinity Inlet	16°14'S, 146°03'E
Mackay, Queensland	21°05'S, 149°14'E

When sampling intertidal areas within easy access to the laboratory, samples of sessile fauna and/or algae were collected in plastic bags with fresh seawater and left in trays in the laboratory for examination. After a few hours, sea spiders would start to move to the surface for oxygen (Bamber and Davis, 1982), and could then be collected and preserved. When possible, habitat samples were washed and sorted on a 0.5 mm size mesh straight after collection and sea spiders were preserved in 70 or 90% ethanol. Only a few species were found by naked eye when using SCUBA or amongst trawl samples.

The identification of species depends mainly on external morphology but dissection of appendages (legs, palps and ovigers) is sometimes necessary. The simple morphology of the animals makes the identification at least to genus a fairly uncomplicated task. The terminology used here is one commonly applied by modern specialists in the field (see Arnaud and Bamber, 1987; Child, 1992). The formula for the ovigers refers to the number of spines in the last four segments starting with the most proximal (e.g. 5:6:7:9). These segments are sometimes referred as the 'strigilis' when they are strongly curved in a sickle shape and the spines are large and denticulate (see Child, 1979). Measurements of trunk length are from the anterior end of the cephalon to the most distal corner of the last pair of crurigers or lateral processes, trunk width is measured across the second pair of crurigers to their distal margins. All measurements are given in mm.

Material was collected and identified by the author unless otherwise specified. Dr. C. A. Child carried out taxonomic verification of species. Complete descriptions and illustrations are given for the new species and most of the known species except when specimens were damaged. The synonymical bibliographies under the taxonomic headings include synonyms and additional papers dealing with the species. Descriptions of genera in this report are mostly based on the material presented and a revision of literature and material from other areas, but in most cases this information can also be found in Child (1998b) and Stock (1954). Holotypes and voucher specimens are deposited at the Museum of Tropical Queensland, Australia (MTQ S105773-S105865).

2.4 Results

Family Ammotheidae Dorhn, 1881

Genus *Ascorhynchus* Sars, 1877

Segmentation lines swollen, sometimes with dorsomedian tubercles. Proboscis pyriform, generally with proximal and distal sutures; carried ventrally. Chelifore scape one- or two-segmented, chelae reduced. Palps of nine segments. Eight or nine-segmented ovigers, several rows of denticulate spines on terminal segments; with terminal claw. Legs long, slender, propodus cylindrical, without heel or spines, auxiliary claws absent. Cement glands multiple dorsal pores or raised cones. —Remarks. Few species known from shallow waters, usually smaller than their counterparts from deeper waters.

Ascorhynchus tenuirostris Carpenter, 1892 [Fig. 2.1]

Ascorhynchus tenuirostris Carpenter, 1892: 555-557, pl. 7 –14.

Ascorhynchus tenuirostre (sic.). — Child, 1990: 311 [literature].

Material examined.— Turtle Bay, 14 May 1999, 1 juv; Swain Reefs, 46 m, collected by trawl, found amongst the green alga *Halimeda* sp. and rubble, 22 Nov 1999, 1♂ [Department of Primary Industries (DPI) sta. DW P422, coll. C. P. Arango & DPI Seagrass Monitoring Project].

Description.— Trunk 2.16 mm long, 1.05 mm wide, finely granulate, fully segmented, segment lines marked with cowlings, single dorsomedian tubercle per segment, tubercle on fourth segment as tall as ocular tubercle, small spines on each tubercle; crurigers separated by their own diameter, all with single distal tubercle covered in very fine granules and 2-3 short spines. Ocular tubercle tall, conical, few spines on tip; eyes at midpoint, pigmented but not dark. Abdomen horizontal, reaching far from distal margin of first coxae of last pair of legs. Proboscis long, narrow, pyriform, carried ventrally, with proximal and distal sutures. Scape of chelifores one-segmented, small palm lacking fingers, in juveniles chelae small, smooth, with pincers gaping when closed. Palps nine-segmented arising laterally from neck, second segment longest, densely setose segments from five to nine. Ovigers ten-segmented, fourth and fifth segments subequal, three rows of compound spines on seventh segment, two rows on eighth ninth and tenth; ectal spines longer, formula 6:3:3:5. Legs slender, smooth; dorsodistal spur on femur; second tibiae with dorsal row of long setae; tarsus small with three short distal spines; propodus long, slender, slightly curved, without heel, rows of fine setae on both margins; main claw robust and curved, almost half of propodus length; auxiliary claws absent.

Distribution.— Previously known only from its type locality in Torres Strait, North Queensland.

Remarks.— Morphology of the adult specimen agrees with the description of the holotype (Carpenter, 1892) except for fewer rows of spines on the eighth segment of the oviger. Another four species of *Ascorhynchus* are known for Australia (Clark, 1963); *A. longicollis* Williams, 1941 and *A. compactum* Clark, 1963 lack the conspicuous median tubercles on the trunk, these structures are much shorter in *A. minutum* Hoek, 1881, and there are longer dorsal coxal spurs. *A. melwardi* Flynn, 1929 has no trace of a palm and the body is more setose; it is known from Cape York and Singapore and is probably the closest relative of *A. tenuirostris*. *Ascorhynchus tenuirostris* can be easily recognized by the narrow shape of the proboscis, the one-segmented scape and the armature of the dorsum.

Genus *Achelia* Hodge, 1864

Trunk discoid, crurigers short and touching along most of their margins; proboscis pyriform, constricted basally and inflated at midpoint; chelifore scapes one-segmented, chelae reduced without fingers; palps of seven or eight segments, legs short and robust, propodus curved, heel spines present, auxiliaries long. Cement gland a dorsodistal tube. —Remarks. The genus

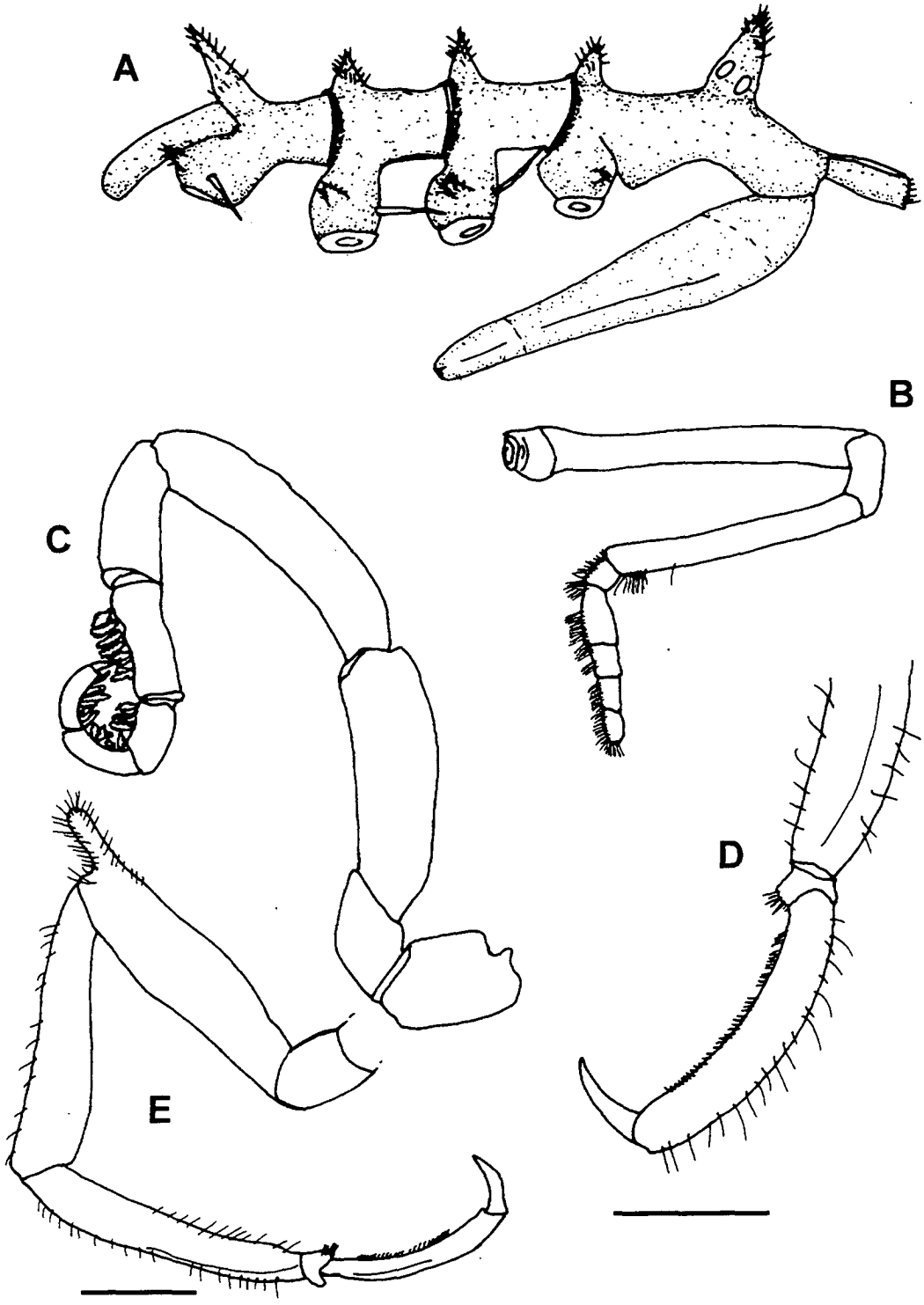

Figure 2.1. *Ascorhynchus tenuirostris*, ♂. A. Lateral view of the trunk and proboscis. B. Palp. C. Oviger. D. Tarsus and propodus; scale bar=0.6mm. E. Third leg from the third coxa; scale bar=0.8mm.

Achelia appears to be an artificial grouping of species with high intraspecific variation in their morphological characters. It is in a strong need of revision being one with a worldwide distribution and of relatively high abundance especially in littoral zones.

Achelia assimilis (Haswell, 1884) [Fig. 2.2]

Ammothea assimilis Haswell, 1884: 1026-1027, pl. 55, fig. 5-9; Loman, 1908: 59-60.

Achelia assimilis. — Flynn, 1919: 89-70, pl. 22, fig. 22-26; Stock, 1954:97-100, figs. 45-46; 1994: 32-33, fig. 9 [literature]; Child, 1977: 440-441; 1988a: 289-290; 1988b: 2; 1991: 138; 1996: 541; Bamber 2000: 621.

Material examined.— Turtle Bay, collected among the green alga *Cladophora prolifera* from intertidal rocky patches on a sandy beach during low tide (< 0.5 m), 23 Mar 1997, 16♂, 6♀, 11 juv., 1 postlarva (coll. J. Otto); 5 Oct 1998, 18♂, 12♀; 14 May 1999, 1♂, 1 juv, 8 adults (not fixed); 12 Jul 1999, 5♂, 3♀ (19 adults not fixed); 4 May 2000, 2♂, 10♀, 1 Jul 2000, 1♂, 1♀, 1 juv.— Rowes Bay, collected among *C. prolifera* from a rock ledge made of small boulders in a sandy muddy environment, during low tide (< 0.5 m), 17Apr 1999, 1♂ w/eggs, 1♀ (19 adults not fixed); 12 Aug 1999, 1♀; 25 Sep 1999,1♀, 1♂; 1 Jun 2000 24♂ (some ovigerous), 18♀, 1 juv.— Flinders Reef, amongst washings of algae, 12 m depth, 3 Jul 1999, 1♀.— Pandora Reef, southern, windward side of the reef, in rubble with turf algae and macroalgae *Dictyota* sp. and *Laurencia* sp., 5 m depth, 15 Jul 1999, 3 juv. Great Palm Island, Cannon Bay, reef flat, on rubble and algae, 2 m depth, 4 Feb 1999, 1 juv. Goold Island, reef flat, in *Sargassum* spp., 4 m depth, 15 Mar 2000, 1♂ (coll. G. Diaz-Pulido).

Description.— Trunk length 1.0-1.4 mm, width across second crurigers 0.6-0.7 mm, third line of segmentation not clear, dorsum smooth, disc-shaped, crurigers touching. Ocular tubercle twice as tall as wide, inclined forward, eyes dark-pigmented. Abdomen slightly swollen at the base then curved upwards at the distal half. Anterior and posterior corner of each cruriger armed with one spine-bearing tubercle. Scape one-segmented, with 3 dorsodistal spines, chelae reduced in adults, only small buds are visible. Palps eight-segmented, last segment twice as long as wide. Ovigers 10-segmented, fifth segment the longest, last four segments with denticulate spines in the formula 2:2:2:2, terminal claw absent. Legs robust, segments relatively short, irregular; first and second coxae of all legs with two pairs of spiny tubercles; femur and tibiae sub-equal in length, with few spines dorsally; propodus slightly curved, no marked heel, three spines on heel, two rows of sole spines; auxiliary claws three-quarters length of main claw. Cement gland dorsodistal tube on femur. Female same as male (description above) but larger in size, femora swollen in adults and spines less strong and

conspicuous. This species is known to be highly variable in diagnostic characters. A variation in the number of spines and size may be found.

Distribution.— *Achelia assimilis* was described by Haswell from Clark Island, Port Jackson in New South Wales Australia. It has been reported from Western Australia (Child, 1975) and more recently from Heron Island and Lizard Island in the southern and northern sections of the Great Barrier Reef respectively (Child, 1990). The records from Townsville and nearby areas add a new site for *A. assimilis* in North Queensland suggesting a possible continuous distribution of the species in diverse shallow-water habitats of the GBR and perhaps the East Coast of Australia. *Achelia assimilis* is mostly a southern hemisphere species. Records exist from Mozambique, Chile, Argentina and from New Zealand to Papua New Guinea to Malaysia, Indonesia and the Philippines, its most northern location in the Pacific.

Remarks.— *Achelia assimilis* was predominantly found among samples from the intertidal, being abundant in some of the samples of *Cladophora prolifera* from Rowes Bay and Cape Ferguson, Townsville. It also occurs in coral reefs but in lower numbers. Two forms of *A. assimilis* described by Stock (1954) exemplify the enormous variability of assumed species in *Achelia*. These differ not only in the general body size but also in a different structure of the palps and different body segmentation lines. The specimens collected in this study agree with the description of the 'large form' but they are smaller than individuals Stock described as the 'large form'.

Achelia nana (Loman, 1908) [Fig 2.4B-C]

Ammothea nana Loman, 1908: 60-61 pl. 1, figs. 1-13.

Achelia nana.— Stock, 1953: 300-301, fig. 14; 1954: 97; 1965: 14-15, figs. 1-3.— Child, 1983: 699.

Material examined.— Holmes Reef, 18 m, collected using SCUBA in fouled rubble with hydroids, sponges and algae mainly *Amphiroa* sp., 17 Sep 1998, 1 ♀ (coll. G. Diaz-Pulido).

Description.— Trunk length 0.64 mm, width 0.52 mm, partially segmented, third line not distinct, cuticle granulate, two antero-dorsal tubercles on each side of cephalic segment, cephalon dorsally swollen; crurigers short, all crowded, with a distal tubercle each. Ocular tubercle low, eyes not pigmented. Abdomen constricted at base, strongly inclined to the front. Proboscis inflated at midpoint in elliptical shape. Chelifores with short scape, one-segmented, dorsal distal spines, palms tiny, non-chelate buds. Palps eight-segmented, last three segments with ventral projections and long setae. Ovigers ten segmented, fifth segment the longest, simple and compound spines on last segments in the formula 1:1:1:2, tenth segment less than half the size of ninth. All legs except for the fourth pair are missing in this specimen. Long femur, dorso-distal spur tipped with spine; first tibia shorter than second, with anterior

constriction; first coxa with large distal-posterior tubercle and two smaller on anterior margin, two tubular spines on second coxa; two heel spines on propodus, claw half the length of propodus, auxiliaries about three-quarters of claw.

Distribution.— This is a common shallow water species found from the shore to 30 meters at different locations from Madagascar to Japan through the Indo-west Pacific to New Caledonia. This record of *A. nana* from an off-shore reef in the Coral Sea extends the distribution to Australian waters, however the species has not been recorded along the coast.

Remarks.— This is a species of the so-called *echinata*-group of the genus *Achelia* (Stock, 1954), characterized by the spiny legs and the shape of the proboscis that is inflated at the middle and distally tapering. As said before, representatives of *Achelia* are very similar and boundaries for the species within the genus are yet to be defined. This specimen fits the description of *A. nana* illustrated by Stock (1965), in the pattern of spines on the legs and ovigers. According to Child (1988a) *A. nana* might have been replaced by *A. variabilis* in Australian and New Zealand waters where *A. nana* had not been collected before. Both *A. nana* and *A. variabilis* overlap in many of the characters. This specimen is not identified as *A. variabilis* because of the smaller size of this adult female compared to the average measurements given by Stock, the spines on coxae two are of different shape and arrangement, and the proboscis is not as tapered as in *A. variabilis* or *A. assimilis*. This is the first record of the species from Australian waters. Bearing in mind the difficulties in the taxonomy of the genus and the lack of sufficient material to justify the validity of one species or the other, this classification of species of *Achelia* should be regarded as tentative until systematic and phylogeographical analyses of the genus are carried out.

Genus Ammothella Verrill, 1900

Slender, completely segmented trunk, crurigers well-separated, often with dorsal tubercles or spines; spines simple or tubular, proboscis ovoid and long. Chelae reduced, palps 9-segmented, long, slender, sometimes with ventral projections on distal segments; ovigers-9 segmented with few denticulate or feathered spines, without terminal claw; legs slender usually with long spines, propodus slender, curved, with auxiliary claws. Cement gland conspicuous dorsodistal tube. Contains ca. 32 species; the genus is commonly found in shallow waters of tropical regions, few species occur in temperate waters.

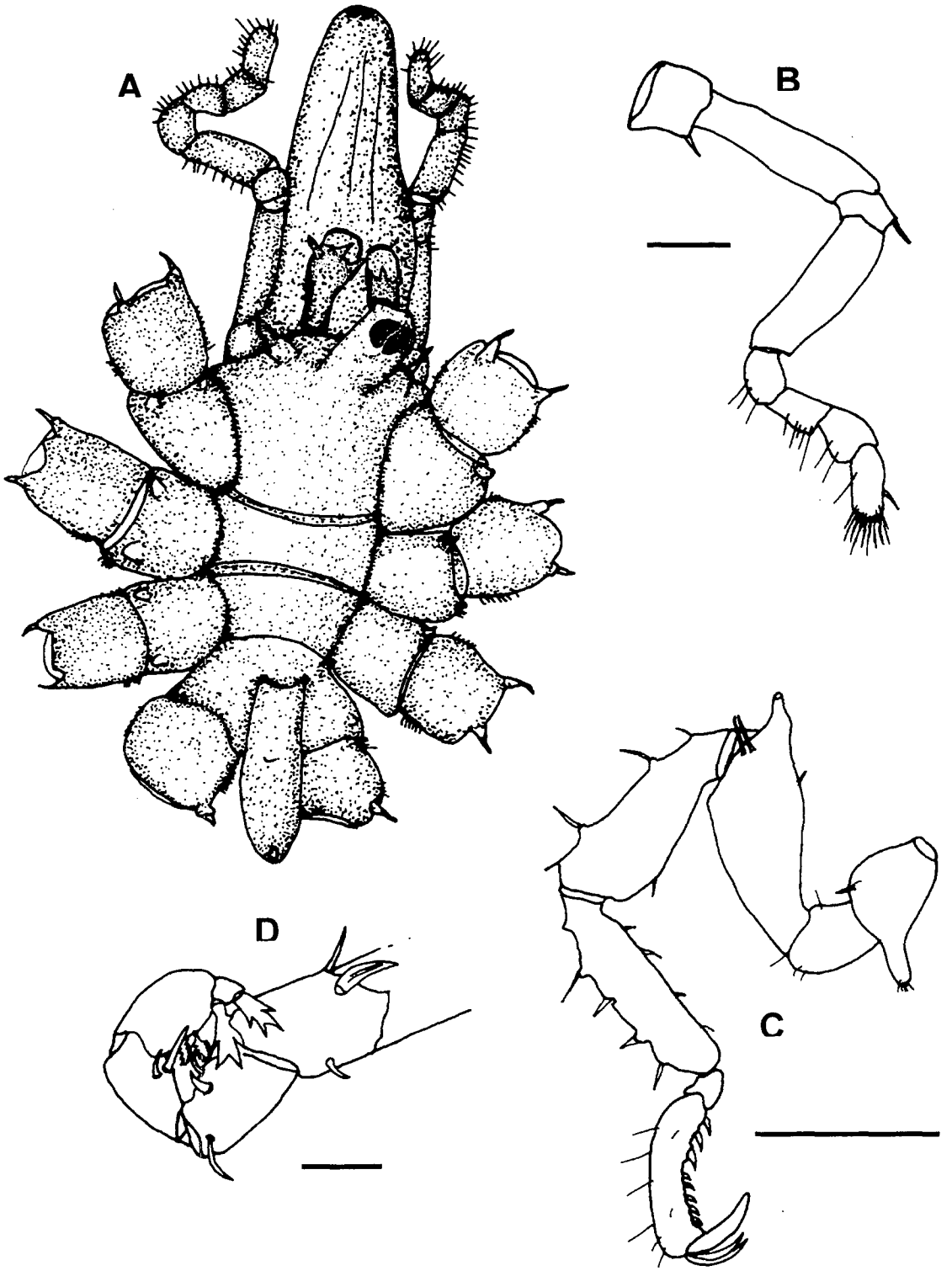

Figure 2.2. *Achelia assimilis*; ♂. A. Dorsal view. B. Palp; scale bar=0.1mm. C. Third leg; scale bar=0.5mm. D. Terminal segments of the oviger with simple and denticulate spines; scale bar=0.02mm.

Ammothella stauromata Child, 1982

Ammothella stauromata Child, 1982: 271, fig. 1; 1996: 544; Nakamura & Child 1988: 809-810; 1988: 5-7; 1990: 316; 1996: 544; Müller 1990: 66; Stock 1994: 29.

Material examined.— Green Island, on reef flat, shallow seagrass beds and algae, 23 Oct 1999, 1 juv.— Turtle Bay, 4 May 2000, 1 juv.

Description.— Juvenile specimens, damaged, lacking ovigers; coincide with description of *A. stauromata* and chelifores illustrated by Child (1982b). Presence of pointed mid dorsal tubercles, especially on distal margin of the cephalic segment.

Distribution.— Three specimens of *A. stauromata* are known from Lizard Island (Child, 1990). This is the second record of the species for Australia from a nearby location in the GBR. This species has a wide distribution through the western Pacific islands, it has been reported from Papua New Guinea to the Marshall Islands, Philippines, Society Islands, Fiji Islands and in the Indian Ocean was collected in Kenya.

Remarks.— A related species found in Australia, *Ammothella theitidis* Clark, 1963, resembles *A. stauromata* in the presence of dorsal tubercles, but the latter species has a medium dorsal tubercle on the cephalic segment; the chelifores are more slender and longer, and the proboscis more globular-shaped in *A. theitidis*.

Ammothella **sp.** 'slender form'[Fig. 2.3]

Material examined.—Turtle Bay, intertidal in *Cladophora prolifera*, 27 Mar 1997 (coll. J. Otto); 33♂, 28♀, 200 juv; 5 Oct 1998, 2♀; 14 May 1999, 3♀, 1 juv., 21 adults (not fixed).— Orpheus Island, Pioneer Bay, shoreline amongst the red alga *Galaxaura rugosa* (Ellis & Solander) Lamouroux, 24 Nov 1998, 2♀, 2 juv. Pandora Reef, in washings of rubble with macroalgae *Dictyota* sp and *Laurencia* sp. 5 Jul 1999, 1♀, 1 juv ?.

Description.— Body ornamented with many conspicuous tubular and simple spines. Trunk 0.62 mm in length, 0.5 mm wide; segments inflated, anterior corners of trunk with short articulated spines; crurigers separated by half their diameters or less, three tubular spines distally on first pair, two or one on other pairs. Ocular tubercle six and half times longer than its distal diameter, rounded apex, eyes large, apical, dark-pigmented. Proboscis typical, with proximal and distal constrictions. Abdomen long, bent backwards at midpoint, armed with two short spines below midpoint, four long tubular spines at bend and four spines on apical half. Scape of chelifores two-segmented, second segment longer, first with one dorsomedian, two laterodistal and three dorsodistal tubular spines; second segment with two dorsal hollow spines and long pointed spines distally. Palm a rounded knob without fingers. Palps nine-segmented, with long ventrodistal setae on the last five segments. Ovigers ten-segmented, seventh segment with two large setae carried laterally; denticulate spines in the formula

1:2:2:2. Legs slender, tibiae longest segments, femur subequal, first coxae with three tubular dorsal spines, second coxae with three dorsomedian tubular spines, femur and tibiae with three to five tubular spines dorsally and few long pointed spines. Cement gland a long dorso-distal tube, as long as diameter of femur. Tarsus very short; propodus curved, with three long dorsal setae, three heel spines, six sole spines; main claw shorter than half the propodus, auxiliaries about three-quarters length of the main claw. Females bear same characters but are larger in body size and have fewer spines on legs.

Distribution.— This particular form of an undescribed species of *Ammothella* is known from the Townsville area and inshore reefs of the Central Section of the GBR.

Remarks.— This species fits in the *appendiculata-rugulosa* group of Child (1990), a complex of small-sized species distinguished by the presence of long dorsal tubular spines on crurigers and first coxae, and very long spiny abdomen and tall ocular tubercle; they are commonly found in tropical shallow waters. These specimens are slightly different from the type material collected in the intertidal *Cladophora* of Rowes Bay being described elsewhere (Lee A. C. and Arango, submitted) and mentioned below as *Ammothella* sp. 'robust form'. I give a brief account of the material examined and the main differences between the two morphs. There is not sufficient evidence to segregate them in two different species and for now differences might be taken as characteristics of separate populations.

***Ammothella* n. sp. 'robust form'**

Material examined.— Rowes Bay, in intertidal *C. prolifera*, 3 Nov 1998, 3♀, 5♂, 1♂ ovg., 1juv; 17 Apr 1999, 1♂, 4♀, 1 juv.; 1 Jun 2000 6♂, 4♀, 5 juv.— Turtle Bay, in *C. prolifera*, 12 Jul 1999 1♂, 1♀, 3 juv.; 8 Sep 1999, 1♂, 1; 4 May 2000, 2♂, 8♀, 4 juv.

Other material.— Rowes Bay , same locality, 17 Sep 1998, 3♂, 3♀ (coll. A. Lee).

Remarks.— Most of the characters as in the above description except these specimens are somewhat larger, more robust in appearance, with wider trunk across the second pair of crurigers (trunk length 0.74 mm; width 0.6 mm). Spines on legs longer and more numerous in the 'robust' form when comparing individuals of the same sex. One or two fewer spines on chelifores and legs of the 'slender form' but the proportions of the segments are the same in the two morphs also for the ovigers (complete description in Lee and Arango, submitted).

Genus *Nymphopsis* Haswell, 1884

Trunk unsegmented, with conspicuous dorsomedian tubercles. Scape two-segmented, trumpet-shaped, chelae reduced to knobs. Palps nine-segmented. Ovigers ten-segmented, no denticulate spines or terminal claw but last segments highly setose. Legs robust, armed with spiny tubercles and arrangements of compound spines, propodus curved with auxiliary claws. Cement gland a short dorsodistal tube.

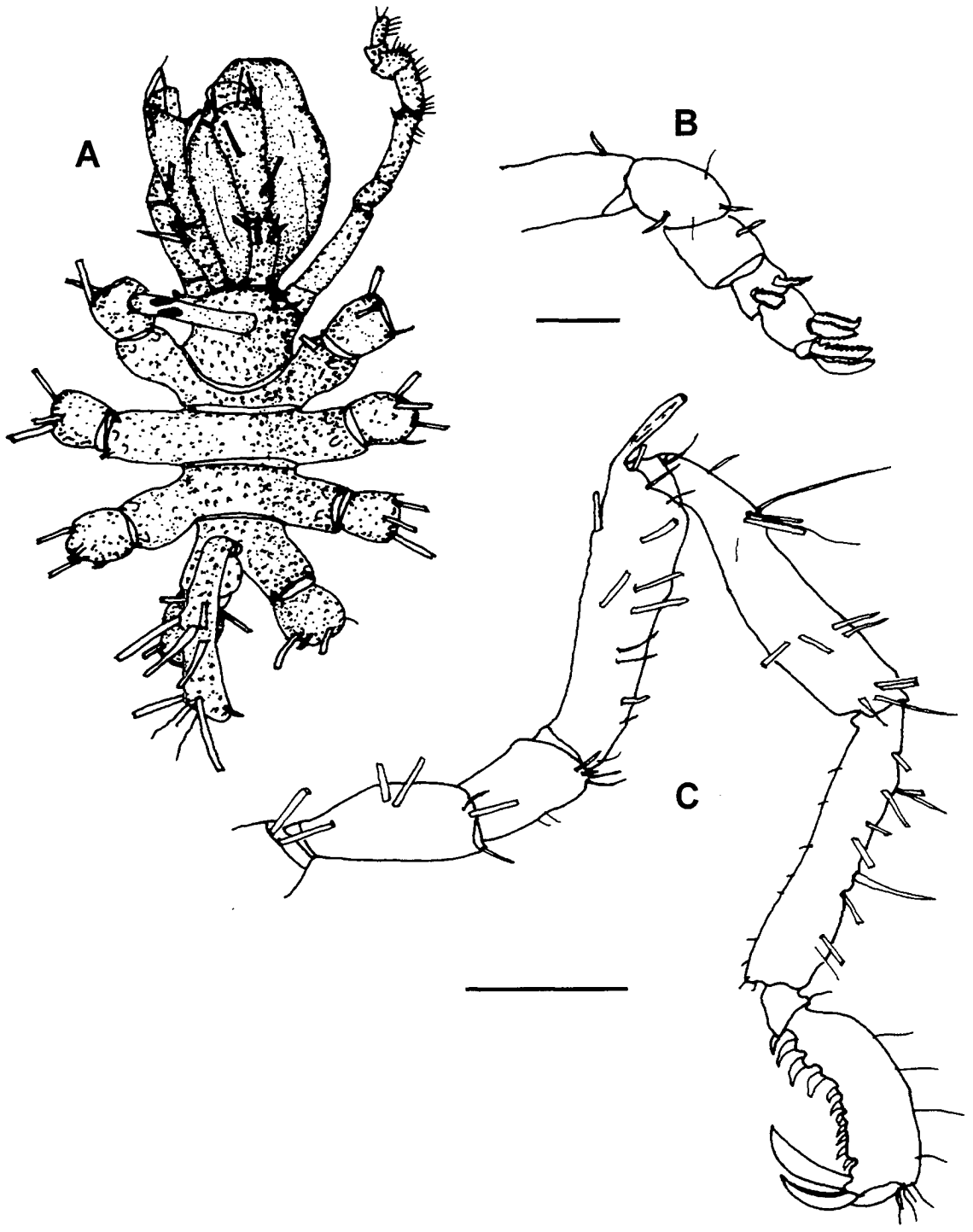

Figure 2.3. *Ammothella* sp. 'slender form' ♂. A. Dorsal view of body and palp. B. Terminal segments of the oviger; scale bar=0.02mm. C. Third leg starting in the second coxa; scale bar=0.3mm.

Nymphopsis acinacispinata Williams, 1933 [Fig 2.4A]

Nymphopsis acinacispinatus Williams, 1933: 173-180, figs. 1-5.

Nymphopsis acinacispinatus acinacispinatus.— Clark, 1963: 5 [list].

Nymphopsis acinacispinata.— Stock, 1992: 82.

Material examined.— Turtle Bay, intertidal amongst the algae *Cheilosporum spectabile* (Harvey ex Grunov) and *C. prolifera*, 12 Jul 1999, 5♂, 2♀, 4 juv.; 14 May 1999, 1 juv.

Description.— Trunk length 2.35 mm, width 2.30 mm, segmentation not evident, massively ornamented dorsally, with three tubercles taller than ocular tubercle bearing comb rows of spines and long pointed apical spine; second and third pair of crurigers length three times their width, separated by less than ½ their own diameter, all crurigers with low, distal, spiny, tubercles. Ocular tubercle erect, pointed tip. Abdomen very long, bent downwards from base, spiny tubercles in pairs from base to apex, largest spines on most basal and most apical pairs. Chelifore scape two-segmented, second segment longer, two compound spines and 3-4 simple spines distally; chelae tiny knobs inside scape. Palps nine-segmented, second segment longest, third to ninth segments with array of conspicuous spines and setae. Ovigers ten-segmented with simple long spines on terminal segments. Legs robust, very ornamented, mostly second and third coxae, with five and three anterior compound spines respectively and two or three on posterior margin; propodus curved and stout, with dorsal row of five to six simple spines; main claw almost as long as propodus, auxiliaries three-quarters length of main claw. Cement gland short duct located on distal swelling on femur. Males distinguished by long ventral spurs on second, third and fourth coxae bearing single genital pore each.

Distribution.— This species had only been reported once for Australia when first described from Port Curtis in Queensland (Williams, 1941). This record extends the distribution of the species to tropical North Queensland. The species is also known from Vizhingom Bay in India (Kurian, 1953 in Stock, 1992)..

Remarks.—. The three tall spiny tubercles carried dorsally in this species are a characteristic shared with *N. bathursti* Williams 1940, first considered a subspecies of *N acinacispinata* (see Stock, 1992), and the only Australian *Nymphopsis* related to this species. In this study *N. acinacispinata* was mostly collected among the alga *C. spectabile*, that has a spinose pattern and displays a similar pink colouration to that of the sea spiders, which allows a good camouflage for *N. acinacispinata* on the alga.

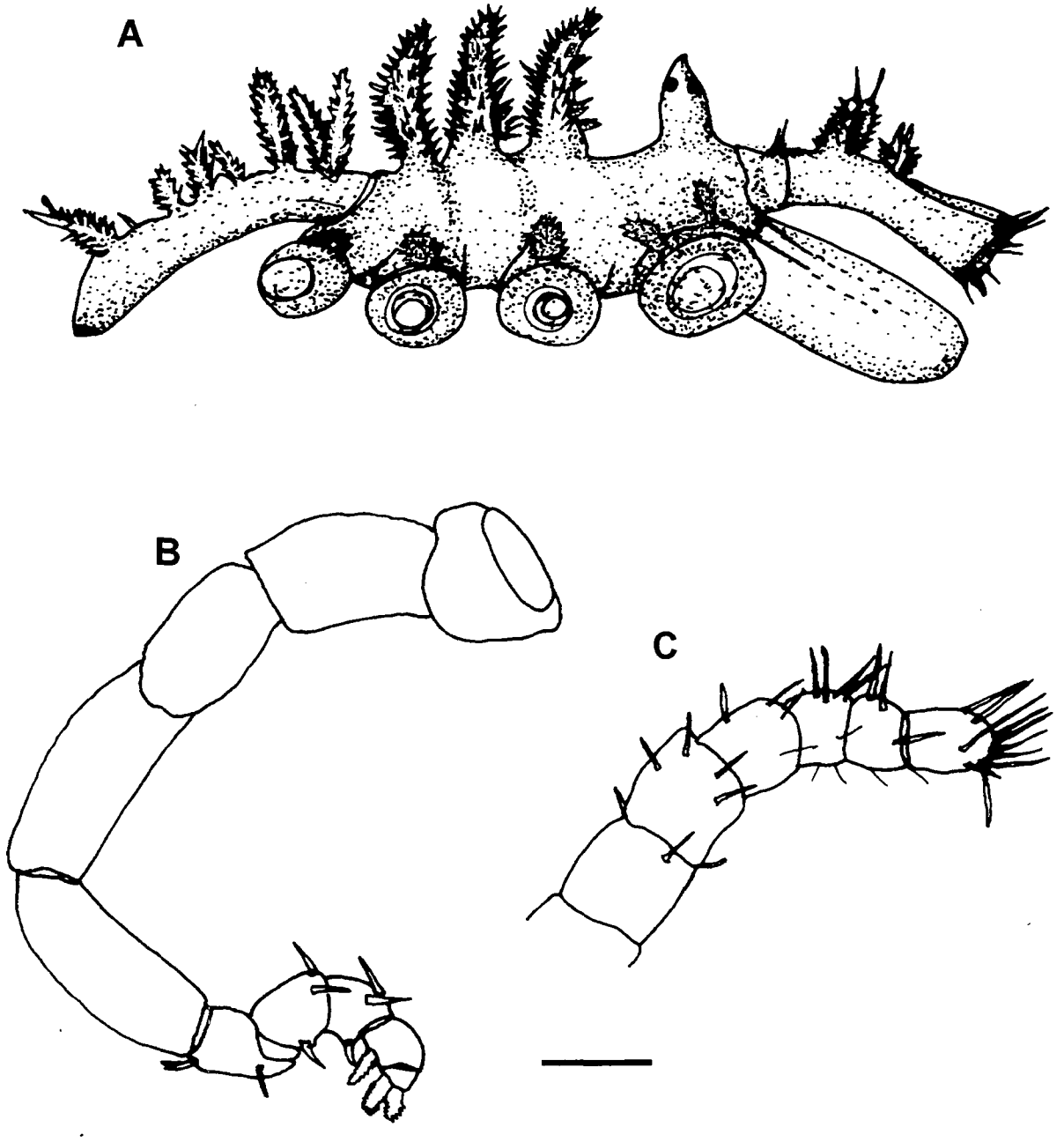

Figure 2.4. *Nymphopsis acinacispinata*, ♂. A. lateral view.— *Achelia nana*, ♀. B. oviger. C. terminal segments of the palp; B-C scale bar=0.02mm.

Genus *Tanystylum* Miers, 1877

Trunk circular, crurigers crowded. Ocular tubercle very short. Proboscis ovoid, barrel-shaped, tapering or down-curved. Chelifores one-segmented, very short, without chelae. Palps four to seven-segmented. Ovigers ten-segmented, last segments with simple spines, no terminal claw. Legs with irregular margins, propodus large, well curved, with large heel spines and large auxiliary claws. Cement gland a small dorsodistal duct.

Tanystylum haswelli Child, 1990 [Fig. 2.5]

Tanystylum haswelli Child, 1990: 317-319, fig. 2.— Stock, 1994: 37-38, fig. 12.

Material examined.—Turtle Bay, in *Cladophora prolifera*, 27 Mar 1997, 2♀, 1♂. Holmes Reef, 18 m, collected using SCUBA amongst the coralline alga *Amphiroa* sp., sponges and hydroids, 18 Sep 1998, 1♂ (coll. G. Diaz-Pulido).

Description.— Trunk length 0.62 mm, width 0.54 mm, not segmented, with granulate surface; body circular-shaped, crurigers very wide and crowded, with very small, rounded distal tubercle. Ocular tubercle pointing forward, tip rounded, eyes not pigmented. Abdomen placed horizontally, apex curved upwards. Proboscis slightly tapering distally. Chelifores small buds, one-segmented. Palps four-segmented, second segment the longest. Ovigers ten-segmented, first and second segments wider. Legs short; tibiae subequal in length, femur longer than tibiae; long distal spur with spines on first coxa, two or three smaller spurs along distal margin; second coxa with short spines and setae; femur with single row of spines distally; both tibiae with dorsal nodes, nodes with short spines; propodus robust, curved without heel, with three heel spines, double row of sole spines; claw half length of propodus; auxiliaries more than half length main claw.

Distribution.— This is the second Australian record of the species originally described from a male collected in Lizard Island, northern GBR (Child, 1990). The female and juveniles were described from material collected around Papua New Guinea (Stock, 1994).

Remarks.— *Tanystylum haswelli* has some similarities with *T. hooperi* Clark, 1977 from New South Wales in the general appearance of proboscis, legs and abdomen, but the males of *T. hooperi* have an apophysis on the seventh segment of the ovigers. It is also similar to *T. bredini* Child, 1970 from the Indo-west Pacific sharing the lack of apophysis on ovigers, four-segmented palps and shape of ocular tubercle but differs in a more tapering proboscis, two anterior and posterior tubercles on crurigers and shorter terminal palp segment *in T. bredini*.

Tanystylum rehderi Child, 1970[Fig. 2.6]

Tanystylum rehderi Child, 1970: 305-306, fig. 5; 1983: 705; 1988: 53-54.

Material examined.— Turtle Bay, intertidal in C. prolifera, 12 Jul 1999, 1 juv.; 5 Oct 1998, 1♀.—Pandora Reef, in rubble with turfs and the macroalgae Dictyota sp and Laurencia sp., 4-6 m depth, in rubble, 28 Oct 1999, 2 juv.; 19 Apr 2000, 2♂.

Description.— Trunk length 0.86 mm, width 0.6 mm, not segmented; crurigers crowded giving discoid shape to body, single anterodistal tubercle on all crurigers. Ocular tubercle short, stout, with tiny pointing tip, eyes not well pigmented. Abdomen relatively long for genus, pointing upwards, with few setae near tip. Proboscis styliform, tapering and

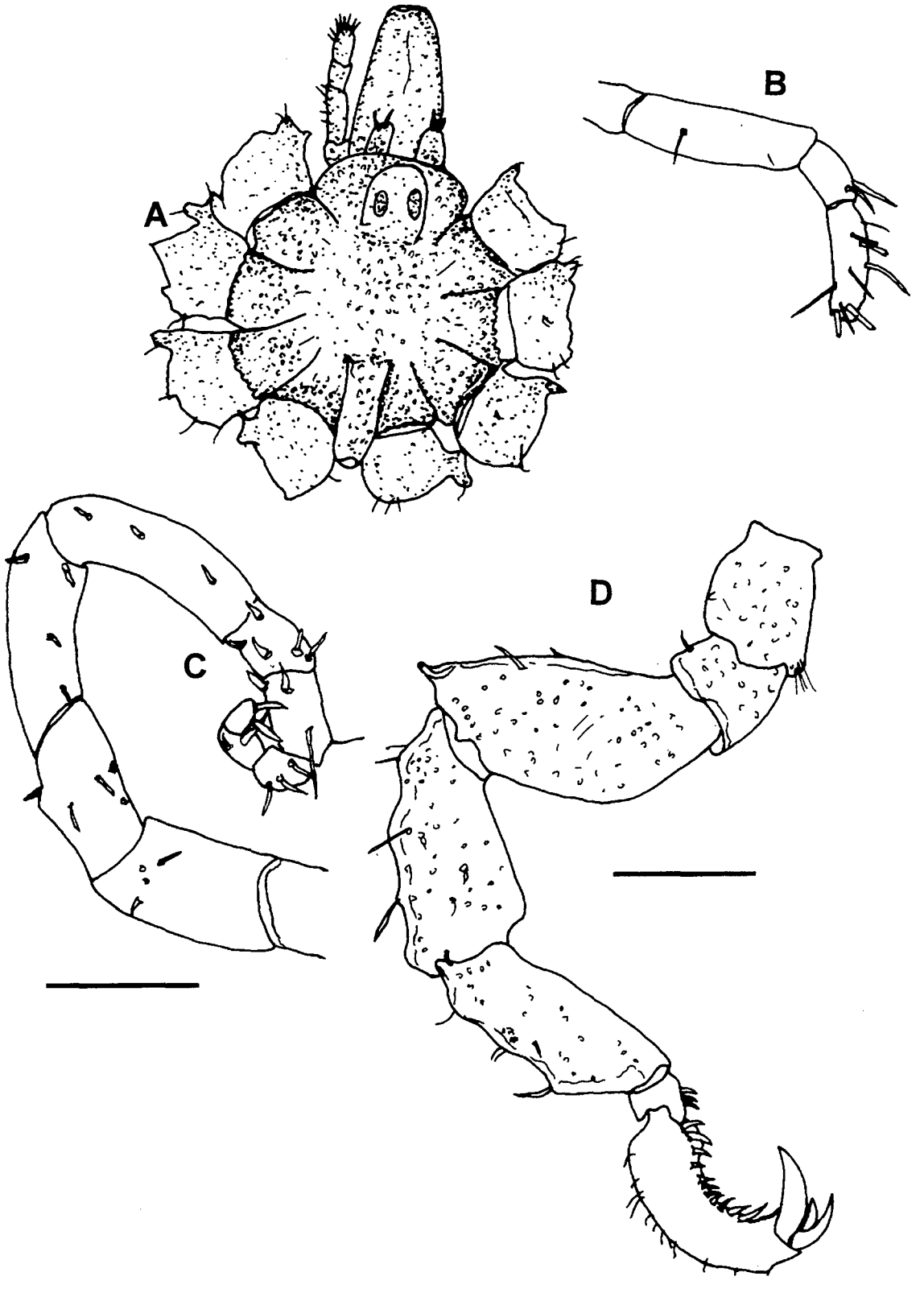

Figure 2.5. *Tanystylum haswelli*, ♂. A. Dorsal view. B. Palp. C. Oviger; scale bar=0.15mm. D. Second leg; scale bar=0.3mm.

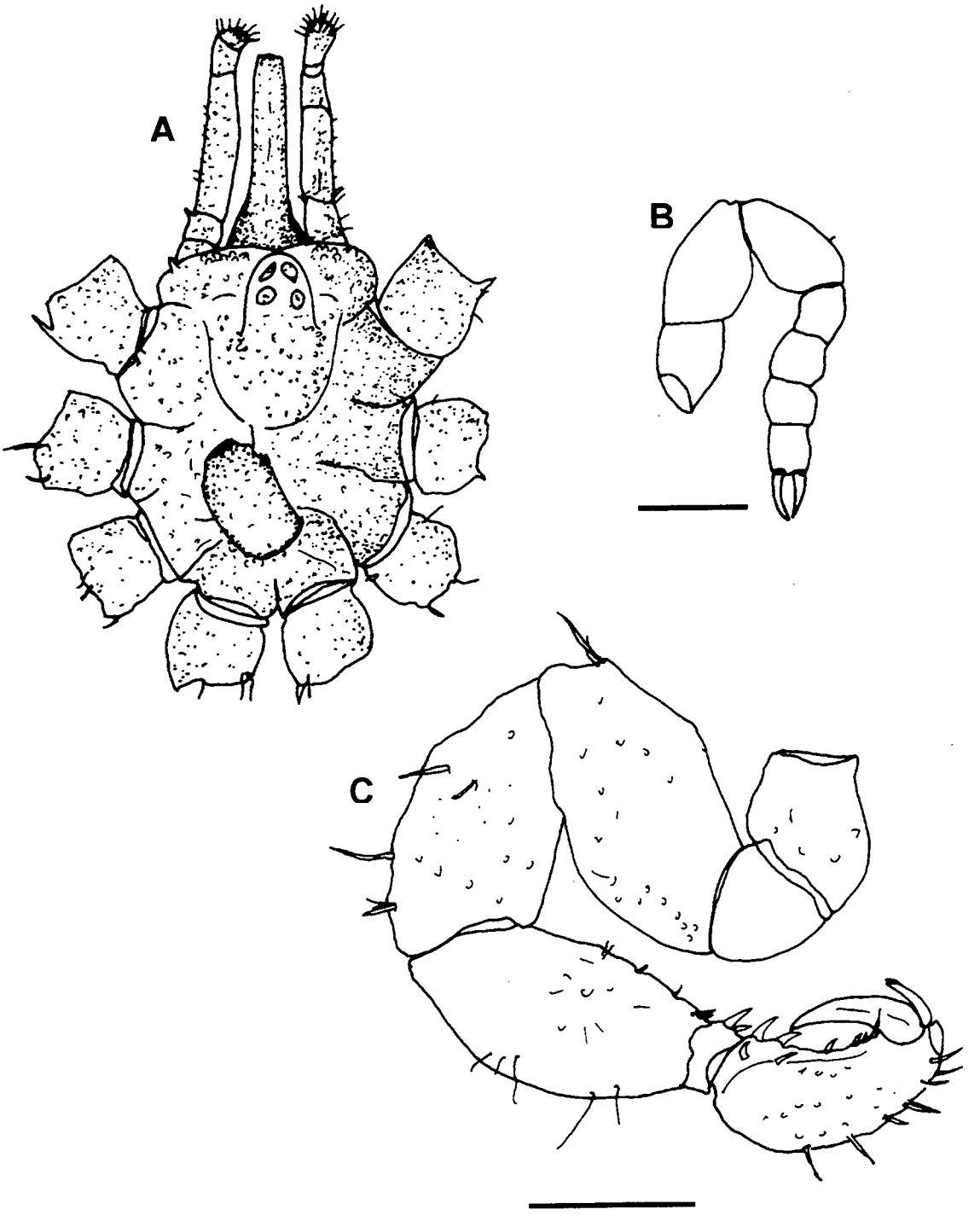

Figure 2.6. *Tanystylum rehderi*, ♀. A. Dorsal view of body and palps. B. Oviger; scale bar=0.1mm. C. Third leg; scale bar=0.3mm.

downcurved. Chelifores tiny knobs, on anterior margin of cephalic segment. Palps of six segments, fourth longest, last segment small, setose, placed right in front of oral surface. Ovigers ten-segmented, all segments short, inflated, last segment with two simple spines. Legs short, robust; femur and both tibiae subequal, single spine distally on first coxa and three or four on femur, in female all femora swollen. Propodus curved, without heel, with two heel spines and six sole spines. Main claw three-quarters of propodus, with small auxiliaries.

Distribution.— This is the first record of this species for Australia. It was described from shallow waters of the Society Islands (Child, 1970) and subsequently reported from the Palau Islands (Child, 1983), the type locality on Mooréa (Müller, 1989) and the Aldabra Atoll in the Indian Ocean (Child, 1988a). It is a rather shallow water species, the deepest record being from 11 meters depth.

Remarks.— This species is one of the few *Tanystylum* characterised by the styliform shape of the proboscis. The height and the shape of the ocular tubercle and the basal swelling of the abdomen are the main features that differentiate this species from closely related *Tanystylum* species.

Family Austrodecidae Stock, 1954

Genus *Austrodecus* Hodgson, 1907

Small forms, leg span less than 10 mm. Trunk segmented, with or without dorsomedian tubercles; crurigers very short. Ocular tubercle tall, pointing anteriorly. Proboscis always a downcurved pipette-like structure with close annulations all along except for the base. Chelifores lacking. Palps four- or six-segmented. Ovigers very reduced or tiny, nonfunctional, one- to six-segmented, without terminal claw. Legs slender, propodus long, no heel, claw usually short, with or without auxiliaries. Cement gland a ventral cone or tube.

***Austrodecus* n. sp.** [Fig. 2.7]

Material examined.— Pandora Reef, 3-6 m depth among rubble and algae, 15 Jul 1999. 2 juveniles, 19 Apr 2000, 1♂. Other material examined.— Townsville Port, piling scrapings, 3 m, ?/2001, 2♀ (coll. Staff JCU).

Description.— Trunk length 0.9 mm, width 0.42 mm, trunk fully segmented, lines of segmentation distinct; body with granulate surface; crurigers distanced by less than half their diameter, each segment with dorsomedian slender tubercle half the length of the ocular tubercle. Ocular tubercle very tall, pointing obliquely towards the front, with rounded tip housing dark pigmented eyes. Abdomen horizontal, somewhat curved downwards, with dorsal row of small granules. Proboscis long, thin, strongly downcurved, joined to basal stalk, typical pipette-like, with about 25 annulations. Palps slightly longer than proboscis, with six

segments, all granulose, segmentation line between second and third almost indistinct, third segment the longest, with two dorsal tubercles, one at midpoint another distally, few ventral setae on fourth segment, gland openings visible on second segment. Ovigers not found in any of the specimens. Legs not very long; femur longest segment, tibiae subequal; two distal, slender tubular spines forming a V-shape dorsally on first coxa, third coxa with small dorsal tubular spine, femur with longer spine of the same type and two long simple spines, distally and ventrally; both tibiae with long distal spine; tarsus short, propodus curved, with no heel, feeble sole spines; main claw long, robust. Auxiliaries lacking.

Distribution.— Only known from the inshore Pandora Reef and the Townsville Port.

Remarks.—This previously unknown species fits into the *gordonae*-section (Stock, 1957), appearing related to *A. stocki* Child, 1988 from the Indo-Pacific, *A. palauense* Child, 1983 from the Palau Islands and *A. staplesi* Stock found in New South Wales (Stock, 1990). They all have a similar armature in the last palpal segments, mid to low median dorsal tubercles and are found in shallow tropical waters. This species differs from *A. stocki* in the lack of distal femoral spur (instead it has a mid-dorsal long tubercle not present in any other species) and the shape of the trunk is not as compact as *A. staplesi* or elongate as *A. palauense.* The dorsal spurs on first coxae are longer than in any of the other species. Remarkably, males of this species do not show signs of ovigers, absence that has been attributed only to those *Austrodecus* species placed in the subgenus *Tubidecus* (Stock, 1991)

Family Colossendeidae Hoek, 1881

Genus *Rhopalorhynchus* Wood-Mason, 1873

Moderately large species. Trunk slender, fully segmented; crurigers widely spaced. Abdomen reduced, semi-ventral. Proboscis spindle-shaped, with or without a single dorsal tooth. Palps ten-segmented. Ovigers ten-segmented, last segments strongly coiled, with multiple rows of denticulate, spatulate spines; terminal claw forming a subchelate structure. Legs long, slender, bare.

Rhopalorhynchus tenuissimum (Haswell, 1884) [Fig. 2.8]

Colossendeis tenuissima Haswell, 1884: 1029-1030, pl. 56, 5-8.

Rhopalorhynchus tenuissimus.— Flynn, 1919: 71-72, pl. 53, 1-3.

Rhopalorhynchus tenuissimum.— Stock, 1958: 114 (table), 117 (key).

Material examined.— Geoffrey Bay, 21 Jul 1982, 1 ♂ (coll. J. Collins).

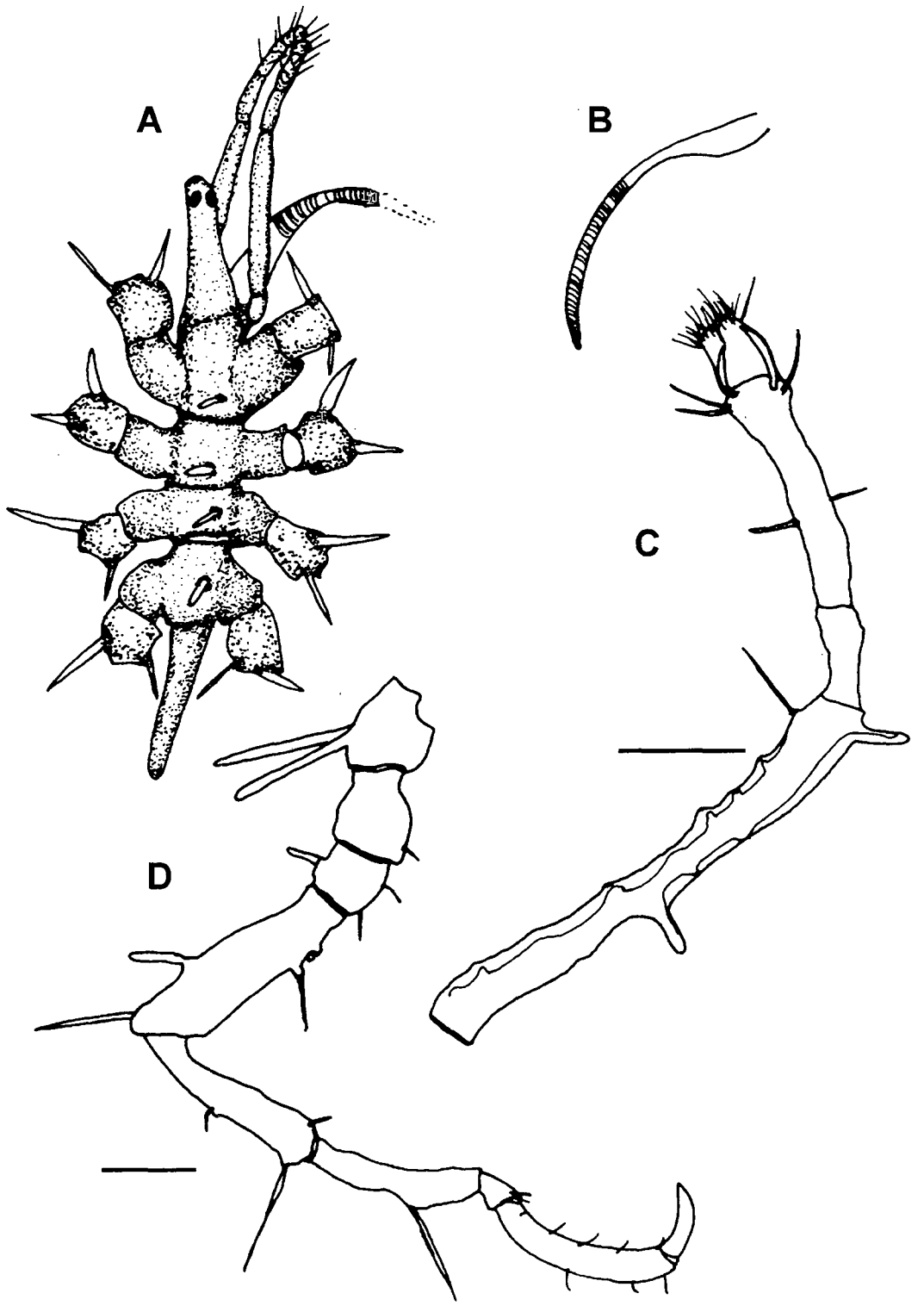

Figure 2.7. *Austrodecus* sp. ♂. A. Dorsal view. B. Lateral view of proboscis. C. Palp; scale bar=0.12mm. D. Third leg; scale bar=0.2mm.

Description.—Trunk length 2.10 mm, width 0.52 mm, fully segmented, marked segmentation lines, very elongate and slender form, completely glabrous, smooth cuticle, second and third segments longer than first and fourth; crurigers short, constricted at base, separated by 3 to 4 times their diameter except third and fourth pairs being much closer. Ocular tubercle on mid-cephalon, swollen on top, with pointed conical apex, small eyes, not dark-pigmented. Abdomen very reduced, horizontal, anus seen ventrally. Proboscis very long, anterior half long, thin peduncle, distal portion inflated, dorsal tooth near the anterior side of the inflated portion. Base of cephalic appendages crowded ventrally. Palps ten-segmented, third segment longest, few setae on sixth to tenth segments, all similarly short. Ovigers of ten segments, fifth and third segments very long and slender, wider distally; two rows of large pointed spines on terminal segments (strigilis). Legs very long and slender, all segments glabrous except for short setae in coxae, femur and first tibia subequal, with distal swelling; tarsus, propodus and claw make 100% of the length of second tibia, propodus straight, unarmed, claw about two-thirds the length of propodus.

Distribution.— *Rhopalorhynchus tenuissimum* was described by Haswell from a single specimen collected at Port Denison, Western Australia. There are unreported records at the Museum of Victoria of the species found at Lady Julia Percy Island, Victoria.

Remarks.— This specimen fits well the description by Haswell (1884). The several species of this genus with a toothed proboscis can only be differentiated by subtle variations of a few characters. *Rhopalorhynchus tenuissimum* was segregated from closely related *R. kroeyeri* by the more anterior position of the dorsal tooth on the proboscis according to Stock's key (Stock, 1958).

Family Nymphonidae Wilson, 1878

Genus *Nymphon* Fabricius, 1794

All sizes, cosmopolitan distribution. Trunk fully segmented; crurigers crowded to very separated, rarely adorned, usually glabrous. Ocular tubercle posteriorly on cephalon. Chelifore scape one-segmented, chelae fully functional, most with long, denticulate fingers, chelae positioned to oppose each other. Palps five-segmented, longer than proboscis. Ovigers with strong 'strigilis', terminal claw with teeth. Legs various sizes and different proportions of the segments, propodus with large heel spines, some species without, claw from very reduced to very long, auxiliaries of various sizes or absent. Cement glands ventral, multiple pores or cones.

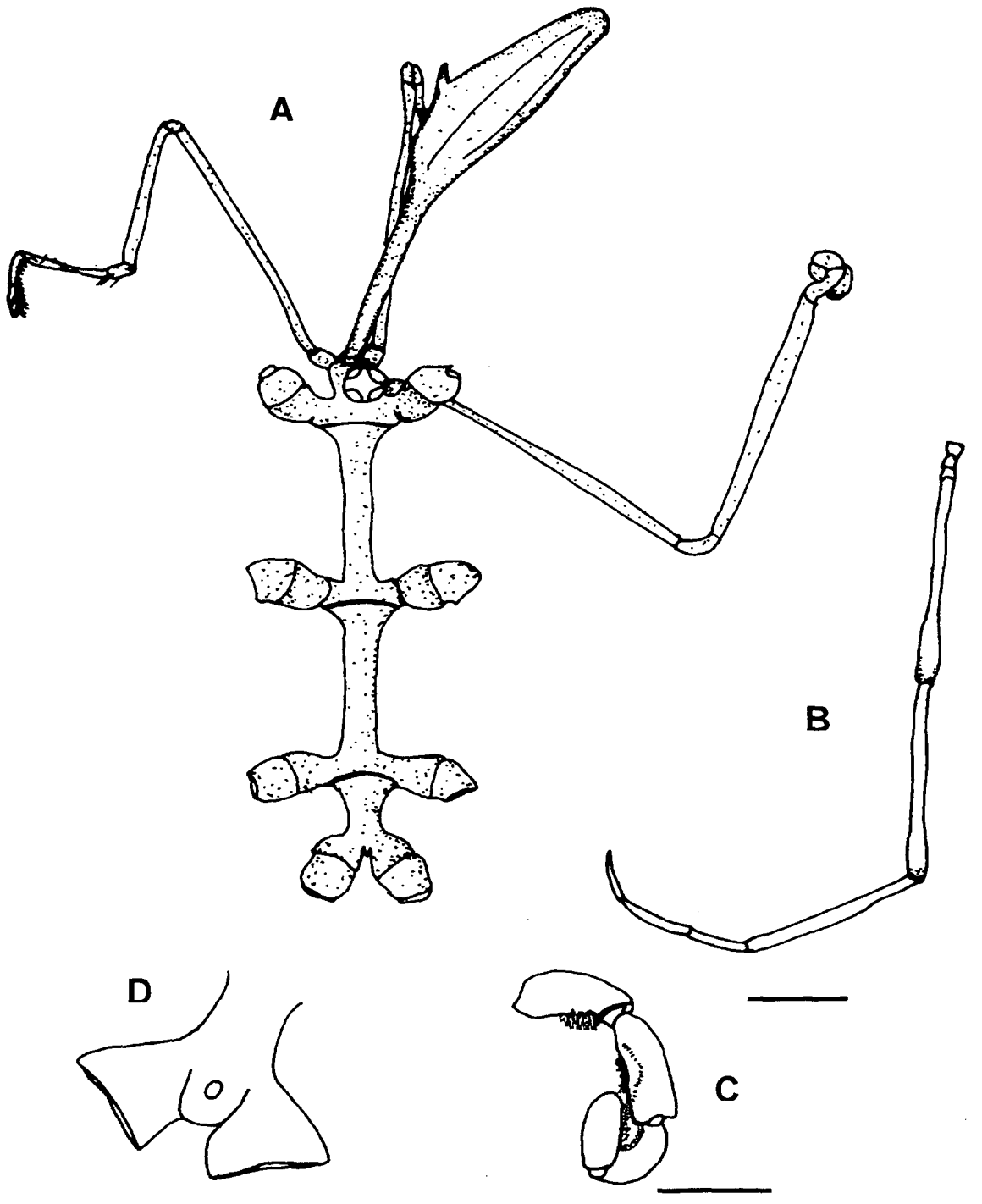

Figure 2.8. *Rhopalorhynchus tenuissimum*, ♀. A. Dorsal view of trunk and proboscis, right palp underneath; with oviger. B. Third leg; scale bar=2.5mm. C. Terminal segments of the oviger; scale bar=0.2mm. D. Ventral view of the fourth pair of crurigers with abdomen and anus in the middle (sketch by hand).

Nymphon micronesicum Child, 1982 [Fig. 2.9]

Nymphon micronesicum Child, 1982: 277-280, fig.3; 1988: 68 [key].

Material examined.—Pandora Reef, 5 m depth, on fouled rubble, 28 Oct 1999, 1 juv.; 6 m depth, 19 Apr 2000, 22♂, 24♀, 11 juv.

Description.— Trunk length 1.68 mm, width 0.6 mm, fully segmented, well defined annulated lines; body smooth, elongated; crurigers separated by almost their own diameter, all crurigers the same size, glabrous; neck constricted. Ocular tubercle of medium size, with two tiny tips on each side distally; abdomen erect and long; proboscis cylindrical, straight but slightly tapering distally. Scape of chelifores one-segmented, glabrous except for longitudinal row of setae, palm massive, globular in males, elongated in females, fingers short, stout, only a small gap at base when closed, both fingers denticulate with 10-12 bifurcate teeth each, fingers placed at right angle with the palm. Last palpal segment longer than third, subequal to second. . Ovigers ten-segmented, fifth segment the longest (single mass of eggs attached), denticulate spines in formula 12:12:11:10; ninth segment with dimorphic spines; terminal claw with no denticulations. Legs long, slender, second tibia with rows of fine setae; propodus straight, elongate without heel, with few spines, claw small, auxiliaries longer than main claw by one-fifth of its length. Cement glands not found.

Distribution.— This species was described from Micronesia but it is part of a complex of very similar species, the *aequidigitatum*-group (Child, 1988a) with a wide distribution, mainly in shallow waters of the Indo-Pacific and the Caribbean. *Nymphon micronesicum* has not been reported from Australia before and appears as a coral reef species so far.

Remarks.— This species agrees with all the characters that identify the *aequidigitatum*-group (Child, 1988a) and it might be difficult to differentiate the species within this group. *A. micronesicum* can be separated by the combination of the terminal palp segment longer than other segments, oviger claw smooth, rugosities on main and auxiliary claws and chelae fingers with bifurcate teeth. Geographically, its closest relative would be *N. draconis* Child, 1990 from Lizard Island, characterized by shorter trunk and neck and conspicuous femoral cement glands.

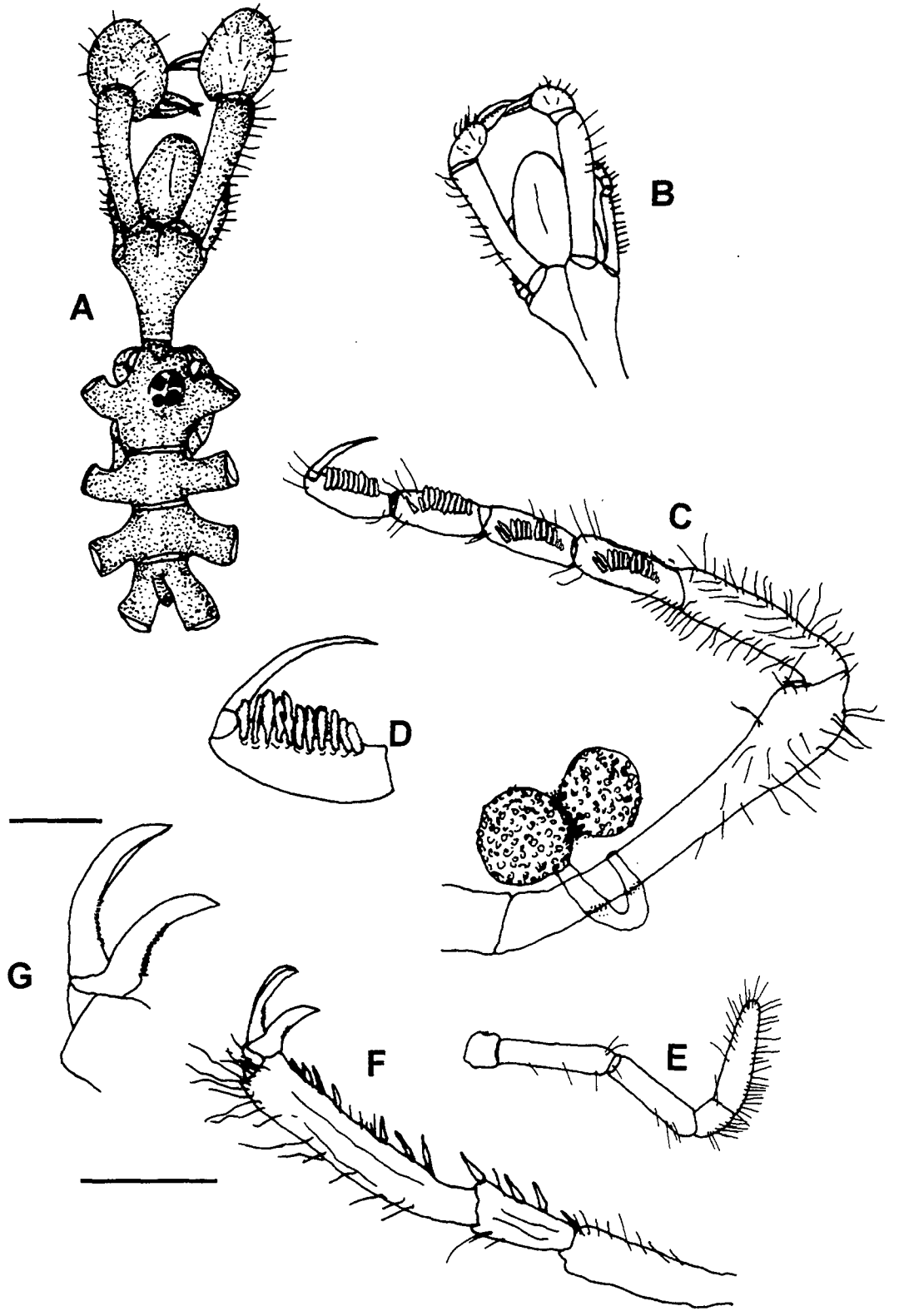

Figure 2.9. *Nymphon micronesicum*, ♂. A. Dorsal view. B. Neck and chelifores of female. C. Oviger. D. Last segment of the oviger with denticulate spines and terminal claw. E. Palp (reduced 10x). F. Tarsus and propodus of third leg; scale bar=0.25mm G. Detail of main claw and auxiliary claws; scale bar=0.1mm.

Nymphon molleri Clark, 1963 [Fig.2.10]

N*ymphon molleri* Clark, 1963: 10-12, figs. 6a-h.

Material examined.— Goold Island, reef slope, 7 m, found in rubble, 18 Apr 2000, 1♂ with eggs. Pandora Reef, in rubble fouled with algae and hydroids, 5 m depth, 15 Jul 1999, 1♀. — Rib Reef, 19 Apr 2000, 6♂, 8♀, 7 juv. Rib Reef, slope, 8 m, among turf algae, 16 Apr 2000, 1♂.

Description.—3.5 mm trunk length, 0.9 mm width, fully segmented, very long and narrow neck, broader anteriorly in joint with chelifores. Body smooth, very slender; crurigers separated by almost three times their diameter, first pair of crurigers largest, others subequal, all glabrous. Ocular tubercle tall, broad with two pointing tips; eyes at midpoint, well pigmented. Abdomen long, obliquely positioned pointing backwards. Proboscis cylindrical, short, glabrous. Scape one-segmented, as long as proboscis, few dorsal setae, palm half the length of scape, long setae laterally and near the base of the fingers; fingers longer than palm, slender, movable finger with 14 fine teeth, two innermost smaller, 11 teeth on immovable finger. Palps five-segmented, longer than proboscis; second segment the longest, fifth segment very setose at the tip, fourth segment joins third pointing upwards. Ovigers with ten segments, fifth the longest, a crown of distal short setae, sixth segment with a row of dorsal short spinules, spines denticulate, well developed, most distal spine the longest in all four segments, oviger spines in the formula: 14:11:9:10 in females, 7:7:4:4 in males. Legs very long, slender, glabrous; second tibia the longest followed by first tibia and femur, no tubercles or spines, few small setae on legs, tarsus half the propodal length; propodus long, slender, without heel or heel spines, sole spines in a single row with distal ones longer; main claw small and auxiliaries almost as long as the main claw. Females are larger and more robust.

Distribution.— An Australian species described from material collected in Port Jackson, not reported from anywhere else so far. This is the most northern record and expands the habitat from temperate to tropical waters.

Remarks.—These specimens fit well with the description and illustrations by Clark (1963) except for a difference in the formula of the ovigers of females and a longer neck in Clark's illustration. The species seems abundant at Pandora Reef where several specimens were seen by naked eye crawling over coral rubble and algae.

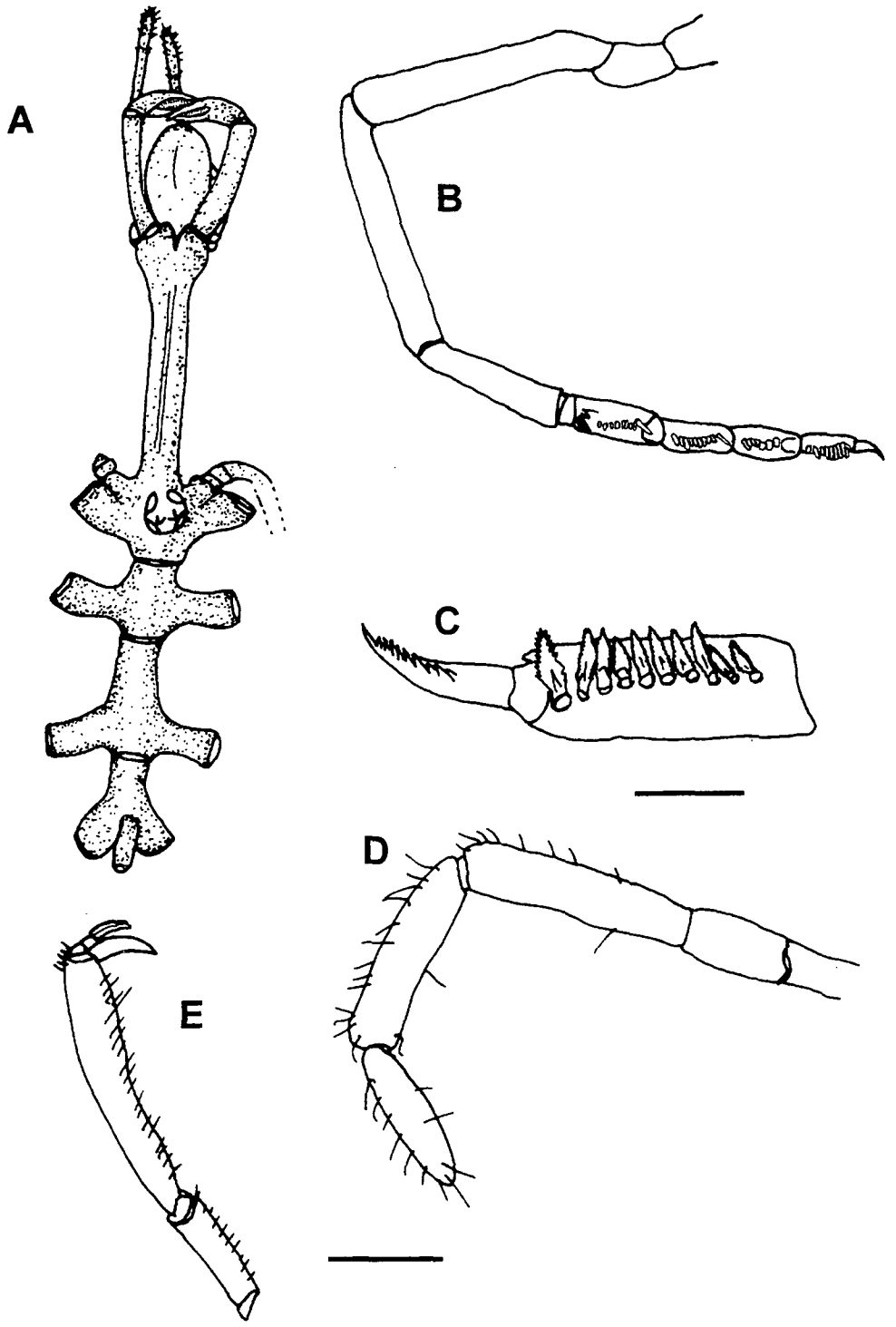

Figure 2.10. *Nymphon molleri* ♂. A. Dorsal view. B. Oviger. C. Last segment of the oviger of a ♀ with denticulate spines and terminal claw; scale bar=0.1mm. D. Palp. E. Tarsus and propodus; scale bar=0.2mm.

Family Uncertain (?)

Genus *Pallenopsis* Wilson, 1881

Species moderately large in size. Trunk fully segmented, crurigers short, crowded or well separated, anterior pair slightly erect. Proboscis short, cylindrical. Scape one- or two-segmented; chelae fully chelate, finger short, placed at right angle to palm, pointing downwards, with serrate edges, setose pad on base of movable finger. Palps one-segmented knobs. Ovigers ten-segmented, sometimes nine-segmented in females, simple spines, no terminal claw. Legs usually long, slender, propodus well curved, with heel spines, with auxiliaries. Cement gland a mid-ventral tube. Two recognised subgenera, *P.* (*Pallenopsis*) and *P.* (*Bathypallenopsis*), which include more tenuous deep-sea forms. Familial rank of *Pallenopsis* uncertain. Females have ovigers and males show vestigial palps, which make the genus similar to callipallenid forms (Child, 1979), however, the position of the ocular tubercle and general body shape resemble more the phoxichilidiid type (Stock, 1978) (see discussion Chapters 3 and 4).

Pallenopsis hoeki Miers, 1884 [Fig. 2.11]

Pallenopsis hoeki Miers, 1884: 324, pl. 35, fig. B.

? *Phoxichilidium hoeki*.— Haswell, 1884: 1022.

Pallenopsis (*Rigona*) *rigens*.— Loman, 1908: 68-69, pl. 9, figs. 128-133.

Pallenopsis (*Rigona*) *hoeki*.— Flynn, 1929: 257-258.

Pallenopsis hoeki.— Stock, 1954: 8; Clark, 1963: 42, fig.24E; Child, 1975: 19.

Material examined.—Pandora Reef, 5 m depth, among rubble, 29 Oct 1999, 1 ♀ (coll. G. Diaz-Pulido); 19 Apr 2000, 1 ♀, 2 juv.

Description.— Trunk length 1.86 mm, width 0.84 mm; with distinct segmentation lines, neck projected; medium-compact body shape, crurigers close to each other but not touching, the first pair larger and erect anteriorly. Ocular tubercle anterior on cephalon, medium sized, rounded; housing large eyes, not dark pigmented. Abdomen long, erect, with three rows of spines, proboscis cylindrical, with shallow constriction on distal half. Scape of chelifores long, robust, one-segmented; palm setose, perpendicular to scape, as half as long; fingers stout, short, movable finger with serrate margin, pad at base of fingers typical of subgenus *Pallenopsis*. Palps one-segmented, near insertion of chelifores. Ovigers nine-segmented in females, ten-segmented in males, with distal spinules on fourth and last segments. Legs slender, with regular margins, smooth; tibiae with row of dorsal setae, single spine on tarsus, propodus slender, row of setae; three heel spines, tiny sole spines; main claw curved, half size of propodus; auxiliary claws about half size of main claw.

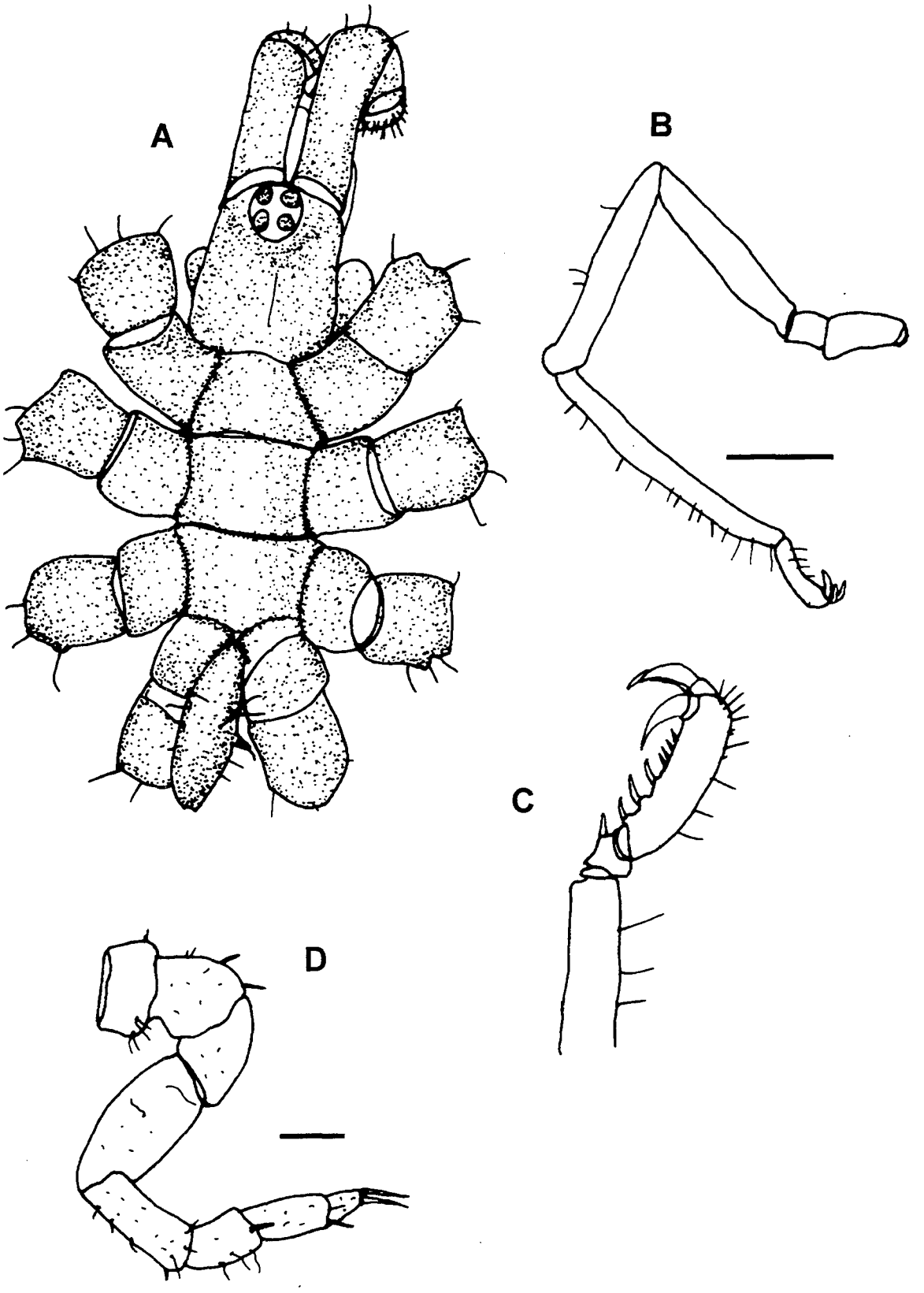

Figure 2.11. *Pallenopsis hoeki*, ♀. A. Dorsal view. B. Third leg; scale bar=1mm. C. Tarsus and propodus third leg. D. Oviger; scale bar=0.1mm.

Distribution.— This species is known from locations in Indonesia and the Philippines, several localities in Western Australia (specimens in the WAM collection), and it has been reported from islands off Cape York.

Remarks.— It is not common to find *Pallenopsis* species in such shallow localities. This is the shallowest record for the species reported from 7-134 m depth. Contrary to Child's comment that *P. hoeki* is seldom collected (Child, 1975), unreported material deposited at the WAM and the present material suggest the species to be more common than supposed.

Family Callipallenidae Hilton, 1942

Genus *Propallene* Schimkewitsch, 1909

Moderately small species. Trunk not markedly segmented, smooth. Proboscis cylindrical. Chelifores scape one-segmented; chelae denticulate and *Callipallene*-like. Palps very short, two-segmented, in males only. Oviger ten-segmented in both sexes, fifth segment with setose laterodistal apophysis in males; short denticulate spines on terminal segments; without terminal claw. Propodus with large heel spines, auxiliaries absent. Cement gland openings numerous (5-22) ventral ducts on femora and sometimes tibiae.

Propallene saengeri Staples, 1979 [Fig. 2.12]

Propallene saengeri Staples 1979: 90-93, fig. 2d, fig. 4; 1982: 456-457.

Material examined.— Rowes Bay, intertidal in *C. prolifera*, 17 Aug 1998, 3♂ with eggs, 2♀, 5 post larvae; 3 Nov 1998, 1♂, 2♀, 2 post larvae; 1 Jun 2000, 1♂, 2♂ with eggs, 2♀, 2 juv.— Picnic Bay, intertidal amongst *Cladophora vagabunda* (L.) van de Hoek, 3 Oct 1998, 4♂, 1♀.— Turtle Bay, 4 May 2000, 1♂ with eggs and protonymphs.

Description.— Trunk 0.8 mm in length, 0.6 mm wide, smooth; crurigers not touching but separated by less than half their own diameter; neck almost twice as long as wide at midpoint. Ocular tubercle low, rounded, placed posteriorly on neck. Abdomen stout, horizontal, as continuation of trunk, tapering distally. Proboscis short, cylindrical. Chelifore scape of one segment, glabrous, endal and distal setae on palm; both fingers curved, gaping when close, immovable finger with four teeth and movable with four or five smaller teeth. Palps in males only, very short, two-segmented, with three long distal setae. Ovigers of ten segments, a setiferous apophysis on fifth segment of males, two recurved long spinules on seventh segment. Denticulate spines present on terminal segments conforming to the formula 7:6:6:6, the most distal spine multifurcated. Legs slender, smooth margins, femur the longest segment, short setae on all coxae, two spines dorsally on tarsus; propodus with heel, two crenulate

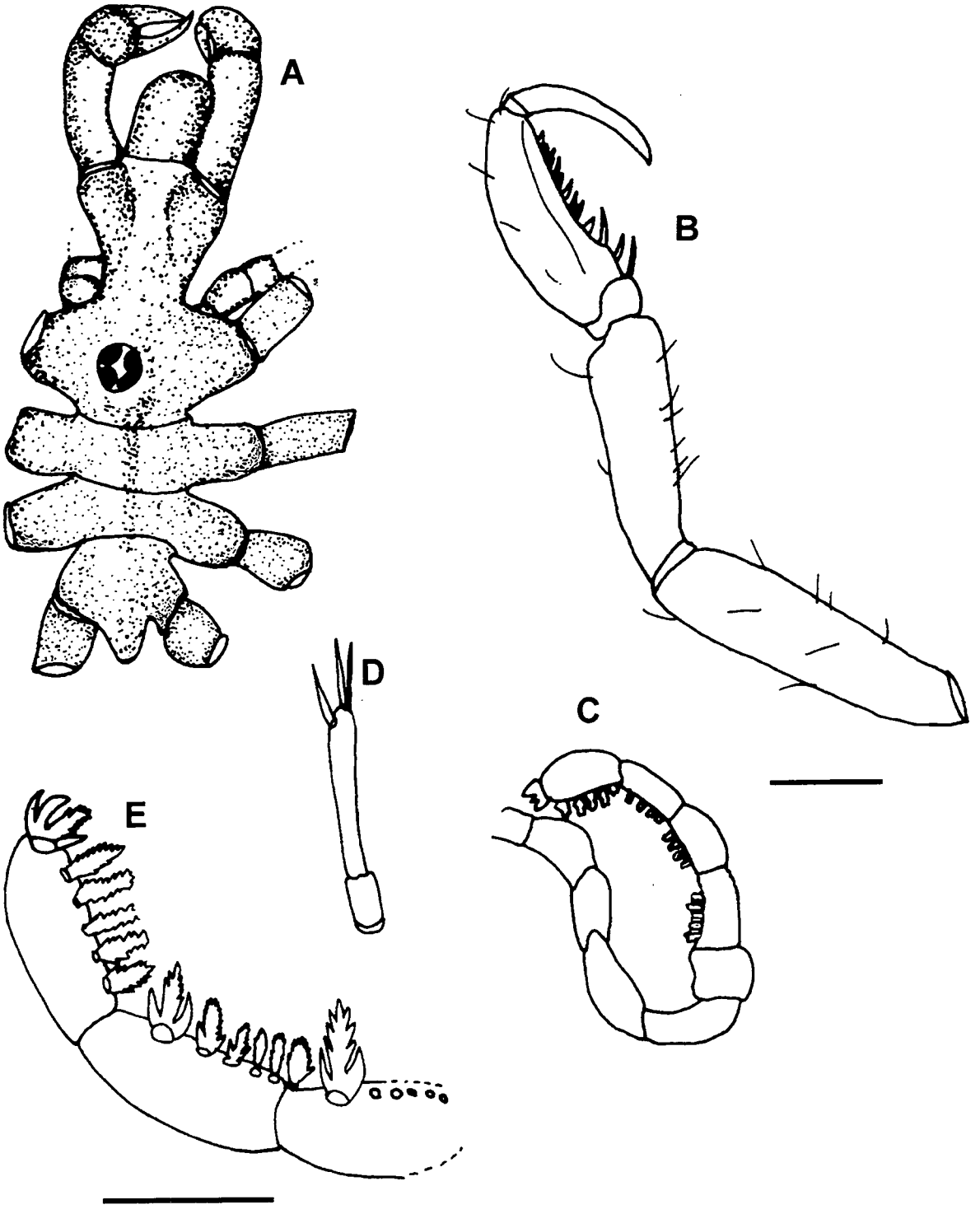

Figure 2.12. *Propallene saengeri*, ♂. A. Dorsal view. B. Tibiae, tarsus and propodus of third leg. C. Oviger of ♀. D. Palp; scale bar=0.2mm. E. Denticulate spines in the terminal segments of the oviger; scale bar=0.03mm.

spines on heel, sole with eight or nine spines; no auxiliary claws. Cement glands numerous short ducts placed ventrally on femur and tibiae (4-6 in femur, 4 in tibia 1, 3-4 in tibia 2).

Distribution.— *Propallene saengeri* was described from the mouth of the Calliope River in south Queensland. It was found in soft mud upstream at 1-2 m depth. This is the second record for the species in Australia. It has been found once in shallow waters of Sagami Bay, Japan (Nakamura and Child, 1983).

Remarks.— *Propallene saengeri* is one of the three species of the genus known for Australia together with *P. cyathus* Staples, 1979 and *P. vagus* Staples, 1979, the latter recorded from southeastern Australia. *Propallene saengeri* is differentiated by its very small size and the greater length of the second palp segment (six times longer than wide). I found *P. saengeri* in relative high abundance in some of the samples at Rowes Bay and in one sample at Turtle Bay in the alga *C.prolifera*. *Propallene saengeri* is characteristic species from shallow sandy-muddy environments. Ovigerous males were collected on several occasions and in some of them the protonymphs had already hatched. This is another species of Callipallenidae in which larval development occurs while attached to the male, a feature that provides good opportunities for studies on development and larval morphology.

Genus *Callipallene* Flynn, 1929

Trunk fully or partially segmented, without tubercles. Proboscis short. Chelifores robust, one-segmented; chelae functional with serrate or denticulate long fingers. Palps absent. Ovigers ten- segmented, fifth segment with laterodistal apophysis only in males, well-developed denticulate spines on last four segments, no terminal claw. Legs usually slender, tarsus and propodus short, with heel spines, claw with large auxiliaries. Cement gland, where known, ventral pores or tiny tubes.

***Callipallene* n. sp.** [Fig. 2.13]

Material examined.—Townsville, Rowes Bay, in *C. prolifera*, 1 Jun 2000; 3 ♂ 1 ♀.

Description.—Trunk length 0.64mm, width 0.27 mm; first two segmentation lines complete, third lacking; body smooth, glabrous; neck moderately long; crurigers separated distally by twice their diameters. Ocular tubercle large, low, placed on raised base; eyes large, filling tubercle, not well pigmented. Proboscis typical, short, tapering to small oral surface. Abdomen glabrous, short, cylindrical. One-segmented scape, 2.5 longer than wide, armed with 3-4 lateral and distal long setae; chelae with similar dorsal setae, immovable finger serrate with six teeth, movable finger without teeth, fingers overlap at tips. Oviger bases placed lateroventral on neck; ten-segmented, fifth segment longest, with small apophysis with two setae; last segments with rounded denticulate spines in formula 6:4:4:5; terminal claw

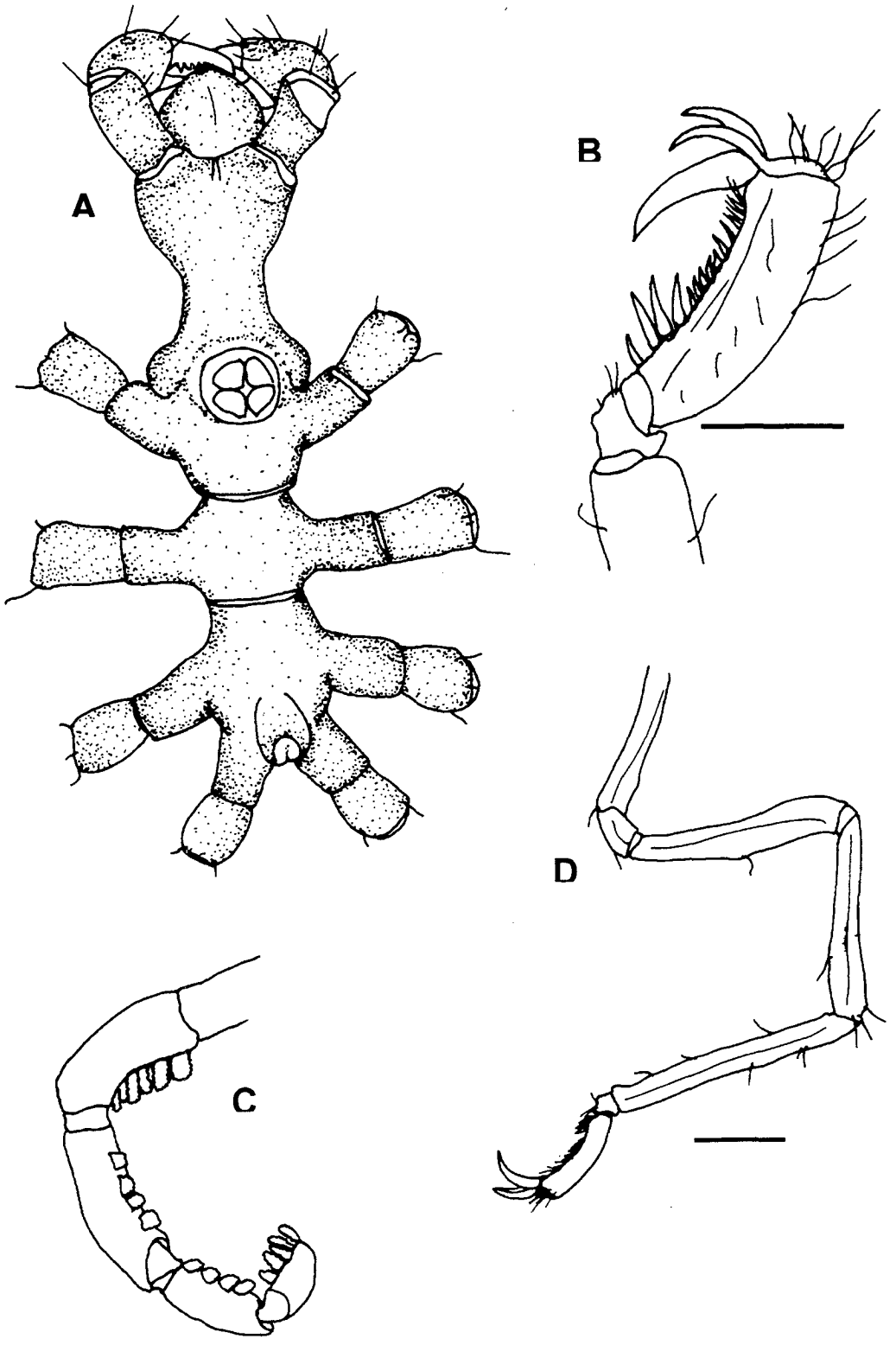

Figure 2.13. *Callipallene* sp. ♀. A. Dorsal view. B. Tarsus and propodus. C. Terminal segments of the oviger scale bar=0.05mm. D. Third leg; scale, bar=0.2mm.

lacking. Legs slender, with few short setae except dorsodistal long setae; femur and second tibia subequal, first tibia a bit shorter. Tarsus very small, with one spine and few setae; propodus moderately curved, slender, with four heel spines, 10-12 sole spines, setose. Claw slightly longer than half the length of propodus, auxiliaries about three-quarters the propodal length. Cement gland apparently a single pore on ventral midpoint swelling. Female slightly larger in all measurements; neck longer, ovigerous females with femora greatly swollen, otherwise same characters as male.

Distribution.—*Callipallene* n. sp. is only known from the intertidal algae around Townsville, Queensland.

Remarks.—This species is being described in Lee and Arango (submitted). *Callipallene* n. sp. is very similar to *C. tridens* Nakamura & Child, 1986 in the general body shape, the ocular tubercle occuring on a slight elevation and the segmentation line between third and fourth segments is indistinct in both. The undescribed species lacks the trident-shaped auxiliary claws of *C. tridens* and has longer and more slender appendages. The extremely small size is also shared by these two species.

Callipallene novaezealandiae Stock, 1954 [Fig. 2.14]

Callipallene brevirostris ssp. *novae-zealandiae* Stock, 1954: 48-50, fig. 21.

Callipallene novaezealandiae.— Child, 1983: 277 (liter.), 1988: 21; 1996: 554.

Material examined.— Rib Reef, reef slope, 9 m depth, in *Galaxaura* sp. and rubble washings, 26 Nov 1998, 1 juv. Pandora Reef, found in rubble, 8 m depth, 7 Mar 2000, 1♂; 19 Apr 2000, 1♂ with eggs, 1♂. — Turtle Bay, in *C. prolifera*, 14 May 1999, 1♀.— Rowes Bay, in *C. prolifera*, 1 Jun 2000, 4♂ with eggs.— Lucinda, in piling scrapings in the Lucinda jetty, 1 Dec 1999, 1♂ with eggs (coll. J. Cruz).

Description.—Trunk length 1.1 mm, width 0.42 mm; fully segmented; body elongated; neck long, very constricted; crurigers apart by more than their own diameter but less than twice the diameter, all crurigers small and smooth. Ocular tubercle rounded, broad, with pointing tip, eyes not pigmented. Abdomen erect, short. Proboscis small, with subtle, lateral projections. Chelifores robust, scape one-segmented, inflated palm, scape and palm subequal in length, both with setae, movable finger as long as the palm, immovable finger shorter, both with serrate margins. Ovigers ten-segmented, fifth segment longest, apophysis in males, row of four setae; last segments with denticulate spines in the formula: 566:6:7 in females, 5:5:5:5 in males. Legs long, slender, femur the longest, tibiae with long setae, other segments glabrous; propodus slender, not very curved, without heel, long setae dorsally, 3-4 heel spines, five sole spines; long main claw, auxiliaries three-quarters of the main claw length.

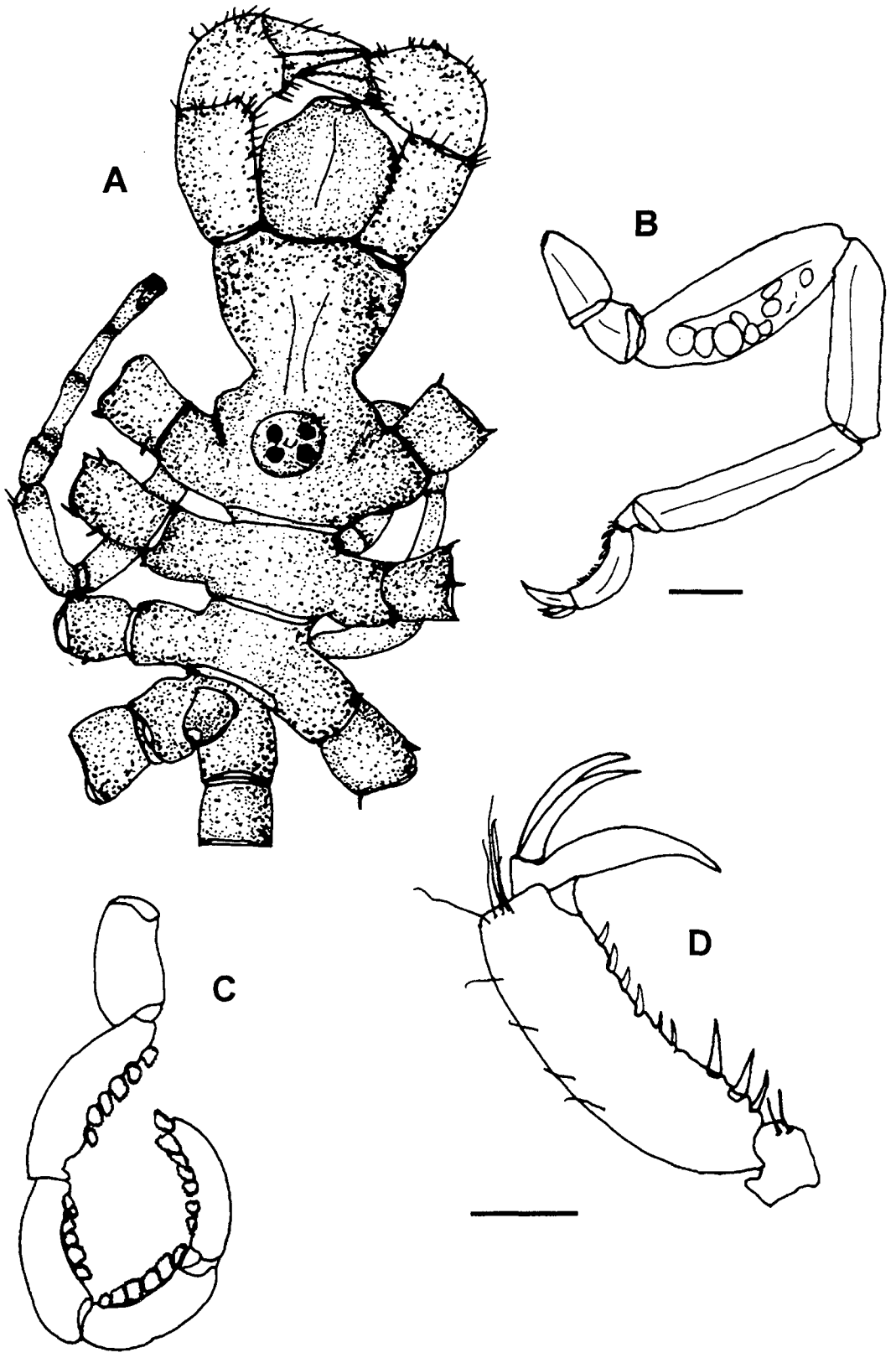

Figure 2.14. *Callipallene novaezealandiae*, ♀. Dorsal view. B. Third leg scale bar=0.2mm. C. terminal segments of the oviger. D. Tarsus and propodus; scale bar=0.03mm.

Distribution.— This species had been found before in South Australia (Stock, 1954) and there is one unpublished record of *C. novaezealandiae* from New Year Island in the Northern Territory (records of the AM). This is a widely distributed Indo-Pacific species taken from East Africa to Japan in 2-247 m (occurs mostly in shallow waters) so it can be expected to have a wide Australian distribution.

Remarks.— *Callipallene* is a difficult genus to work with considering the high intraspecific variation of the few characters known to differentiate species. Length of the neck and shape of the ocular tubercle seem to change according to age and sex. The number of spines on the ovigers also appears to be variable at least within this species. The specimens reported here agree for the most part with the description and figures in Stock (1954), but have fewer denticulate spines on the ovigers, as also noted by Child for his specimens from the Marshall Islands (Child, 1982b). *Callipallene novaezealandiae* is recognized by monomorphic denticulate spines, fingers short and not so curved, and usually four heel spines. However, these characters might change or be found in closely related species. This is one of the genera in urgent need of taxonomic revision.

Genus *Parapallene* Carpenter, 1892

Medium-sized species. Trunk segmented, with long neck and sometimes a 'collar'; crurigers short. Proboscis cylindrical, with mid-constriction, lips projecting. Chelifore scape one-segmented, chelae small, inflated, fingers short, smooth. Palps absent. Ovigers ten-segmented, with few denticulate spines or none, terminal claw. Legs long, slender; auxiliary claws present or absent. Cement glands ventral in *P. exigua* (Stock, 1954).

Parapallene famelica Flynn, 1929 [Fig. 2.15]

Parapallene famelica Flynn, 1929: 258-260, figs.6-9.— Clark, 1963: 28-29, fig.14a-g.

Material examined.—North Fitzroy Reef, 52 m depth, trawled with bryozoans and algae, 23 Nov 1999, 1♂ (DPI sta. DW P422, coll. C. P. Arango & DPI Seagrass Monitoring Project). Mackay, 11 m depth, in rubble and algae, 2 Feb 2001, 1♂, 2♀ (coll. DPI Seagrass Monitoring Project).

Description.—Trunk 8.71mm long, 2.21 mm wide; elongate, thin body, smooth; neck twice as long as wide; crurigers short, separated by about three times their diameter. Ocular tubercle low, pointed, eyes below midpoint, not well pigmented. Abdomen short, erect. Proboscis straight, with expanded anterolateral angles. Scape one-segmented, robust, as long as proboscis, with a small distal tubercle topped with a short spine; palm just shorter than the scape, globular, placed in right angle pointing downwards; large fingers, movable longer, both non-denticulate. Ten-segmented ovigers, third and fourth segment fused, fifth segment longest, distal apophysis in males; no visible spines on terminal segments; long terminal claw

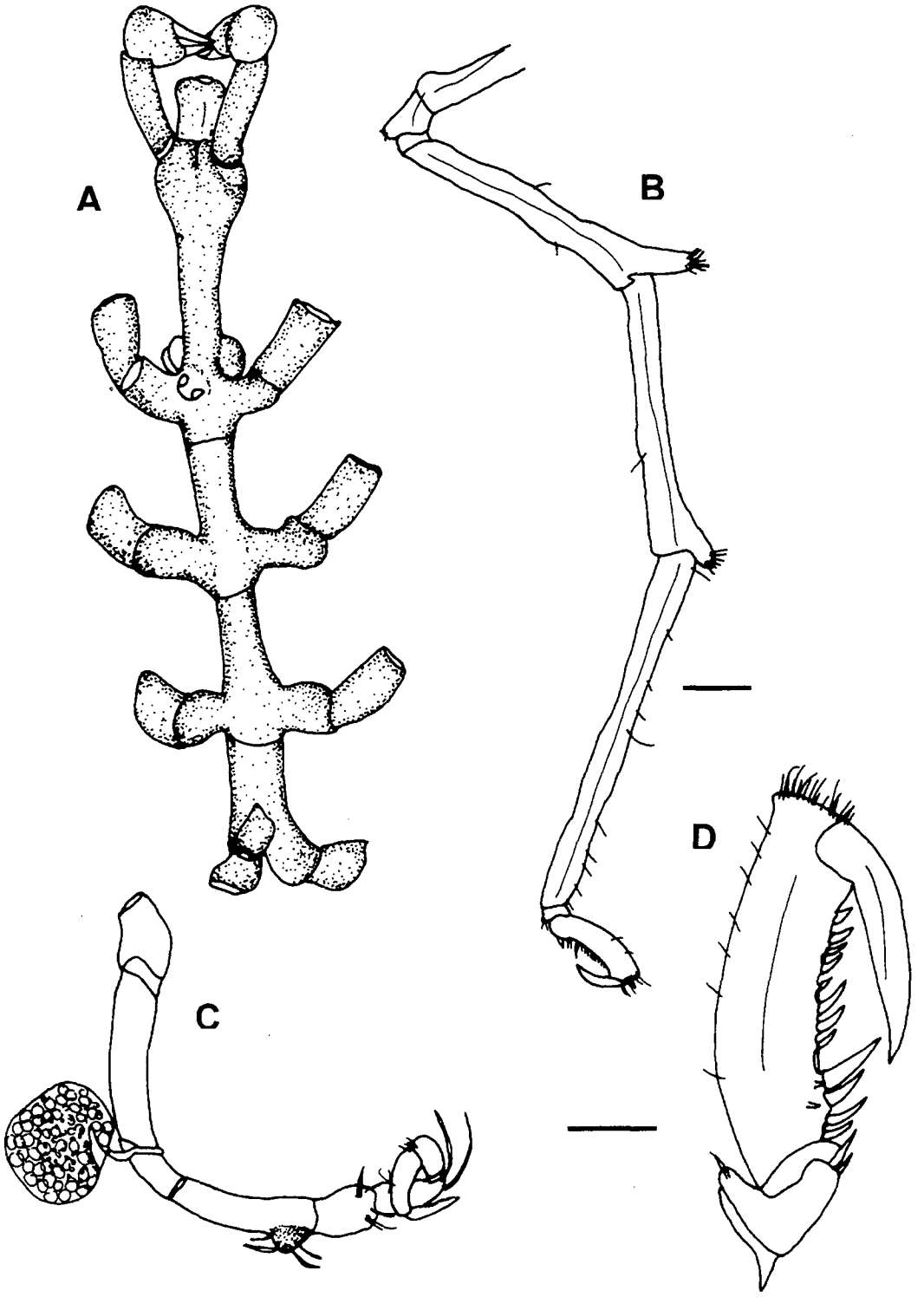

Figure 2.15. *Parapallene famelica*, ♂. A. Dorsal view. B. Third leg; scale bar=1mm. C. Oviger with mass of eggs attached. D. Tarsus and propodus; scale bar=0.4mm.

with denticulations, larger distally. Legs slender but strong, genital pores on low protuberances in all legs of females, only on third and fourth pairs of legs in males; distal tubercle on femur and first tibia; cement gland not seen, although four ventral darker spots were noticed in males. Four heel spines on propodus, six to eight sole spines; main claw more than 2/3 length of propodus.

Distribution.— To my knowledge this species is known only from Lindeman Island where it was first found, and from Port Philip in Victoria, both Australian localities, one tropical, the other temperate. This record from Mackay, Queensland fits within the range of geographical and bathymetrical distribution known.

Remarks.—This is the largest species of the present collection. Eight species of *Parapallene* are known for Australian waters. *P. australiensis* Hoek 1881, *P. haddoni* Carpenter 1892 and *P. nietstrazi* Loman 1908, seem related to *P. famelica*, which can be distinguished by its more elongate form, with long neck and widely separated crurigers, and the absence of denticulate spines in the ovigers.

Genus *Pigrogromitus* Calman 1927

A monotypic genus. Small tropical species. Trunk ovoid, with low mediandorsal knobs, crurigers short, crowded, glabrous. Proboscis short, barrel-shaped. Chelifores tiny, lateral to proboscis, scape two-segmented, fingers simple. Palps absent. Ovigers ten-segmented, distal spines simple, with terminal claw. Legs robust, short, claw large, curved, without auxiliaries. Cement gland unknown. Remarks.—This is a peculiar mono-specific genus, which closely resembles the *Pycnogonum* species in the shape of trunk and proboscis, however it can be differentiated by the three-segmented chelifores, completely absent in *Pycnogonum*.

Pigrogromitus timsanus Calman, 1927 [Fig.2.16]

Pigrogromitus timsanus Calman, 1927: 408-410, fig.104a-f.— Hedgpeth, 1948: 214-216, fig.23.— Child, 1979, 46-47 [early literature] .— Staples, 1982: 457, fig. 2g-j.

Material examined.—Lucinda jetty, in piling scrapes, 3m depth, ?-1999, 1♂ with eggs (coll. J. Cruz)

Description.— Compact species, 1.48mm in trunk length, 1.04 mm wide; fully segmented, bumpy dorsum, mid dorsal swellings on all segments; crurigers wider than long, touching each other distally. Ocular tubercle rounded, placed anteriorly on cephalon, small eyes, pigmented. Abdomen horizontal, between crurigers of fourth segment. Proboscis inflated, wider distally, with characteristic middle constriction (remnant of segmentation ?). Chelifores on each side of proboscis, second scape segment longer than first, palm and fingers small, unarmed. Ovigers ten-segmented, fifth segment longer, spare setae, last segments decreasing

in size, unarmed except for short setae, pointed simple spine on last segment forming a subchelate structure with the curved terminal claw.Legs short, robust, femur longest segment, followed by first tibia, few short setae on coxae, femur with two ventral spines and distal tubercle, mid-dorsal low swellings on tibiae. Propodus longer than second tibia, curved, with no heel and few sole spines. Main claw abouth half the length of propodus.

Distribution.— This is a pantropical-temperate species, frequently collected in shallow habitats distributed widely over the world, including Indo-Pacific, Caribbean and Mediterranean localities. In Australia the species is known from the vicinity of Brisbane and this record from Cleveland Bay, both in Queensland.

Genus *Seguapallene* Pushkin, 1975

Small-sized callipallenids. Trunk segmented, robust appearance, neck short, wide. Proboscis short, rounded at tip. Chelifore scape one-segmented, chelae large, immovable finger denticulate. Palps absent. Ovigers ten-segmented, with denticulate spines and terminal claw. Legs typical, propodus short, with heel spines, auxiliaries present. Cement glands unknown.

Remarks.— *Seguapallene* is a rare genus of six recognised species, one known from the sub-Antarctic, the others collected in Indo-Pacific localities (Child, 1991). It is characterized by a long denticulate terminal claw and large, robust chelifores.

Seguapallene* cf. *micronesica Child, 1983

Seguapallene micronesica Child, 1983: 709-711, fig.4; 1991: 145.

Material examined.—Chilcott Island, 1m depth, in *Halimeda* sp, *Amphiroa* sp. and rubble washings, 10-15m depth, 14 Sep 1998, 1 ♀; Willis Reef, 15-16 m depth, 2 juv. (coll. G. Diaz-Pulido).

Description.— The material collected was not in good condition and only allowed determination of similarities with the Indo-West Pacific species *Seguapallene micronesica.* The specimens all have separated crurigers, short main propodal claw, triangle-shaped teeth of chelae, different from sister species *S. crassa* Child, 1990 described from Lizard island. The two juveniles show same characteristics as female.

Distribution.— This species was described from the intertidal at Palau Islands in Micronesia followed by a record from Guam (Child, 1991), that expanded its Pacific distribution to the Northeast Pacific. The genus is known for its Indo-west Pacific distribution and this record suggests a wider distribution confined to littoral habitats of the tropical Pacific.

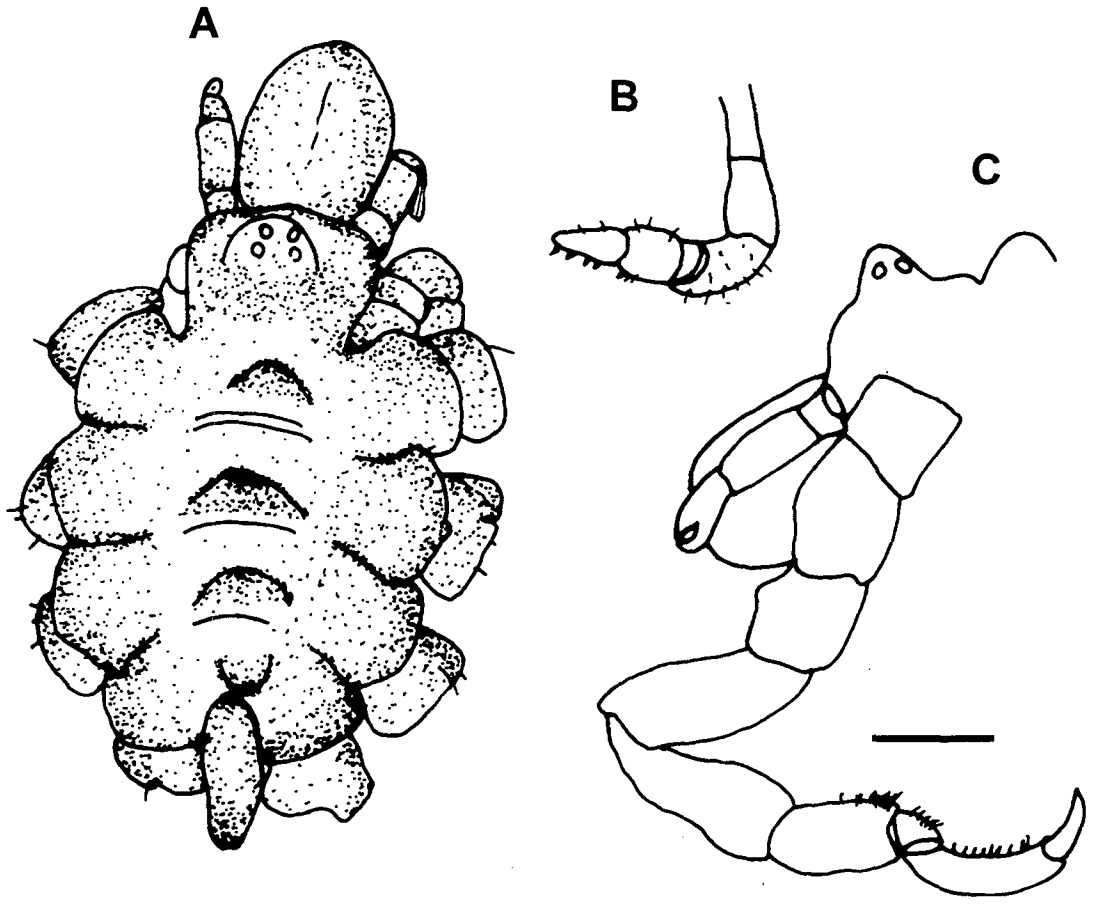

Figure 2.16. *Pigrogromitus timsanus*, ♂. A. Dorsal view. B. Oviger. C. Lateral view of the cephalon with chelifore and first leg; scale bar=0.3mm.

Family Phoxichilidiidae Sars, 1891

Genus *Anoplodactylus* Wilson, 1878

From tiny to medium-sized species. Trunk well-segmented or partially segmented, body compact or very slender, cephalic segment prolonged forward with distinct neck. Ocular tubercle usually high. Proboscis cylindrical or slightly tapering distally (one species pipette-like upcurved). Chelifore scape one-segmented, chelae small, fingers plain or toothed. Palps lacking. Ovigers six-segmented, slender, in males only, distal segments setose. Legs slender or robust, propodus long, with heel and long heel spines, sole with setal lamina, with tiny auxiliary claws or lacking. Cement glands multiple or single tubes, pores or slits, at mid femur or more distal.

Anoplodactylus batangensis (Helfer, 1938) [Fig. 2.17]

Pycnosoma batangense Helfer, 1938:174-176, fig. 6 a-c.

Anoplodactylus batangensis. —Stock, 1953: 39-41, fig. 4; 1954: 54; 1968: 54 [previous literature].— Child, 1988: 14; 1992: 41-42, fig. 18; 1996: 549.

Material examined.—Turtle Bay, intertidal *C. prolifera*, 27 Mar 1997, 1♀, 44 juv. (coll. J. Otto); 5 Oct 1998, 2♂,7♀, 1 juv.; 12 Jul 1999, 1♂,1♀; 4 May 2000, 3♀; 1 Jul 2000, 1♂ with eggs. .—Central Section, Orpheus Island, Pioneer Bay, intertidal seagrass bed and filamentous algae, 7 Sep 1998, 1 juv.

Description.— Trunk length 0.82 mm, width 0.36 mm; lines of segmentation just visible in dorsal view; body compact, crurigers separated by about half their diameter. Ocular tubercle inclined forward, rounded tip, eyes well pigmented; proboscis upturned, tapering distally. Abdomen erect, same height as ocular tubercle. Chelifore scape one-jointed, smooth, touching each other, palm with some short setae, fingers right in front of mouth, in downward diagonal position. Ovigers six-segmented, typical of *Anoplodactylus*. Legs robust, with irregular margins, ventral genital spur on second coxa of fourth pair of legs in males, one dorsodistal long spine on femur and tibiae; propodus large, curved, robust, strong heel, two heel spines, five to six sole spines and propodal lamina; auxiliary claws absent. Cement gland mid dorsal tube. Females rather similar to males but smoother in appearance, femora swollen.

Distribution.— This species had been reported once from Lizard Island (Child, 1990) in Australia. It has been found in almost every tropical collection of pycnogonids worldwide. It is considered a pantropical species in littoral and shallow depths.

Remarks.— This small species is recognised within *Anoplodactylus* due its slender styliform, upcurved proboscis, unique in the genus. Stock re-described the type specimen annotating the tricuspidate tip of the ocular tubercle housing no pigmented eyes. The specimens described above do not agree with these features. *Anoplodactylus batangensis* has been reported with a dorsal chalky stripe from the base of the ocular tubercle to the base of the abdomen (Child, 1982a). The specimens in this collection do not display such colouration pattern but have a pale pink cuticle. Surprisingly, the colouration described by Child for *A. batangensis* resembles more the pattern I observed in *Anoplodactylus* sp. B (see below). The wide distribution of the species and such morphological variation mentioned suggest the need of population-level studies.

***Anoplodactylus* n. sp. A** [Fig 2.18]

Material examined.—GBR, Orpheus Island, Pioneer Bay, reef flat, in *Galaxaura rugosa*, 27 Jul 1998, 3♀, 1juv; 24 Nov 1998, 5♂, 17♀; 14 Apr 2000, 1♀.— Great Palm Island, Cannon Bay, reef flat 2 m depth, in *Laurencia* sp. and *G. rugosa*, 28 Nov 1998, 1♂.—Picnic Bay, intertidal, found in *Laurencia* sp. attached to rock, 3 Oct 1998, 1♂ with eggs, 1♀.Turtle Bay,

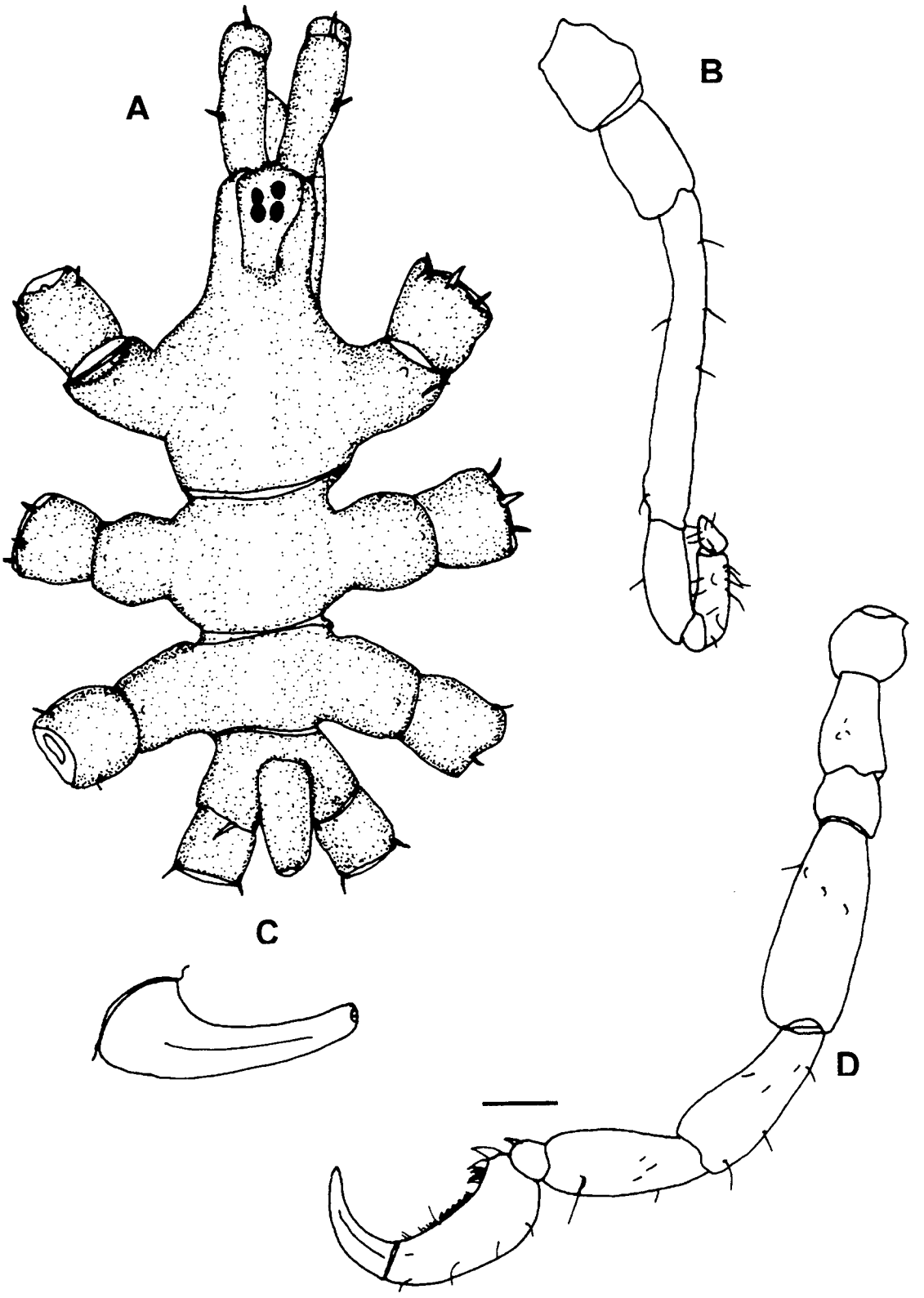

Figure 2.17. *Anoplodactylus batangensis*, ♂. A. Dorsal view. B. Oviger. C. Lateral view of the proboscis. D. Third leg; scale bar=0.07mm.

intertidal in *C. prolifera*, 27 Mar 1997, 3♂, 4♀, 3 juv. (coll. J. Otto); 12 Jul 1999, 1♂; 4 May 2000, 2♂, 2♀; 1 Jul 2000, 1♀.

Description.— Leg span 2.5 mm. Trunk length 0.64 mm, width 0.49mm; ovoid in dorsal outline, glabrous, neck short, with concave sides, anterior pair of crurigers extends slightly forward beyond the oviger bases; crurigers crowded together, armed with low rounded distal tubercles. Ocular tubercle moderately tall, large well-pigmented eyes, low apical cone. Abdomen erect, as tall as ocular tubercle. Proboscis short, cylindrical, slightly tapering distally. Chelifore scape one-segmented, smooth surface, almost straight and touching. Ovigers with six segments, third segment longest, last segment pointed, twice as long as wide. Legs short, with swellings, second coxae of fourth pair of legs with ventral genital spurs in males, a spine distally on femur and tibiae; propodus large, curved, strong heel, two heel spines, main claw more than three-quarters the length of the propodus, no auxiliary claws visible. Cement gland a mid-dorsal tube. Females with swollen femora and smoother appearance, with same pattern of spination as males.

Distribution.— This species is known from localities near Townsville and coral reefs in the central section of the GBR.

Remarks.— This species is described in Arango (in press). Given its wide distribution in the area studied, it is expected the species had been overlooked before due to its small size. One of the tiniest sea spiders known, it is similar to *A. viridintestinalis* Cole 1904 and also *A. crassus* Child 1988. These share the ovoid trunk and small robust appearance with crowded crurigers, short proboscis and short legs but the characteristic shape of the neck is not found in any other species. Two specimens of *Anoplodactylus* found on a boulder of the beach at Picnic Bay in Magnetic Island were identified as this species. Although the measurements and characteristics fit with those of the type specimens, their colouration was more greenish than in the holotype.

Anoplodactylus digitatus (Böhm, 1879) [Fig. 2.19A-D]

Phoxichilidium (Anoplodactylus) digitatum Böhm, 1879: 184-185, pl. 1, fig. 2-2b.

Anoplodactylus digitatus.— Stock, 1965: 28-28 [synonymy & literature].— Stock, 1992: 94; 1994: 57. — Müller, 1992: 164-166, figs. 18-26. — Child, 1996: 551-552.

Material examined.—Turtle Bay, intertidal in *C. prolifera*, 14 may 1999, 1♀.—Lucinda Jetty, 3 m, 1♂ (coll. J. Cruz).

Description.— Trunk length 1.56 mm, 0.72 mm wide; fully segmented, smooth, medium to elongate shape; crurigers set apart by own diameter, all same size. Ocular tubercle anterior on cephalon, erect, not projecting forward, with low protuberance on top. Abdomen erect, longer than ocular tubercle. Proboscis long, cylindrical, four ventral protuberances in females.

intertidal in *C. prolifera*, 27 Mar 1997, 3♂, 4♀, 3 juv. (coll. J. Otto); 12 Jul 1999, 1♂; 4 May 2000, 2♂, 2♀; 1 Jul 2000, 1♀.

Description.— Leg span 2.5 mm. Trunk length 0.64 mm, width 0.49mm; ovoid in dorsal outline, glabrous, neck short, with concave sides, anterior pair of crurigers extends slightly forward beyond the oviger bases; crurigers crowded together, armed with low rounded distal tubercles. Ocular tubercle moderately tall, large well-pigmented eyes, low apical cone. Abdomen erect, as tall as ocular tubercle. Proboscis short, cylindrical, slightly tapering distally. Chelifore scape one-segmented, smooth surface, almost straight and touching. Ovigers with six segments, third segment longest, last segment pointed, twice as long as wide. Legs short, with swellings, second coxae of fourth pair of legs with ventral genital spurs in males, a spine distally on femur and tibiae; propodus large, curved, strong heel, two heel spines, main claw more than three-quarters the length of the propodus, no auxiliary claws visible. Cement gland a mid-dorsal tube. Females with swollen femora and smoother appearance, with same pattern of spination as males.

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Anoplodactylus digitatus (Böhm, 1879) [Fig. 2.19A-D]

Phoxichilidium (Anoplodactylus) digitatum Böhm, 1879: 184-185, pl. 1, fig. 2-2b.

Anoplodactylus digitatus.— Stock, 1965: 28-28 [synonymy & literature].— Stock, 1992: 94; 1994: 57. — Müller, 1992: 164-166, figs. 18-26. — Child, 1996: 551-552.

Material examined.—Turtle Bay, intertidal in *C. prolifera*, 14 may 1999, 1♀.—Lucinda Jetty, 3 m, 1♂ (coll. J. Cruz).

Description.— Trunk length 1.56 mm, 0.72 mm wide; fully segmented, smooth, medium to elongate shape; crurigers set apart by own diameter, all same size. Ocular tubercle anterior on cephalon, erect, not projecting forward, with low protuberance on top. Abdomen erect, longer than ocular tubercle. Proboscis long, cylindrical, four ventral protuberances in females.

Distribution. — This is a widely known species from the tropical Indo-Pacific and the Caribbean, not recorded for Australia before [in Lee and Arango (submitted)].

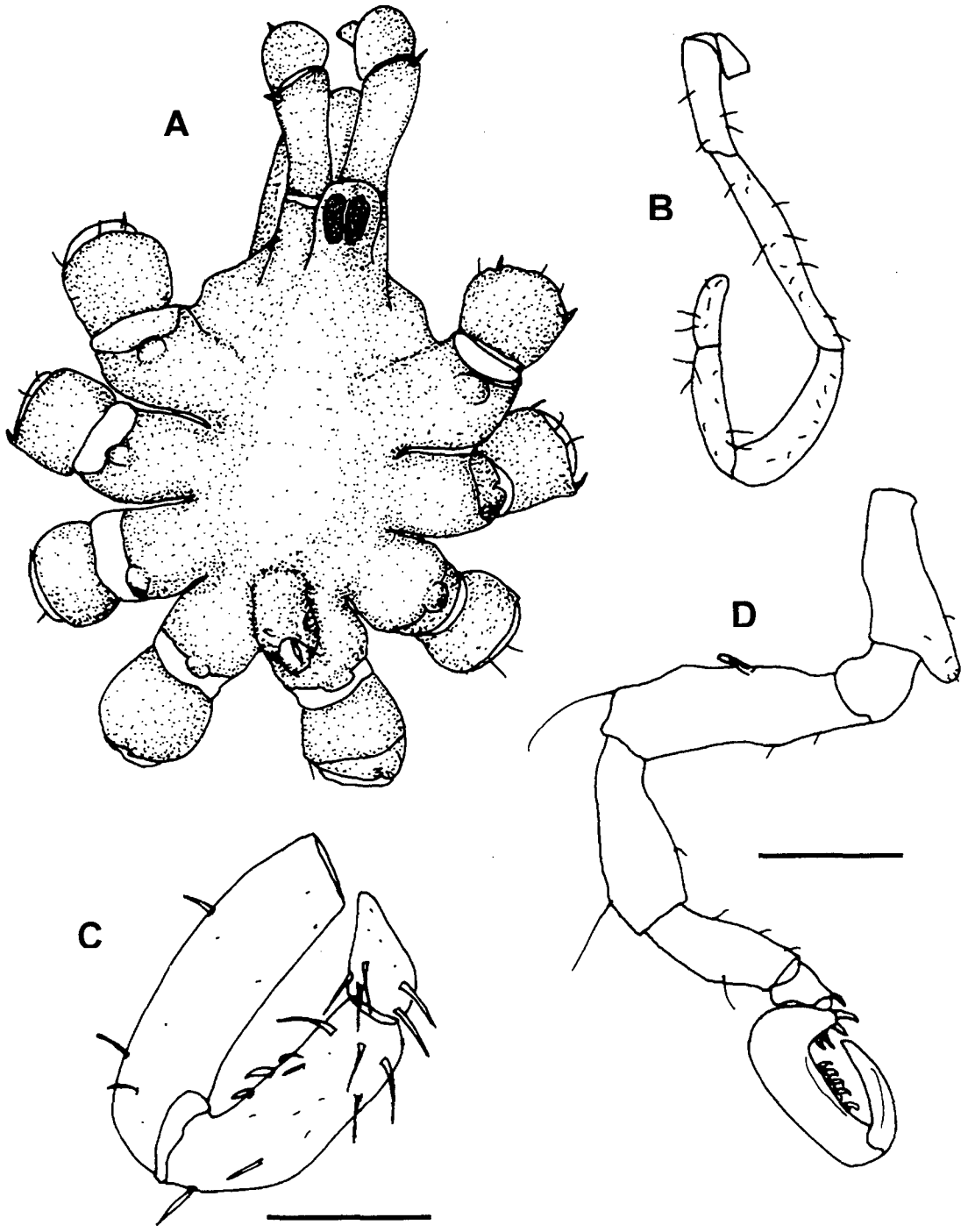

Figure 2.18. *Anoplodactylus* sp. A. ♂. A. Dorsal view. B. Oviger. C. Terminal segments of the oviger; scale bar=0.05mm. D Third leg; scale bar=0.2mm.

Remarks.— There is variation in the number of cement gland cups in this species. *Anoplodactylus glandulifer* was described from specimens having two or three openings (Stock, 1954) but now the range is known from 2–5 (Müller, 1992a; Child, 1998a). As for most of the *Anoplodactylus*, the absence of males in a collection makes identification at species level difficult. *Anoplodactylus glandulifer* can be distinguished by the combination of presence of tiny palp knobs, propodal lamina and multiple cement gland cups.

***Anoplodactylus longiceps* Stock, 1951** [Fig. 2.21]

Anoplodactylus longicollis.— Williams, 1941: 36-38, figs. 2-5 (preoccupied).

Anoplodactylus longiceps Stock, 1951: 16; 1954: 83; 1956: 97-98, fig.14c-d.— Child, 1975: 20, fig. 9f; 1990: 331.

Material examined.—Turtle Bay, intertidal *C. prolifera*, 14 May 1999, 2♀, 2♂; 12 Jul 1999, 2♂, 1♀.

Description.— Trunk 1.56 mm long, 0.23 mm wide, fine segmentation lines, elongated body; crurigers separated by more than half their diameter, smooth, all the same size. Ocular tubercle tall, pointing upwards, tip very acute; eyes apical , not well pigmented. Abdomen erect, slightly swollen distally. Proboscis cylindrical, with constriction on distal half. Chelifores long, scape one-segmented, touching at base then widely separated, about three-quarters length of proboscis; palm and chela half the size of scape, few short setae and spinules distally on scape and palm. Ovigers six-segmented, in young males collected only four segments had been differentiated. Legs smooth except for long distal tubercle on femur; propodus long, with heel, one large heel spine and two or three smaller ones, six sole spines; no propodal lamina visible; main claw long, well curved; auxiliaries absent.

Distribution.— This species is found in eastern and western Australia, Kei Islands in Indonesia and other western Pacific islands. It was described by Williams (1941) from Lindeman Island at the GBR and reported from Lizard Island by Child (1990). These intertidal specimens are the shallowest record (known bathymetrical range 2-12 m).

Remarks.— *Anoplodactylus longiceps* might be related to *A. simplex* Clark 1963 (synonymized with *A. cribellatus* Calman 1923, see Bamber, 1997a), but the crurigers in *A. longiceps* are more widely separated, the distal tubercle on femora are more prominent and the genital spurs of the males of *A. longiceps* are larger and pointed. In males, the number of cement glands ranges from two in *A. longiceps* to 10 or more in the *cribellatus* –complex (Bamber, 1997b). These specimens coincide with the green colouration mentioned by Child (1998a). Two specimens of *A. longiceps* were observed feeding upon the dorid nudibranch *Okenia* sp. also found in the *Cladophora* tufts (Arango and Brodie, in press).

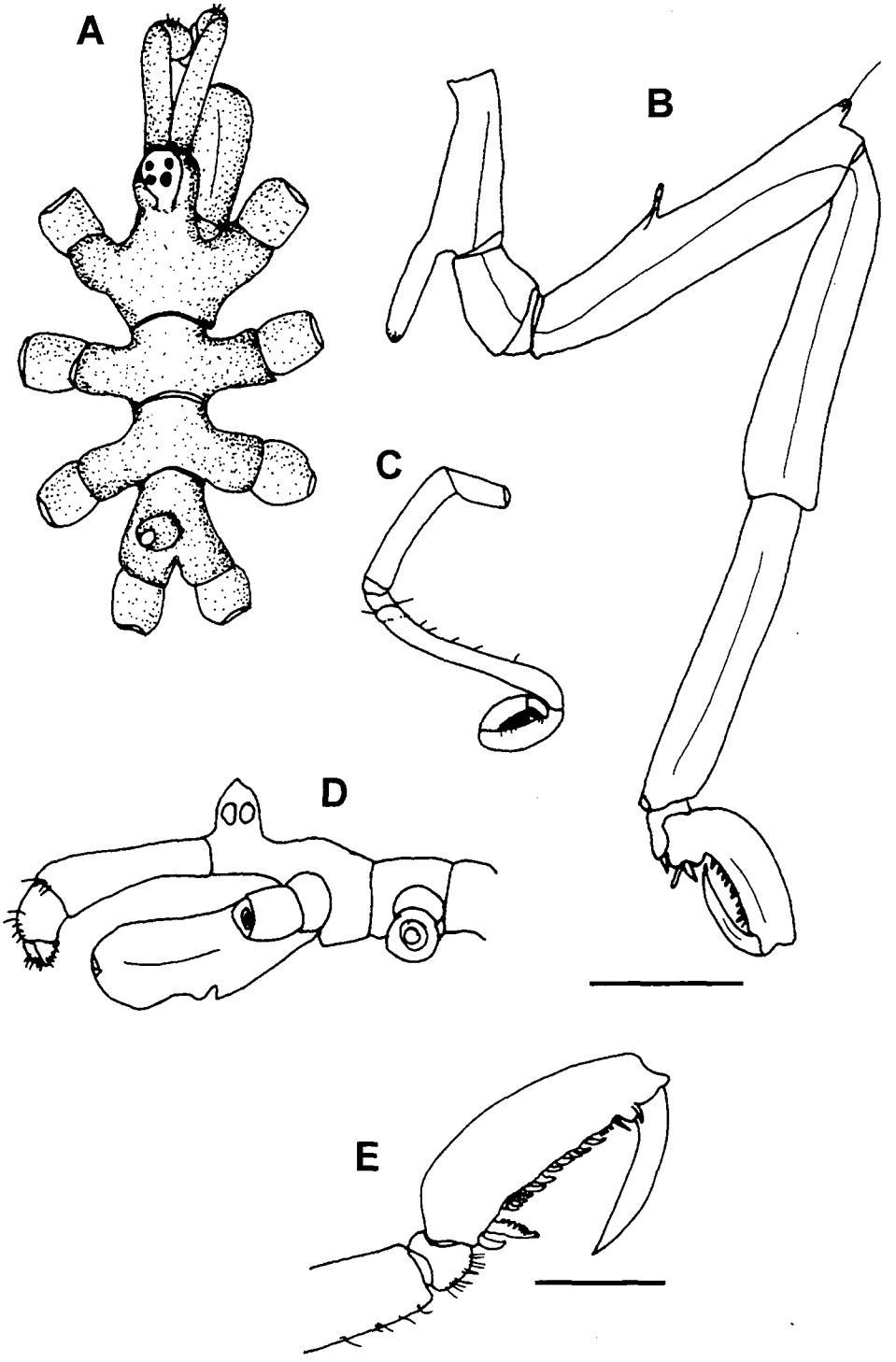

Figure 2.19. *Anoplodactylus digitatus*, ♂. A. Dorsal view. B. Third leg. C. Oviger. D. Lateral view of the cephalon of a ♀ showing ventral protuberances on the proboscis; scale bar=0.6mm.— *Anoplodactylus pectinus*. E. Tarsus and propodus; scale bar=0.25mm.

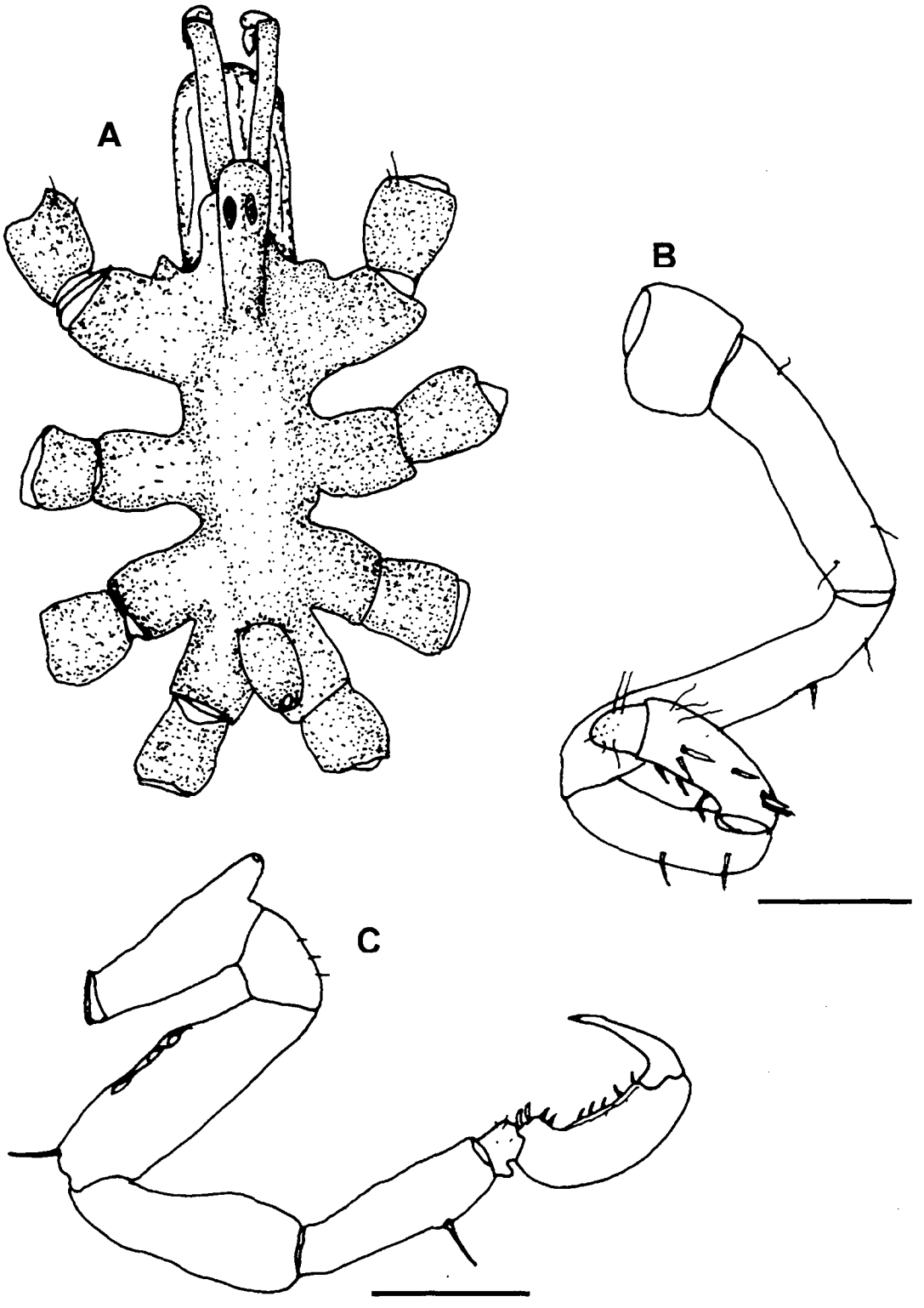

Figure 2.20. *Anoplodactylus glandulifer*, ♂. A. Dorsal view. B. Oviger; scale bar=0.15mm. C. Third leg; scale bar=0.3mm.

Anoplodactylus pectinus Hedgpeth, 1948 [Fig 2.19E]

Anoplodactylus pectinus Hedgpeth, 1948: 234-236, fig.34.— Stock, 1955: 235, fig. 11; 1975: 1050, 1052, fig. 41a.— Child, 1979: 47[key], 58.

Material examined.—Great Palm Island, Cannon Bay, reef flat, 2 m depth, in *Sargassum* sp., 4 Feb 1999, 1 ♂ with eggs.

Description.— Trunk 0.96 mm long, 0.62 mm wide; fully segmented, fine segmentation lines, all body smooth and glabrous, crurigers separated by their own diameter except the third and fourth which are separated by less than half their diameter, fourth pair of curigers smaller. Ocular tubercle slightly projecting forward, blunt tip, eyes on top, dark-pigmented. Abdomen long, curved upwards. Proboscis cylindrical, constricted at midpoint. Chelifores long, slender, as long as proboscis; small, delicate chelae in front of oral surface. Ovigers typical, third segment with basal constriction, mucous ring placed right at constriction holding mass of eggs. Legs slender, short ventral setae all along legs, single dorsal row of setae on tibiae, single long distal spine on femur and tibiae; short tarsus with ventral spinules; propodus cylindrical, with a dorsal long spine, the most distal heel spine the largest, pectinate, with six or seven denticulations; one smaller heel spine proximally; small propodal lamina; claw about half the length of the propodus; tiny auxiliary claws.

Distribution.— This common shallow-water (0-34 m depth) pantropical species had not been recorded from Australia previously. It is known from many Indo-Pacific, western Atlantic and Caribbean localities.

Remarks.— *Anoplodactylus pectinus* is easily recognizable by the evident pectinate heel spine, otherwise it displays all basic features of mid size species of the genus. This specimen from Great Palm Island in the GBR shows a distinctive green colouration even after preservation.

***Anoplodactylus* n. sp. B** [Fig. 2.22]

Material examined.— Turtle Bay, intertidal in *C. prolifera*, 2♂, 4♀; 5 Oct 1998, 7♀, 2♂ with eggs; 14 May 1999, 1♂, 1♀; 7 Oct 1999, 1♀; 4 May 2000, 20♂, 31♀.—GBR, Orpheus Island, intertidal in *G. rugosa*, with cyanobacteria and sponges, 7 Sep 1998 1♂. Rib Reef, reef flat, 2 m depth, in rubble washings, 26 Nov 1998 1♀.— Townsville, Rowes Bay, intertidal in *C. prolifera*, 3 Nov 1998, 1♀; 1 Jun 2000, 28♂, 34♀.

Description.— Trunk of compact form, 1.34 mm in length, 0.9 mm wide; segmented, lines not strongly marked, dorsum smooth; crurigers separated by half their diameter, pointed tubercles on distal margin of all crurigers, those on fourth pair smaller. Ocular tubercle

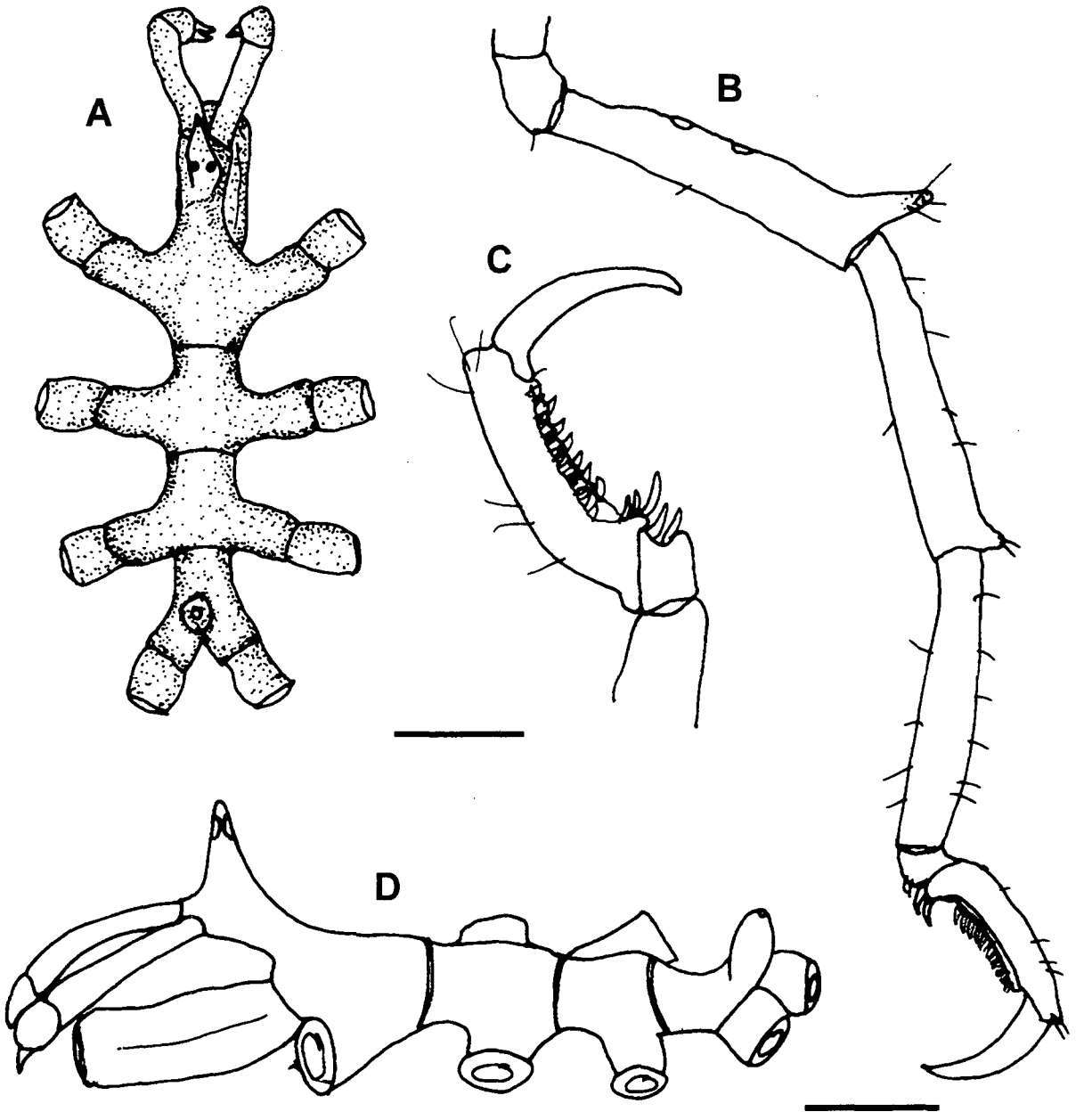

Figure 2.21. *Anoplodactylus longiceps*, ♂. A. Dorsal view. B. Third leg scale bar=0.4mm. C. Tarsus and propodus; scale bar=0.2mm. D. Lateral view of the trunk.

pointing anteriorly, well-pigmented eyes. Abdomen erect. Proboscis cylindrical, slightly tapering, upturned distally, two proximoventral protuberances in females. Chelifores as long as proboscis, scape one-segmented, single dorsodistal spine on scape, chelae right in front of oral surface, palms with short spines and setae, fingers slender and curved, gaping when closed. Ovigers six-segmented, third segment longest, with basal constriction, short setae on third and fourth segments, row of long setae on fifth segment. Legs slender, small setose anterior and posterior tubercles on first coxa, second coxa of third and fourth legs of males with ventral spurs, those on fourth pair larger, single dorsodistal spine on femur and tibiae,

femora swollen in females. Cement gland a dorsal tube on midpoint of femur; propodus strong, with heel, two robust heel spines, propodal lamina absent; long claw, no auxiliary claws visible.

Distribution.— This undescribed species has been found in localities of Townsville and coral reefs in the Central section of the GBR.

Remarks.— The specimens of this collection agree with *A. evansi* in the shape of the cement gland, the genital spurs and the ventral protuberances of the female proboscis but differ from Clark's description (Clark, 1963) in the trunk not as clearly segmented, with a narrower neck and ocular tubercle slightly inclined forward; the propodus is not as curved as in Clark's drawing, auxiliary claws are not visible, proboscis is more tapered in my specimens, and second tibia is longer; also there is a significant difference in size of the animals, these from North Queensland are less than half the size of *A. evansi* (Length 3.3 mm). *Anoplodactylus erectus* Cole, 1904 differs in the subcuticular extension of the cement gland and a lamina, otherwise is very similar.This is a remarkable species due to its exclusive high abundance in the green alga *Cladophora prolifera* from intertidal areas of Townsville. All the specimens have a broad dorsal chalky-white stripe contrasting with the green colour of the diverticula (Arango, in press).

Anoplodactylus tenuicorpus Child, 1991 [Fig.2.23]

Anoplodactylus attenuatus Child, 1988a: 12-14, fig 5; 1988b: 56 [preoccupied *Phoxichilidium attenuatum*.— Hodge, 1864].

Anoplodactylus tenuicorpus n. comb.— Child, 1991: 142-143.— Stock, 1994: 67.

Material examined.—Rib Reef, slope, 8 m depth, 6 September 1998, 1♂ with eggs. Pandora Reef, 3-6 m in rubble with algae, 15 Jul 1999, 1♀; 28 Oct 99, 1♂; 19 Apr 2000, 1♂, 2♂ with eggs, 5♀, 1 juv.

Description.— Trunk length 2.10 mm, width 1.14mm; fully segmented, segmentation lines very fine; body smooth, glabrous, elongated, tenuous; crurigers separated by almost six times their diameter. Ocular tubercle low protuberance anteriorly on cephalon, eyes on top of tubercle, not dark pigmented. Abdomen erect, of medium size. Proboscis short, straight, slightly constricted distally. Chelifore scape one-segmented, very slender, with distal short spine; chelifores longer than proboscis; palm small, as slender as scape, with few setae, immovable finger with 4-5 fine teeth. Ovigers six-segmented. Legs very long, slender, femur and second tibia subequal, first tibia shorter, about same length as coxae and tarsus. Legs glabrous except for long spinule distally on femur and tibiae; propodus cylindrical, long, without heel, with short dorsal spinule, three heel spines, pectinate, fine sole spines. Long

main claw more than three-quarters the length of the propodus, auxiliaries absent. Cement glands five low cups dorsally on femur.

Distribution.— This very slender species has been found at the Aldabra Atoll, Indian Ocean, Indonesia, Philippines, Papua New Guinea and Guam. The deepest records are this report and that from Guam of 10m depth. Most of the records describe very similar reef habitats for this species.

Remarks.— *Anoplodactylus tenuicorpus* is easily recognized by its tenuous shape. A similar species known in Australia might be *A. longiceps*, which shares the presence of two cement gland cups but is not that elongate; *A. pectinus* have also a pectinate heel spine but that is a more robust and smaller species. *Anoplodactylus exaggeratus* Stock, 1994 is probably the most similar, it is known from Indonesia and differs mainly in bearing a single cement gland.

Anoplodactylus tubiferus (Haswell, 1884) [Fig. 2.24]

Phoxichilidium tubiferum Haswell, 1884: 1032, pl. 57, figs. 1-5.

Anoplodactylus tubiferus.— Cole, 1904: 288.— Loman, 1908: 72.— Flynn, 1920: 79-81, pl. 10, figs. 12-14, pl. 11. Fig. 15.— Williams 1941: 35.— Clark, 1963: 49.

Material examined.— Cleveland Bay, 15 m depth, dredged, ?/9/99, 3♀, 1♂ with eggs, 2 juv (coll. J. Cruz).

Description.— Trunk length 1.84 mm, width 1.26 mm; completely unsegmented, smooth; crurigers separated by almost twice their diameter, twice longer than wide, with long fine distal spines. Ocular tubercle extremely tall (0.64 mm), slender, straight, pointing upwards; eyes not pigmented. Abdomen subequal to ocular tubercle, erect, slender, slightly inclined downwards from the base. Proboscis long, straight, placed at 45° degrees, with minor middle-constriction, somewhat similar to *Endeis* but not inflated. Chelifore scape one-segmented, long, slender, with long dorsal setae and spinules; small chelae, fingers well curved and smooth. Ovigers six-segmented, slender, first and second segments more robust than others, third segment the longest, glabrous. Legs slender, not long, femur thicker than other segments, all with dorsal fine spines, longer spines distally on femur and tibiae. Cement gland more than twice the length of segment diameter, protruding diagonally from femur; short tarsus, propodus as long as half of the second tibia, without prominent heel, one large heel spine and small uniform sole spines, no propodal lamina; main claw almost as long as propodus; minute auxiliaries on each side of the base of the claw.

Distribution.— This long-known species was first reported from Queensland, Australia by Haswell (1884). It has also been collected in the north of New South Wales according to museum specimens at the AM. It is distributed in a wide area from the Australian coasts to

places in the Indo-west Pacific, Madagascar, the Persian Gulf, the Philippines and Japan. It is known from a wide a range of depths from 2 to 235 m.

Remarks.— Males are fairly easy to identify due to their extremely long cement gland tube. The unsegmented pattern of the trunk and the extremely tall ocular tubercle and abdomen also help to discriminate the species.

Anoplodactylus versluysi Loman, 1908 [Fig.2.25]

Anoplodactylus versluysi Loman, 1908: 73-74, pl. 3, figs. 33-39.— Stock, 1954: 84-85, fig. 38a; 1968: 50 [text].

Material examined.— Pandora Reef, 3-6 m, in fouled rubble, 19 Apr 2000, 3♀,4♂.

Description.— Trunk length 2 mm, 0.9 mm wide; partially segmented, third line not visible dorsally, body smooth, elongate; crurigers separated by twice their diameter, fourth pair smaller; ocular tubercle tall, of conical-shape, acutely pointed, eyes pigmented . Abdomen erect. Proboscis cylindrical with inflation at midpoint; female with four ventral protuberances. Scape long, one-segmented, as long as proboscis, with dorsolateral setae, chelae setose, fingers curved, slender, gaping when closed. Ovigers six-segmented, third segment longest, with basal constriction, with short erect setae; long setae on segments five and six. Legs long, first coxa with one anterior and two posterior spines, two pairs of spines on second coxa, in males third and fourth legs with long ventral genital spurs, in females low protuberance on all legs decreasing in size from the fourth to the first pair; femur and first tibia with single distal spine, short duct dorsally on mid-femur as cement gland, ventral row of setae on second tibia. Propodus of medium size, curved, with heel, setose distally, with three heel spines, one longer than the other two, about 13 sole spines, claw long, curved, with tiny auxiliary claws.

Distribution.— This is a new record for Australia. *Anoplodactylus versluysi* is so far restricted to Madagascar and Indo-West Pacific localities.

Remarks.— These specimens from Pandora Reef coincide with descriptions by Loman (1908) and Stock (1954). The main difference lies in the type of habitat in which they were collected since *A. versluysi* was known from specimens collected in trawls rather than as a reef species.

Genus *Endeis* Philipi, 1843

Medium-sized species. Trunk elongated, segmented, most species with a "collar" at the joint of proboscis and neck. Chelifores and palps lacking. Ovigers seven-segmented, only in males, without spines or with small recurved spinules on distal segments; without terminal claw. Legs long, slender, some species with visible caeca, propodus long, with strong auxiliary claws. Cement gland outlets one or two longitudinal rows of lateral pores.

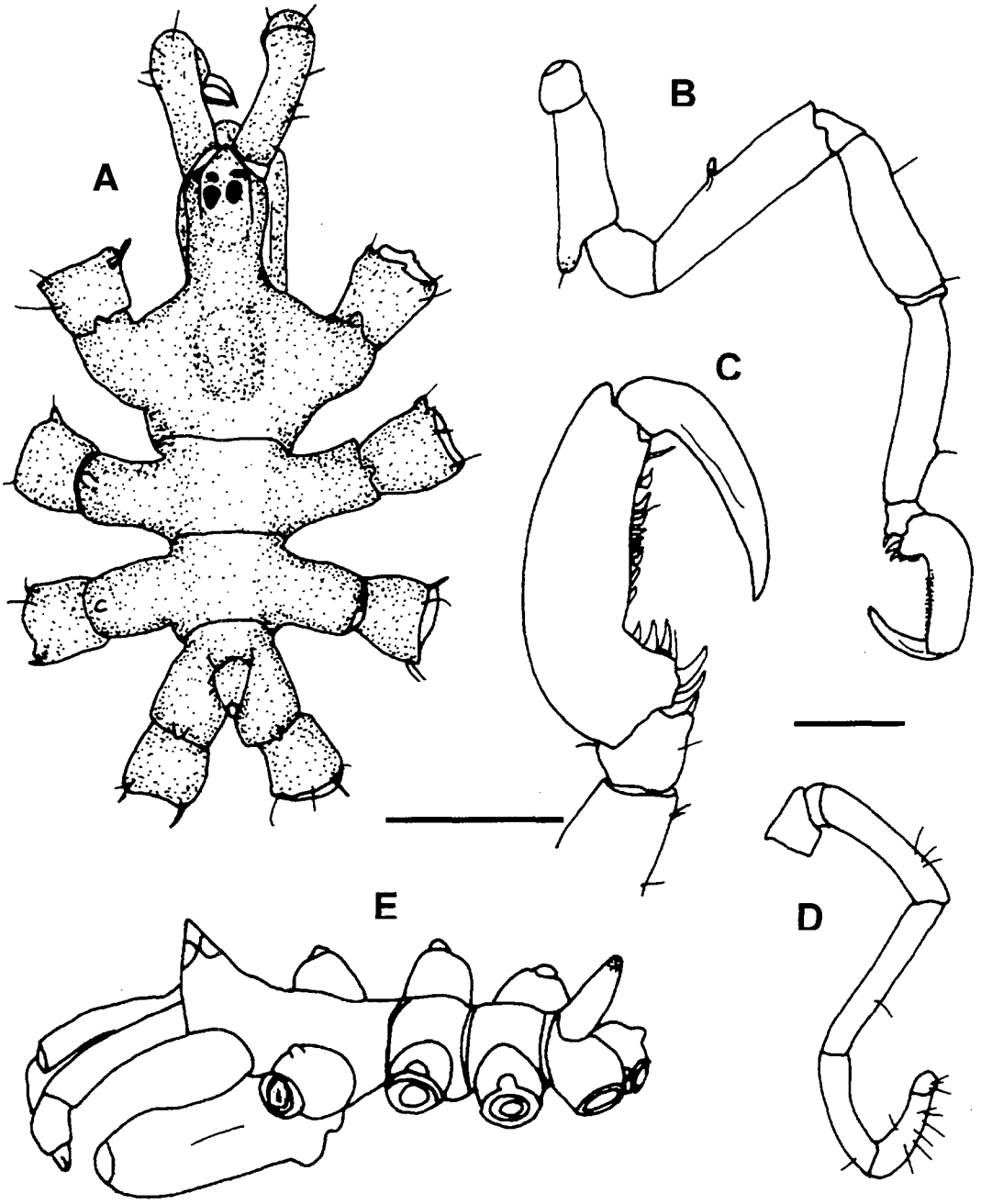

Figure 2.22. *Anoplodactylus* n. sp. B. ♂. A. Dorsal view. B. Third leg; scale bar=0.4mm. C. Tarsus and propodus. D. Oviger; C-D scale bar=0.3mm. E. Lateral view of ♀ (paratype) showing ventral protuberances on proboscis.

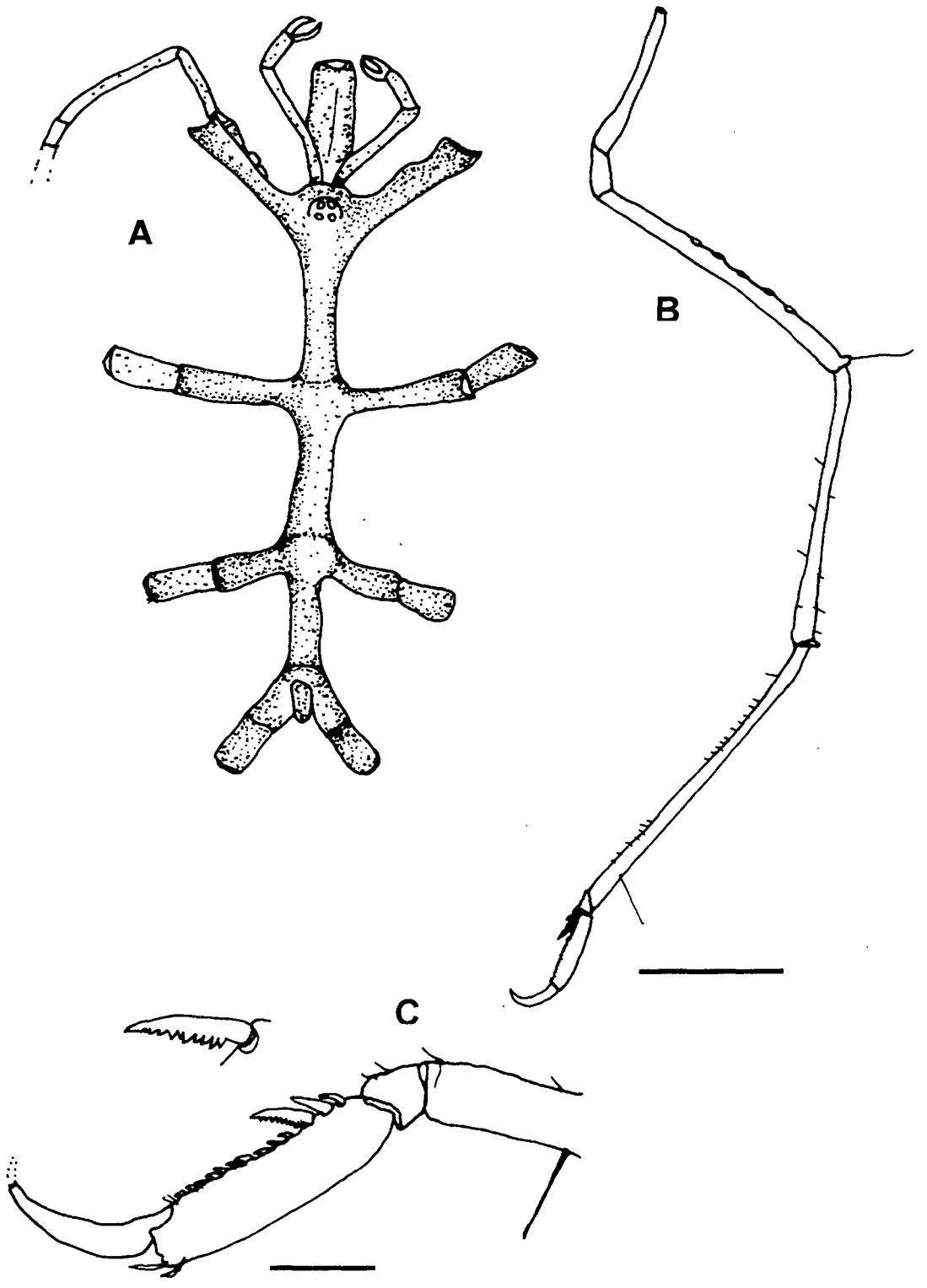

Figure 2.23. *Anoplodactylus tenuicorpus*, ♂. A. Dorsal view. B. Third leg with cement glands; scale bar=0.6mm. C. Tarsus and propodus with detail of pectinate spine (sketch by hand); scale bar=0.2mm.

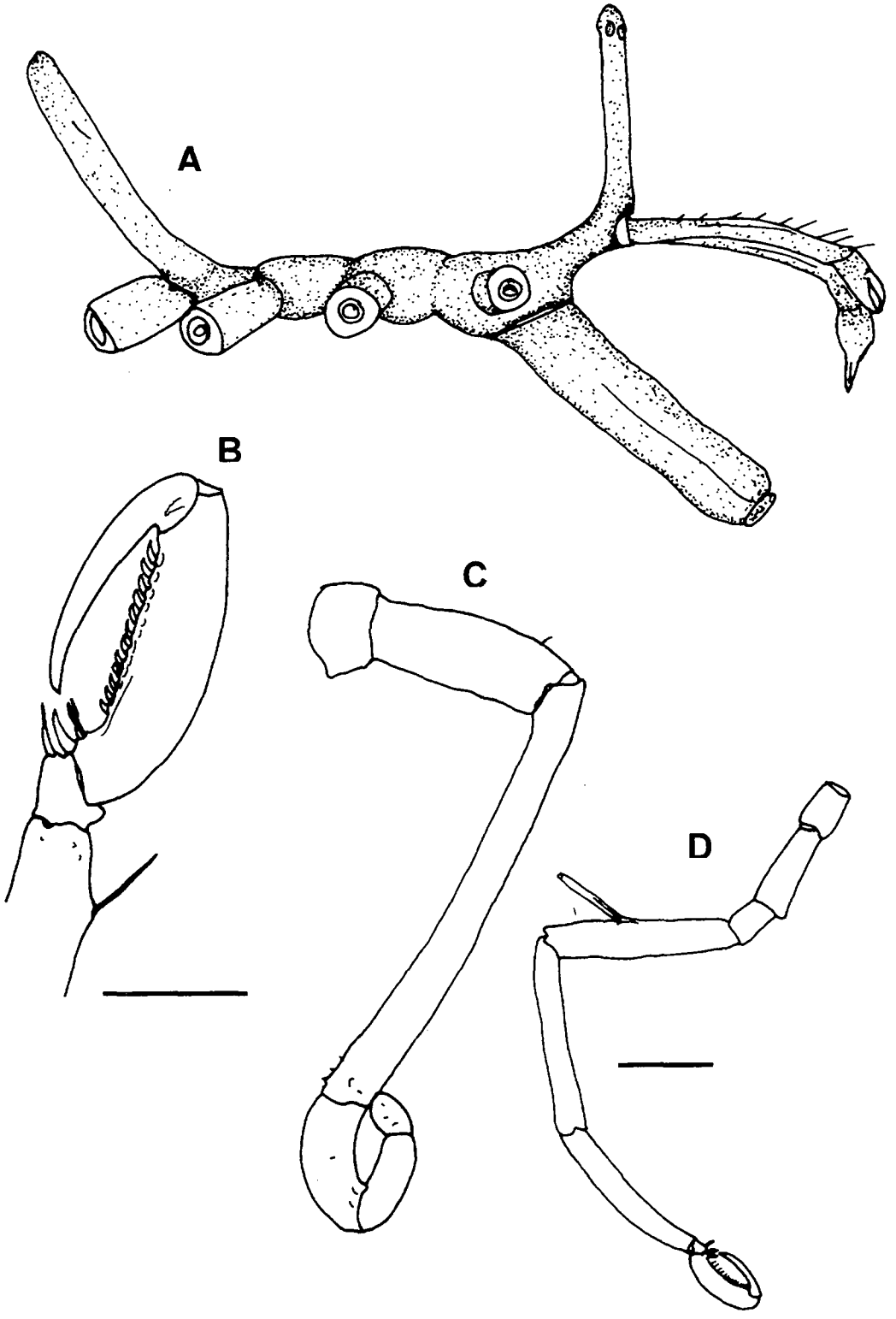

Figure 2.24. *Anoplodactylus tubiferus*, ♂. A. Lateral view. B. Tarsus and propodus. C. Oviger; scale bar=0.3mm. D. Third leg with cement gland; scale bar=0.7mm.

Endeis biseriata Stock, 1968 [Fig. 2.26]

Endeis biseriata Stock, 1968: 57-60, fig. 21; 1979: 28-30, fig. 9; 1992: 134.

Endeis biserata [sic.].— Child, 1988: 20; 1990: 332-333.

Material examined.— Pandora Reef, 4-6 m depth, in rubble, 7 Mar 2000, 1♂; 19 Apr 2000, 1♀,1♂. Goold Island, reef slope, 7 m, in rubble and algae, 18 Apr 2000, 1♀, 1♂; 24 Aug 2000, 6♂, 3♀. Rib Reef, reef slope, 8 m, in *Palythoa* sp, 8 Jul 2000, 1♀.—Lucinda jetty, 3 m depth, in pilings scrapes, ?/9/99, 7♀, 4 ♂ with eggs, (coll. J. Cruz) .— Townsville Marina, on fouling panels with bryozoans and hydroids, 2 m depth, 13 Oct 1999, 1♀ (coll. Staff JCU).

Description.— Trunk length 2.5 mm, width 1.3 mm; fully segmented, elongate shape; cephalon with collar; crurigers separated by twice their diameter, with two tubercles distally. Ocular tubercle tall, conical shape. Proboscis typical of *Endeis*, long, inflated at the middle with short setae around oral surface. Ovigers seven-segmented, fifth the longest, smooth, but small spinules on distal segments. Legs long, second tibia the longest, femur with long distal tubercle enclosed by two low ones on distal margin, both tibiae with dorsal row of setae; propodus curved, without heel, three heel spines, eight sole spines, claw half the size of the propodus, auxiliaries half the size of main claw. Cement glands 40-42 pores distributed in two irregular rows laterally on each femur.

Distribution.— *Endeis biseriata* was first found in Australia by Child (1990) at Lizard Island, in similar conditions to those reported here. This is an Indo-West Pacific species distributed from the Red Sea and Madagascar to Indonesia, the Philippines and Hawaii. It might have a pantropical distribution. These collections are within the depth range known for the species.

Remarks.— These collections from several reefs of the central section of the GBR added to the Lizard Island record suggest that this species could be common and continuously distributed in tropical North Queensland. The size and relative high abundance allowed observations of preference of substrate and feeding behaviour. A total of 22 adults of *E. biseriata* were found exclusively on the zoanthid *Protopalythoa* sp. Some adults were seen inserting the tip of the proboscis into a polyp and remaining attached for about one minute or crawling from one polyp to the other repeatedly inserting the proboscis (Arango, 2001). Concentration profiles of ecdysteroids (ES) in *Endeis biseriata* and *Protopalythoa* sp. were examined in collaboration with Dr. Tomaschko at the University of Ulm, Germany (following methodology in Tomaschko and Bückmann, 1993), but the concentration of ES observed in *E. biseriata* was too low compared to that observed in *P. litorale* to assume any ecological function of these specific compounds. The possibility that palitoxins or other metabolites might be involved in chemical protection cannot be discarded.

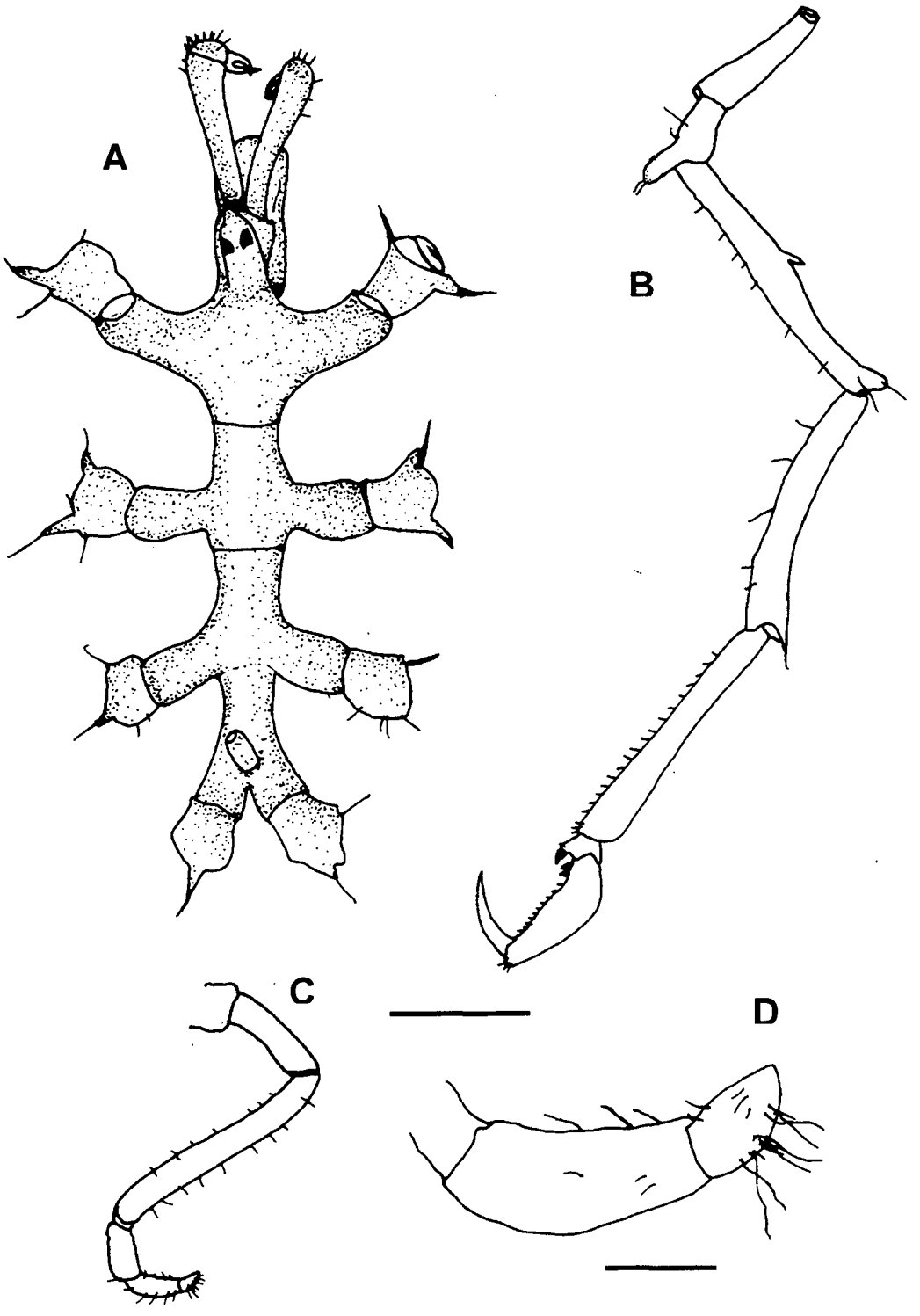

Figure 2.25. *Anoplodactylus versluysi*, ♂. A. Dorsal view. B. Third leg. C. Oviger; scale bar=0.5mm. D. Terminal segments of the oviger; scale bar=0.1mm.

Endeis flaccida Calman, 1923 [Fig. 2.27]

Endeis flaccida Calman 1923: 295, fig. 17.— Child, 1979:66 (refs.).

Material examined.— Cape Ferguson, Turtle Bay, intertidal in *C. prolifera*, 12 July 1999, 1♀.— Cairns Marina, fouling panels with bryozoans and hydroids, 2 m depth, Nov 99, 16♂, 21♀, 9 juv.; Feb 2001, 30♀, 29♂ 17 with eggs, 3 juv. (coll. O. Floerl).

Description.— Trunk 1.04 mm long, 0.4 mm wide; segmented, smooth; crurigers separated by their diameter. Ocular tubercle broad, conical shaped, taller than abdomen, with yellowish-pigmented eyes. Abdomen erect. Proboscis short, slightly tapering distally, crown of short setae around oral surface. Ovigers seven-segmented, sixth and seventh segments with few recurved spinules. Legs not long, femur and second tibia subequal, caeca in pockets along the legs visible through cuticle; first coxa with distal pointed tubercle, three pointed spines distally on femur, one dorsal spine on first tibia and short setae on second tibia. Propodus curved, strong heel, three heel spines, six sole spines. Main claw half the length of propodus, auxiliaries 75% the length of the main claw.

Distribution.— *Endeis flaccida* has not been recorded from Australia before, however, there are unreported records of sea spiders collected in Queensland and New South Wales identified as *E. flaccida* (sic. *E. flaecida*). This is a widely distributed species known from the Indo-West Pacific region, the coasts of South Florida and both sides of the Isthmus of Panama.

Remarks.— The species is recognizable by the combination of visible conglomerate guts especially on femur and tibiae and by the absence of prominences or long spines. Depth and habitat are similar to where the species has been found before. In this study *Endeis flaccida* occurred amongst hydroids on fouling panels settled at Trinity Inlet in the Cairns Marina, North Queensland (O. Floerl –JCU- pers. comm.). It seemed to be seasonally abundant between November and March when most of the males collected were ovigerous.

Endeis mollis Carpenter, 1904 [Fig. 2.28]

Endeis mollis Carpenter, 1904: 182-183, figs. 1-7 .— Stock, 1968: 58-59 (text and key) .— Child, 1979: 66 (refs.).

Material examined.— Rib Reef, 8 m depth, on *Millepora exaesa* , 27 Jun 2000, 2♀; 26 Jul 2000, 2♀; 7 Jul 2000, 1♂; 8 Jul 2000, 3♂, 6♀; 26 Aug 2000, 1♂.

Description.— Trunk 2.8 mm in length, 1.21 mm wide, fully segmented, elongate shape, all individuals show two chalky white dorsal lines from base of ocular tubercle to base of abdomen, white stripes along the legs contrasting with spotted green-coloured diverticula, even after preservation. Ovigers seven-segmented, second segment longest, single spinule on segments six and seven. Legs long, smooth, femur and tibiae subequal, 1-2 distal spines

on first coxa, femur slightly swollen distally; tarsus very small with few spines; propodus cylindrical, blunt, distal margin with four spines, without heel, three heel spines, 9-10 sole spines well-developed; auxiliaries longer than half length of claw. Cement glands 22-24 pores distributed in lateral single row on femur.

Distribution.— First described from the Gulf of Manaar (Sri Lanka), *Endeis mollis* is now known as a circum-tropical species being common in the Indo-west Pacific and the Caribbean. The species has been collected as far as the Izu Peninsula, in Japan (Nakamura and Child, 1983). The apparent difficulties in differentiating the species from *E. meridionalis* or other closely related species may confound the pattern of distribution.

Remarks.— Examining reports and museum samples of *E.mollis* I notice there is some ambiguity differentiating *E. mollis* and *E. meridionalis*. *Endeis mollis* was described by Carpenter as a smooth species, more glabrous and less spiny than *E. meridionalis*. The latter has a strong spine on mid femur, small spinules on the collar and the second tibia is subequal to the femur (Carpenter, 1904; Stock, 1965). These characters are not present in my specimens of *E. mollis* however; identical unreported specimens at the Australian Museum have been identified as *E. meridionalis*. There is variation in the number of cement gland pores between males collected at Rib Reef (24 pores) and Goold Island (22 pores), although only one male is available from Goold Island. The apparent variation of this character between such nearby populations suggests its diminished usefulness at the time of raising new species based on number of cement gland pores.

Family Pycnogonidae Wilson, 1878

Genus *Pycnogonum*, Brünnich, 1764

Small species. Trunk and legs stout, sometimes with overall reticulate or tuberculate pattern, segments short. Ocular tubercle low, rounded. Abdomen short, horizontal. Proboscis barrel-shaped, short. Chelifores and palps lacking. Ovigers reduced in size, eight- or nine-segmented, only in ♂ or lacking entirely, without spines or terminal claw. Legs very short, robust; propodus with spines, main claw large, curved, most with auxiliary claws. Cement glands unknown.

***Pycnogonum* n. sp.** [Fig. 2.29]

Material examined.—Coral Sea, Holmes Reef, reef drop off, 18 m, in *Amphiroa* sp., hydroids and sponges washing, 18 Sep 1998, 1 ♀ (coll. G. Diaz-Pulido).

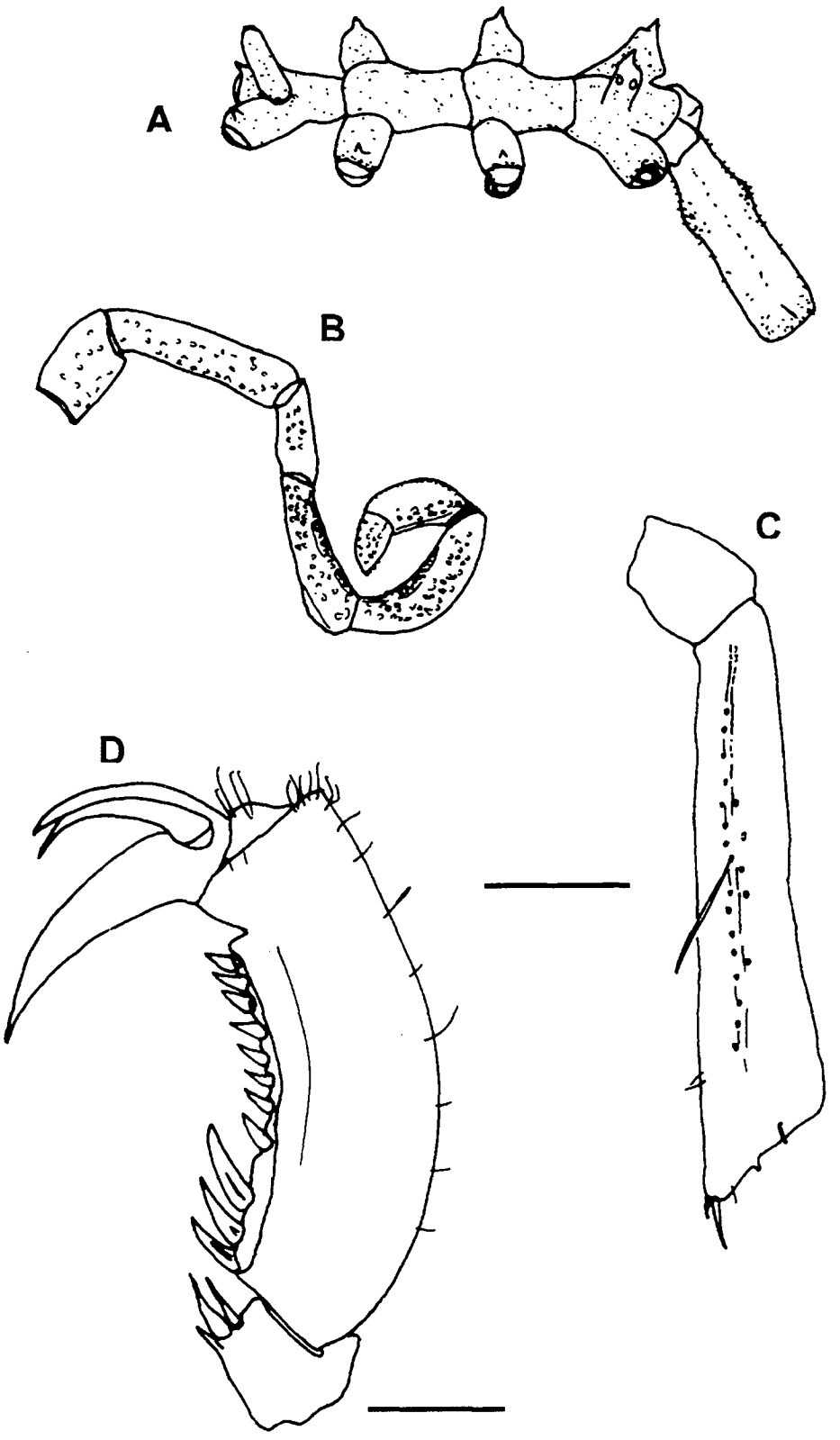

Figure 2.26. *Endeis biseriata*, ♂. A. Lateral view. B. Oviger. C. Femur with cement gland pores; scale bar=0.8mm. D. Tarsus and propodus; scale bar=0.15mm.

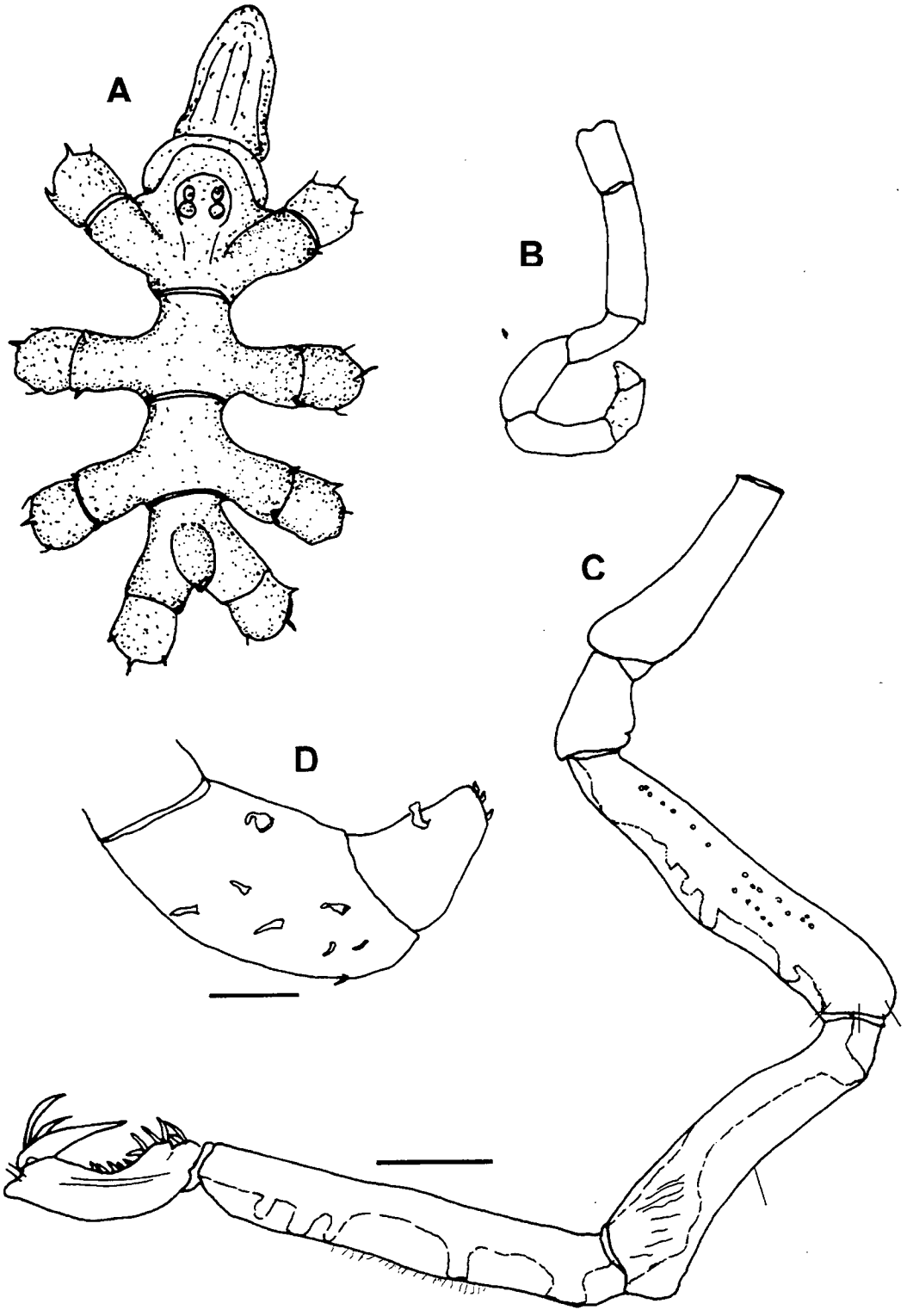

Figure 2.27. *Endeis flaccida*, ♂. A. Dorsal view. B. Oviger. C. Third leg with internal diverticula; scale bar=0.5mm. D. Terminal segments of the oviger; scale bar=0.1mm.

Description.—Trunk 1.26 mm in length, width 0.58 mm; fully segmented, compact, cuticle granulate; segments with cowlings, posterior margins raised over next segment;, three stout dorsomedian tubercles, smooth collar at base of proboscis; crurigers almost touching, fourth pair smaller and pointing backwards, broad distal tubercle on each cruriger, those on last pair larger. Ocular tubercle very low, rounded, anterior eyes not pigmented, granulate. Abdomen horizontal, joined ventrally to last trunk segment, with dorsal tubercle or bump. Proboscis cylindrical, distally tapering, with dorsal tubercle. Legs short, robust, first pair somewhat longer, all coarsely granulated, dorsal row of coarse granules, femur not so densely granulated, single distal long spine on femur and tibiae; propodus as long as first tibia, without heel, robust main claw, half the length of the propodus.

Distribution.—Only known from Holmes Reef in the Coral Sea.

Remarks.— This specimen has similarities with *P. asiaticum* Müller, 1992 described from Malaysian coral reefs in the shape of the trunk and pattern of dorsal tubercles. But the new species does not have auxiliary claws and does not have the papillose pores covering the body. *Pyconogum saxulum* Child, 1998 another West Pacific reef species, resembles *Pycnogonum* sp. in the lack of auxiliaries but it is more compact and the median tubercles are of 'pebbly' appearance (Child, 1998a). The presence of a tubercle on the proboscis and on the abdomen, and the lack of auxiliaries do not allow me to fit the specimen in any of the known species of *Pycnogonum* (Arango, in press).

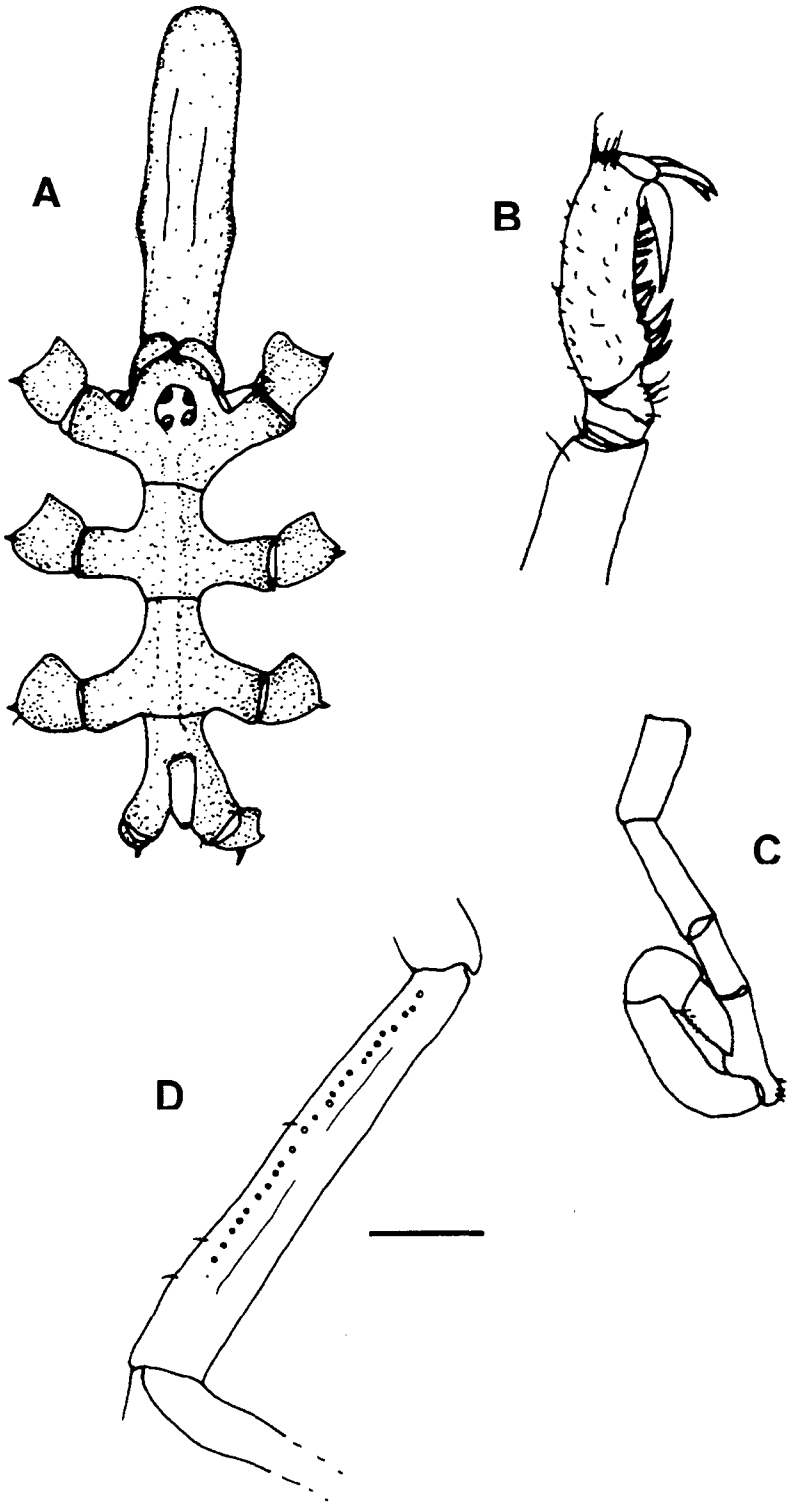

Figure 2.28. *Endeis mollis*, ♂. A. Dorsal view. B. Tarsus and propodus. C. Oviger. D. Femur with cement gland pores; scale bar=0.7mm.

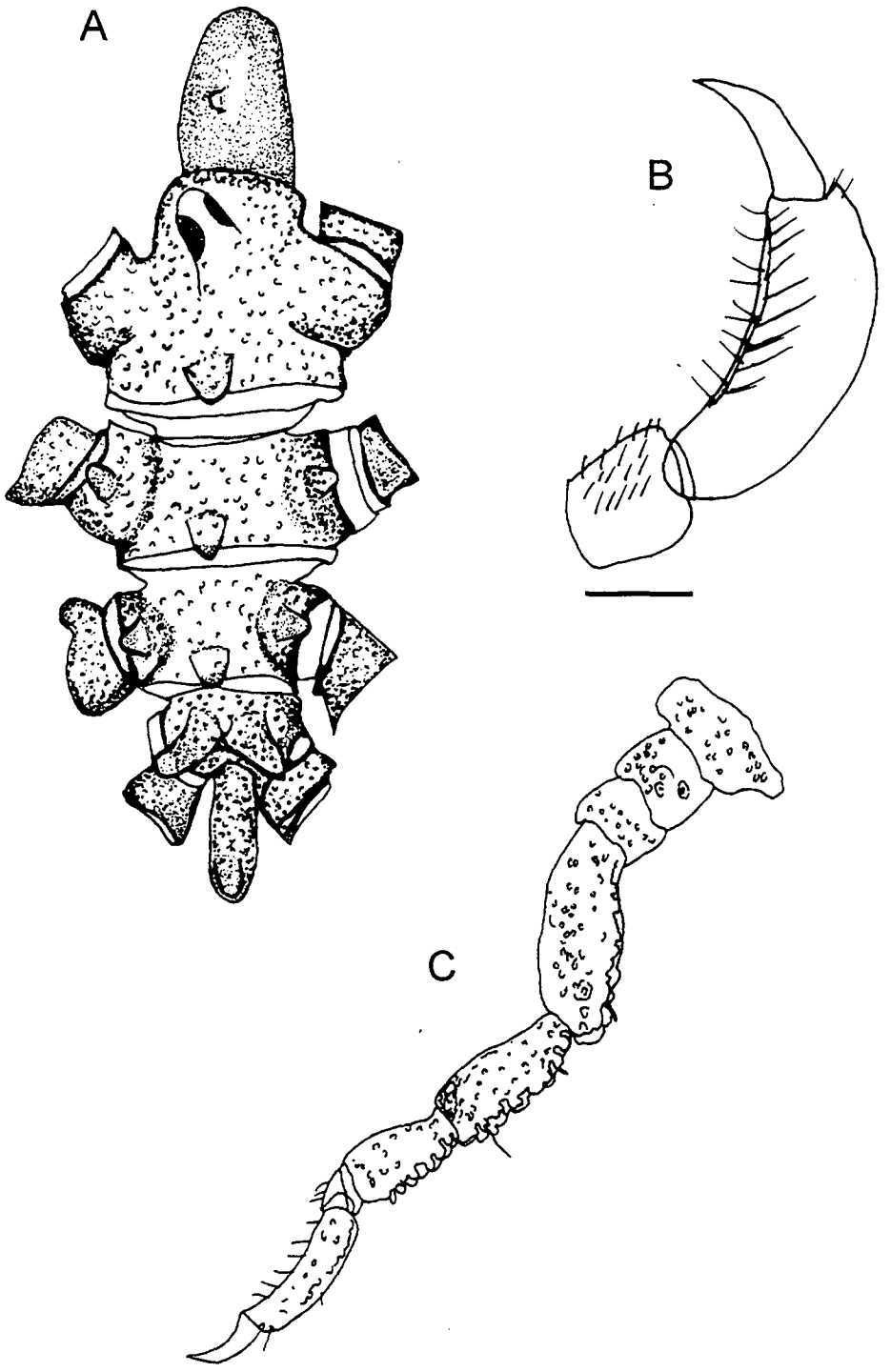

Figure 2.29. *Pycnogonum* sp. ♀. A. Dorsal view showing tubercle on proboscis and abdomen. B. Tarsus and propodus; scale bar=0.03mm.. C. Third leg.

2.5 Discussion

2.5.1 Observations on habitats studied

The green alga *Cladophora prolifera* was shown to shelter a diverse and abundant community of sea spiders at least in the intertidal habitats sampled. Fourteen species were collected amongst *C. prolifera*, four of them undescribed. Algae have been considered a good habitat for sea spiders but most of the studies relate to temperate areas, particularly the Mediterranean (see Arnaud and Bamber, 1987). *C. prolifera* in tropical North Queensland could be taken as a similar case to the brown alga *Halopteris scoparia* (see De Haro, 1978) or *Cystoseira* spp., known to harbor similarly diverse pycnogonid communities in the Marseilles area and French waters (see Arnaud and Bamber, 1987). In the neotropics, Caribbean seagrass beds are known to support a diverse fauna (Child, 1979; Stock, 1975a), however, only a single individual of *Ammothella stauromata* and two possible *Anoplodactylus* larvae (not reported) were found in the few shallow seagrass beds sampled along the coast of Queensland. However, the much lower density and biomass of seagrass beds in the eastern coast of Australia compared to those in the Caribbean should be noted. Other known Caribbean vegetal habitats, such as the macroalgae *Laurencia* sp, and *Sargassum* spp., were also found to be substrata for sea spiders in the Great Barrier Reef and Coral Sea reefs. It remains to be seen if the pattern of diversity and abundance is consistent in *C. prolifera* populations at least in other Queensland localities. The fact that Turtle Bay (or AIMS beach) is an extremely protected area with very restricted human access might have some influence in the diversity observed. Sampling for mites (Acari) was also very successful at the same site (J. Otto, pers. comm.).

In the GBR, a remarkable site sampled was Pandora Reef, an inshore bank reef in the central section of the GBR in which rubble at 4-6 m depth was found to support a diverse and abundant pycnogonid community. Eleven species of pycnogonids representing five of the eight families and including a new species of *Austrodecus*, were recorded from this particular site. All came from a small area on the southern, windward, side of the reef. Coral rubble fouled by coralline algae, zoanthids and other sessile fauna was the substrate sampled at this particular reef. The relative high abundance of larger species at Pandora Reef, compared to other similar habitats drew the attention especially to *Endeis* species, found at this and other reefs such as Goold Island and Rib Reef. Ecological interactions of tropical sea spiders are very little known (but see Varoli, 1994), a few species have been found associated with molluscs (Arnaud and Bamber, 1987) and echinoderms (Stock, 1975a, 1979; Sloan, 1979) but the feeding habits or host specificities are not well known. Most of the ecological information available comes from temperate species that are shown to be predators principally of

bryozoans (Ryland, 1976), actinians (Mercier and Hamel, 1994) and hydroids (Staples and Watson, 1987). The report of the occurrence and feeding activities of *E. mollis* and *E. biseriata* on the fire coral *Millepora exaesa* (Hydrozoa) and the zoanthids *Palythoa caesia*, *Protopalythoa* sp. and *Parazoanthus* sp (Anthozoa) at the GBR (Arango, 2001) suggests that established populations of pycnogonids in coral reefs and other tropical habitats might have ecological significance as predators and possible mediators of chemical interactions as suggested for some species from temperate waters (Sheerwood et al., 1998; Rogers et al., 2000).

2.5.2 Biogeographical comments

Of the 33 species reported here, 24 are known from Indo-West Pacific localities and four are considered pantropical species (see Table 2.2). These numbers support Child's conjecture of similarities between the pycnogonid fauna from the Great Barrier Reef and that from a tropical array north of the Equator (Child, 1990), with very little or no affinity with New Zealand or South American Pacific faunae. Shallow-water pycnogonid communities seem to have similar composition, at least at genus level, in the Indo-West Pacific, tropical Australia and the Caribbean. This is especially evident with the ammotheid genera such as *Ammothella*, *Tanystylum* and *Eurycyde* (the latter not present in this collection but recorded from the GBR by Child, 1990). Species of *Anoplodactylus*, mainly the small, compact forms related to the *pygmaeus*- group (Stock, 1975b), and *Callipallene* species, are also characteristic of shallow tropical habitats. *Achelia* seems to have a wider distribution both latitudinal and bathymetrical, but forms such as *A. assimilis* are frequent dwellers of the tropical coasts.

Seemingly, there has been more collecting effort on pycnogonid fauna (or more reports, at least) to the north than in most Australian waters. The 15 new records for Australia in this regional collection support this notion (Table 2.2). I recognise that some of these species reported as new records might have been collected before and deposited in Museum collections as unidentified material, or may have been identified but not reported. Such is the case of the *Endeis* species and some *Anoplodactylus* (unreported records at AM). Also species such as *Nymphopsis acinacispinata*, a supposedly rare species collected only once (Child, pers. com.), has 17 records at the Australian Museum. This reflects a strong need for systematic revision of the Australian pycnogonid fauna: more than 500 lots of unidentified material have been deposited in museum collections.

Of the species collected here, five were known previously from Western Australia (Table 2.2). Child (1975) suggested similarities between the western and eastern coasts of Australia in terms of composition of the pycngonid fauna. Of the 29 species known from Western Australia, 12 also occur in the eastern coast. However, Child did not mention an evident

segregation between tropical and subtropical faunae or the different bathymetrical ranges of distribution. *Callipallene novaezealandiae*, *Achelia assimilis* and *Nymphopsis acinacispinata* can be considered widely distributed along shallow waters of the Australian coasts while *Pallenopsis hoeki* and *Anoplodactylus longiceps* are species known so far only from tropical localities in both western and eastern coasts. The former seems to prefer shallow water habitats but is rarely collected, while the latter is a broadly known tropical species with a wider bathymetrical range (0 to 134 m) (Child, 1975). This shows the importance of establishing comparable latitudinal and depth boundaries or zones for the aim of fauna composition comparisons and zoogeographical inferences.

Along the Australian eastern coast it is difficult to establish a boundary between a north and a south sub-region of distribution of species, probably due to discontinuous collection effort. *Pigrogromitus timsanus*, *Propallene saengeri* and *Rhopalorhycnhus tenuissimum* are species that are expected to be found continuously distributed along the Queensland coast having been reported from the vicinity of Brisbane (Staples, 1982) and being known from northern Pacific localities. *Anoplodactylus tubiferus*, *Achelia assimilis and Nymphon molleri* are known from as far as South Australia (Clark, 1963; Stock, 1973b). Conclusions about the patterns of distribution and diversity of pycnogonids at least for the Queensland coast would be more complete after examination of a wide range of microhabitats in a continuous covering of the coast. Only then, will the systematic, biogeographical, and ecological scenarios for pycnogonids be better understood.

Table 2.2. Distribution of the species of pycnogonids collected, in Australia and worldwide. QL = Queensland, NSW = New South Wales, WA = Western Australia, SA = South Australia, NZ = New Zealand, S = Pacific coast of South America, NP = North Pacific up from 20°N, IO = Indian Ocean, IW = Indo-West, W = West Pacific Islands, M = Mediterranean, C = Caribbean. * Indicates species not collected in this study but known from North Queensland.

SPECIES	AUSTRALIA QL	NSW	WA	SA	NZ	S	NP	IO	IW	W	M	C
Ammotheidae												
*Ascorhynchus melwardi**	X	-	-	-	-	-	-	-	-	-	-	-
Ascorhynchus tenuirostris	X	-	-	-	-	-	-	-	-	-	-	-
Achelia assimilis	X	X	X	X	X	X	-	X	X	-	-	-
Achelia nana	X	-	-	-	-	-	X	X	X	-	-	-
*Ammothella prolixa**	X	-	-	-	-	-	-	-	-	-	-	-
Ammothella stauromata	X	-	-	-	-	-	-	-	-	-	-	-
Ammothella sp.	X	-	-	-	-	-	-	-	-	-	-	-
*Eurycyde setosa**	X	-	-	-	-	-	-	-	X	-	-	-
Nymphopsis acinacispinata	X	X	X	X	-	-	-	-	-	-	-	-
*Nymphopsis armatus**	X	-	-	-	-	-	-	-	-	-	-	-
Tanystylum haswelli	X	-	-	-	-	-	-	-	-	-	-	-
Tanystylum rehderi	X	-	-	-	-	-	-	X	X	X	-	-
Austrodecidae												
Austrodecus childi n. sp.	X	-	-	-	-	-	-	-	-	-	-	-
Colossendeidae												
Rhopalorhynchus tenuissimum	X	-	X	X	-	-	-	-	-	-	-	-
Nymphonidae												
*Nymphon draconis**	X	-	-	-	-	-	-	-	X	-	-	-
Nymphon micronesicum	X	-	-	-	-	-	-	-	X	-	-	-
Nymphon molleri	X	X	-	-	-	-	-	-	-	-	-	-
Uncertain (?) Family												
Pallenopsis hoeki	X	-	X	-	-	-	-	-	X	-	-	-
Callipallenidae												
Callipallene sp.	X	-	-	-	-	-	-	-	-	-	-	-
Callipallene novaezealandiae	X	-	X	X	X	-	-	X	X	-	-	-
Pigrogromitus timsanus	X	-	-	-	-	-	X	X	X	X	X	X
Propallene saengeri	X	-	-	-	-	-	X	-	-	-	-	-
*Seguapallene crassa**	X											
Seguapallene cf. *micronesica*	X	-	-	-	-	-	-	-	-	-	X	X
*Parapallene australiensis**	X		X	X				X				
Parapallene famelica	X	-	-	-	-	-	-	-	-	-	-	-
Phoxichilidiidae												
Anoplodactylus batangensis	X	-	-	-	-	-	-	-	X	X	-	X
*Anoplodactylus brucei**	X											
*Anoplodactylus chamorrus**	X	-	-	-	-	-	-	-	X	X	-	-
*Anoplodactylus cribellatus**	X	X										
Anoplodactylus sp A.	X	-	-	-	-	-	-	-	-	-	-	-
Anoplodactylus digitatus	X	-	-	-	-	-	-	-	X	-	X	X
Anoplodactylus glandulifer	X	-	-	-	-	-	X	-	X	-	-	X
*Anoplodactylus haswelli**	X	-	-	-	-	-	-	-	-	-	-	-
Anoplodactylus longiceps	X	-	X	-	-	--	-	-	X	X	-	-
Anoplodactylus pectinus	X	-	-	-	--	-	-	-	X	-	-	X
Anoplodactylus sp B.	X	-	-	-	-	-	-	-	-	-	-	-
Anoplodactylus tenuicorpus	X	-	-	-	-	--	-	X	X	X	-	-
Anoplodactylus tubiferus	X	X	-	-	-	--	X	-	X	X	-	-
Anoplodactylus versluysi	X	-	-	-	-	-	-	X	X	-	-	-
Endeis biseriata	X	-	-	-	-	-	-	X	X	X	-	-
Endeis flaccida	X	-	-	-	-	-	-	-	X	-	-	X
Endeis mollis	X	-	-	-	-	-	X	-	X	X	-	X
*Endeis straughani**	X	-	-	-	-	-	-	-	X	-	-	-
Rhynchothoracidae												
*Rhynchothorax vallatus**	X	-	-	-	-	-	-	-	-	-	-	-
Pycnogonidae												
Pycnogonum sp.	X	-	-	-	-	-	-	-	-	-	-	-

CHAPTER THREE

Cladistic analysis of the Pycnogonida based on morphological characters

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3.1 Summary

Phylogenetic relationships among the main lineages of extant sea spiders were studied using cladistic analysis of 36 morphological characters. This is one of the first attempts to analyze the higher level relationships of the Pycnogonida using cladistic techniques. The analysis tested the hypothesis of an evolutionary trend towards the reduction of the head appendages in the sea spiders. An exemplar method was employed, sampling 38 species from all the recognized families. A Devonian pycnogonid fossil was used as outgroup. A single polytomous most-parsimonious tree, obtained under the implied weighting method implemented by the program Pee-Wee, is presented as the preferred hypothesis of pycnogonid phylogeny. Two major clades of extant Pycnogonida are evident, one grouping the Ammotheidae with Austrodecidae, Colossendeidae and Rhynchothoracidae, the other major group including the Callipallenidae, Nymphonidae, Pycnogonidae and Phoxichilidiidae. The genus *Pallenopsis* appears as a basal branch of the latter. Different assumptions of multistate character transformations were used to trace the evolution of the head appendages. In general, unordered characters performed better than when ordered. It is shown that reduction or loss of the head appendages has occurred independently in each of the two main clades as parallel evolution events, not as a global sequential gradual reduction. Comparison of the relationships proposed here to traditional classifications supported most of the clades suggested by Stock. However, traditional taxonomic characters need to be supplemented by different sets of anatomical, molecular and developmental data, among others, to produce more robust phylogenetic hypotheses for both the higher and lower level relationships of the sea spiders.

3.2 Introduction

Monophyly of the Pycnogonida has been suggested in morphological and molecular phylogeny studies of the Arthropoda, in which representatives of at least four different families of sea spiders have been included (Giribet and Ribera, 2000; Giribet et al., 2001). A meagre fossil record indicates that sea spiders have existed since at least the Devonian (Bergström et al., 1980), and unidentified chelicerate larvae from the Cambrian have been

regarded as pycnogonid forms (Walossek and Müller, 1997). Although the Pycnogonida is clearly an ancient group, the age of the extant lineages is completely unknown.

Few published studies have addressed the phylogenetic relationships of the pycnogonids, either above or below family level, and none of them has used explicit cladistic analysis (but see Lovely, 1999). Hedgpeth (1947) proposed a classification of the pycnogonids based on the presence and complexity of the head appendages named chelifores, palps and ovigers. At that time, he stated it would be almost impossible to draw a family tree, referring to the failed attempts in separating some of the families due to the occurrence of 'transitional' genera presenting features of different high taxa. In the same study, Hedgpeth suggested a direction in the evolution of the group based on a gradual reductive trend. He regarded family Nymphonidae as the most 'generalized' lineage. Its members have functional chelae, 10-segmented ovigers and long palps. Pycnogonidae are considered the most 'specialized', due to the absence of all the head appendages in both sexes of some species (Hedgpeth, 1947) (see captions Fig. 3.1).

Fry (1978) examined relationships among pycnogonids and revised the generic classification. However, the unclear outcome of his phenetic analysis, which created about 20 new families, did not receive much support (Arnaud and Bamber, 1987; Munilla, 1999). A more recent attempt to develop a phylogeny of sea spiders resulted in a large number of equally-parsimonious trees, poorly resolved consensus trees and little resolution on the relationships of the known families (Bain, 1992 in Lovely 1999). 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* Broili, and on the assumption that 10-segmented appendages were the plesiomorphic state. He proposed a hierarchy of the pycnogonid families (Fig. 3.1A), reiterating Hedgpeth's ideas of a gradual reduction in the number of segments of the head appendages (Stock, 1994). In his recent summary of the knowledge of the evolution of sea spiders, Munilla (1999) concluded that pycnogonid phylogeny can be derived from the assumption of 'regressive evolution', the gradual loss of appendages segments over evolutionary time (Fig. 3.1B).

So far, the hypothesis of an evolutionary trend of successive reductions in the number of segments of the appendages has not been tested and the validity of the families as monophyletic groups needs to be reviewed. A first attempt using current cladistic techniques on 24 morphological characters of 24 genera showed the paraphyly of Ammotheidae as its most relevant result (Lovely, 1999), however character evolution was not analyzed and the reductive trend was not addressed. In this study, I test the assumption of a reductive trend in

the Pycnogonida and examine the monophyly of the currently recognized families of sea spiders using quantitative cladistic analysis.

Ordered multistate characters can be useful when assuming trends of reduction series (Wilkinson, 1992). For example, instead of assuming that all possible changes have an equal chance to occur, it is assumed that the head appendages went through a gradual reduction from the maximum number of segments to a complete absence. To test this assumption, the characters for the appendages (e.g. number of segments of palps, chelifores and ovigers) are ordered and the outcome of the analysis is compared with that of a unordered analysis, looking for the most parsimonious resolutions. However, although a testable hypothesis of the evolution of the characters can be proposed, it is not possible to address the polarization of the states since additional information, especially fossil descriptions are needed.

When dealing with higher level phylogenies, taxon sampling can be of major importance since it can affect resulting topologies (Bininda-Emonds et al., 1998). The Pycnogonida are characterized by a great morphological plasticity and there are exceptions for almost every morphological character state within families and genera (Thompson, 1904). The approach implemented in this study differs from previous analyses in that, as far as possible, genera polymorphic for the characters coded are represented by more than one species, instead of a single hypothetical supraspecific taxon (Yeates, 1995).

The objective of this study was to evaluate the traditional hypothesis of pycnogonid relationships based on morphological characters. The aim was to reexamine the classical taxonomic features used as synapomorphies to unite the major lineages of the Pycnogonida and test their monophyly. The use of different character coding methods allowed me to test the hypothesis of a reduction series of the head appendages, to examine how informative the characters are and to compare the results to existing classifications.

3.3 Materials and Methods

3.3.1 Taxon sampling

A total of 38 species belonging to 21 genera representing all recognized families of extant Pycnogonida plus the fossil species *P. problematicus*, were included as ingroup taxa in the present analysis (Table 3.1). For a matter of convenience, the traditional family assignments of pycnogonid genera have been used throughout the thesis while their validity is being tested. Ammotheidae and Callipallenidae, the most diverse lineages in terms of morphology and number of genera, are represented here by eight and four genera respectively (*Pallenopsis* included under conditions mentioned in Chapter 2). Colossendeidae is represented by a species of the type genus *Colossendeis* and by a species of *Rhopalorhynchus*. 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*, is represented by three species.

Several factors influenced the selection of species for the analysis. Firstly, most of the taxa included in the analysis were part of the collections made by the author in shallow water habitats of North Queensland and from the Colombian Caribbean (Arango, 2000). Additional taxa were kindly provided by collaborators in Australia and overseas and descriptions from the literature were used for those species not available (all material sources listed Appendix 3). Type genera and those genera abundant and/or of widest distribution were selected from each of the families. The genera for which more than one species were included due to intrageneric 'polymorphism' were *Achelia*, *Ammothella*, *Ascorhynchus*, *Cilunculus*, *Tanystylum*, *Callipallene*, *Austrodecus*, *Nymphon* and *Anoplodactylus*. I included 'transitional' or problematic taxa, such as *Pallenopsis*, *Tanystylum* and *Endeis*, whose taxonomic status had been a matter of debate, to provide a test for competing taxonomic hypotheses.

Most of the phylogenetically informative characters within the Pycnogonida are derived from structures absent in any other arthropod group (e.g. characters of ovigers and proboscis). For this reason it is very difficult to take outgroup relationships into account. *P. problematicus*, a fossil pycnogonid species from the Devonian (Fig. 3.3), was incorporated 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 known (Bergström et al., 1980). Characters of *P. problematicus* were coded according to published descriptions of the fossil specimens (Bergström et al., 1980). In the absence of algorithms that distinguish between inapplicable characters and missing data (Lee and Bryant, 1999), the use of fossil taxa offers some difficulties when coding detailed morphological characters (Kitching et al., 1998). However, it remains as the best option to provide a sister goup to the the extant Pycnogonida.

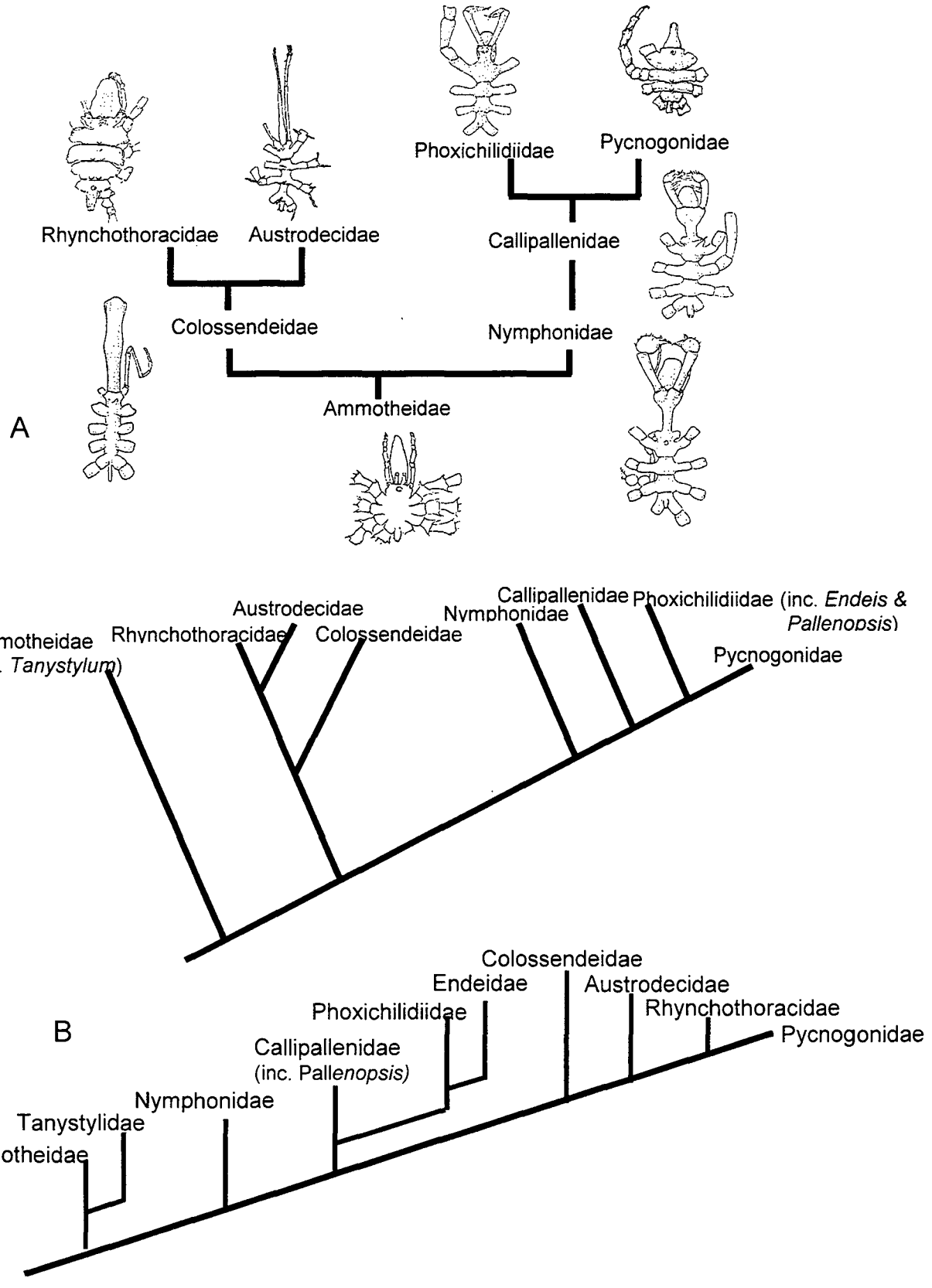

Figure 3.1. Phylogenetic hypotheses of relationships among pycnogonid families. A) Diagram from Stock (1994) with captions representing basic body plans of each of the lineages. Own interpretation of Stock's diagram below. Illustrations from Hedgpeth, 1948; Stock 1989, 1991. B) Phylogenetic diagram by Munilla (1999).

Table 3.1 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	6	7	8	9	0	1	2	3	4	5
Palaeoisopus problematicus	0	0	0	0	0	1	0	1	0	0	0	0	0	?	?	?	?	?	1	0	?	?	1	0	?	?	?	0	1	0	0	2	0	0	2	0
Eurycyde raphiaster	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
Achelia assimilis	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
Achelia australiensis	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
Ammothella sp.	0	1	1	1	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0	2	0	0	0	1	0	0	0	-	-	1	1	0	1	0	0
Ammothella biunguiculata	0	1	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0	0	2	0	0	0	2	0	0	0	-	-	1	1	0	1	0	0
Ammothea hilgendorfi	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
Tanystylum haswelli	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
Tanystylum rehderi	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
Nymphopsis acinacispinata	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
Ascorhynchus glaberrimus	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
Ascorhynchus ramipes	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
Ascorhynchus tenuirostris	0	2	1	0	0	0	0	0	1	0	1	1	0	0	0	0	2	1	0	0	2	0	1	1	2	0	0	1	-	-	0	1	0	1	2	0
Cilunculus armatus	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
Cilunculus sekiguchi	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
Nymphon micronesicum	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	0	1	0	0	1	0	0
Nymphon molleri	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	0	1	0	0	0	0	0
Nymphon surinamense	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	0	1	0	0	0	0	0
Colossendeis megalonyx	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
Rhopalorhynchus tenuissimum	1	-	-	-	0	0	0	0	1	0	1	1	0	1	-	-	-	-	0	0	0	0	1	0	0	0	0	1	-	-	2	0	0	2	0	0
Austrodecus glaciale	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
Austrodecus gordonae	1	-	-	-	0	3	0	3	5	0	4	0	0	0	0	1	2	1	1	0	0	0	1	0	2	0	1	0	-	-	0	3	0	2	0	0
Rhynchothorax australis	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
Callipallene novazelandiae	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	0	1	0	0	0	0	0
Callipallene brevirostris	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	0	1	0	0	0	0	0
Parapallene famelica	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
Propallene saengeri	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	0	1	0	0	0	0	0	0
Pseudopallene ambigua	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	2	0
Pallenopsis schmitti	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
Anoplodactylus sp.	0	2	0	0	1	-	1	-	4	1	-	0	1	0	0	1	0	1	1	0	2	0	0	0	3	1	-	-	1	1	1	0	1	1	0	0
Anoplodactylus batangensis	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
Anoplodactylus tenuicorpus	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
Anoplodactylus glandulifer	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
Anoplodactylus insignis	0	2	0	0	1	-	1	-	4	1	-	0	0	0	0	0	0	1	0	0	2	0	0	0	3	1	-	-	1	1	1	0	1	0	0	0
Anoplodactylus sp.	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
Anoplodactylus longiceps	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
Endeis mollis	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
Pycnogonum litorale	1	-	-	-	1	-	1	-	2	1	-	1	0	1	-	-	-	-	2	1	3	1	1	0	1	1	-	-	-	-	0	1	0	0	0	0

3.3.2 Characters

Thirty-six morphological characters of adult sea spiders were scored across the 38 species selected as exemplar taxa (see list of characters and character states in section 3.3.2.2, data matrix in Table 3.1). 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 (characters 5, 7, 8 and 10), the actual number present in each of the taxa 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 the character in question is absent in some of the taxa. In the pycnogonids, this is a major problem when coding external morphological features, specifically those of the head appendages and cement glands, since these structures are absent from many of the taxa. Reductive coding (Strong and Lipscomb, 1999) was used, denoting inapplicable characters with "-" (e.g. number of palp segments in those taxa having no palps). There are problems associated with the reductive coding, such as nonindependence and redundant absence states (Lee and Bryant, 1999). However, reductive coding, when analyzed under unambiguous optimization settings (default in the Nona and Pee-Wee packages), is the option that best reflects the information content of the data collected when inapplicable values need to be introduced to the matrix (Strong and Lipscomb, 1999).

All the sixteen multistate characters were initially treated as equally weighted and unordered. Even if the unordered analysis is justified by arguing that character transformations should not be assumed but tested by cladistic analysis (Hauser and Presch, 1991), different assumptions of character transformations (e.g. ordered or unordered) should be compared (Wilkinson, 1992). The disagreement between 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 –9-segmented –7segmented, 6 segmented, etc) were coded both as unordered and ordered and the results of the analyses were compared.

3.3.2.1 Character evaluation

The analyses were carried out without assigning any *a priori* polarity to characters. Outgroup relationships and polarization of characters in pycnogonids, are issues difficult to approach due to the lack of clear 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 it was 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.

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

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 (Winter, 1980). The presence of chelifores in adult pycnogonids (character 0) is considered a plesiomorphic state based on outgroup comparison (comparing them to chelicerates) and the ontogenetic criterion, since all pycnogonid larvae and juvenile forms have chelifores. Chelifores are completely functional in adult pycnogonids of all members of the families Nymphonidae, Phoxichilidiidae [excluding *Endeis* following Child (1992)], and most species of Callipallenidae. When present, chelifores of living 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 Ammotheidae, which contains a range from a few fully chelate species to species having no chelifores but just a single short segment on the front of the cephalon. The presumably 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 of teeth on the chelae (character 28) might have phylogenetic importance and have been coded among those forms bearing chelifores. The former is an apparently stable character within some genera of Ammotheidae (*Nymphopsis*, *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 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 grouped based on the presence-absence of chelifores, but it is unlikely, or at least not yet shown, that all the forms that have lost the chelifores before adulthood are phylogenetically related.

Palps

Considered homologues to pedipalpi in arachnids (Winter, 1980). Similarly to the chelifores, the absence of palps is a feature used to group 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 state of the palps in the fossil outgroup cannot be determined with complete certainty, but apparently it shows nine segments (Bergström et al., 1980) (Fig. 3.3). Ten- segmented palps are coded for some Ammotheidae (e.g. *Eurycyde* 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 and six segments are found in Ammotheidae, Austrodecidae, Rhynchothoracidae and some callipallenids. The number of segments of palps 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, their presence and number of segments have been coded separately for each sex to include differences observed in the callipallenid *Propallene* (characters 4, 5, 6, 7). A transformation series of the palps towards reduction is tested by coding the characters of number of segments as ordered.

Ovigers

Unlike the chelifores and the palps, there is no counterpart or possible homologous structure 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 have been assumed 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 in males and females were coded separately since they are sexually dimorphic. Females of Pycnogonidae, Phoxichilidiidae and *Endeis* lack ovigers completely. The terminal claw of nymphonids and some ammotheids and callipallenids, could be derived from the eleventh segment of the ovigers observed in the fossil. However, an ancestral condition within the group could be the presence of a terminal claw as remnant of the main claw of the propodus of the legs, retained during the modification of the ovigers, if ovigers are to assumed to be modified legs (Aranud & Bamber, 1987). Thus, the loss of the terminal claw could be seen 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 some 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 defend either a primitive condition or a specialized feature 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 distinguish 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 structures such as tridactyl claws believed to be ancestral and occurring in some chelicerate taxa (e.g. *Nothrus* sp., Acari) (Der Hammen van, 1986). The presence of heel spines is included to examine the phylogenetic informativeness of this character (character 22). The absence of heel spines in both clades A and B (Fig. 3. 4) appears as a parallel event of secondary loss.

Cement gland openings on the femora of males (in *Propallene* occurring on tibiae as well) are present in most pycnogonid taxa (character 13) suggesting it is the plesiomorphic state although it being uncodeable for the fossil *P. problematicus*. Absence of these structures in the unrelated taxa Colossendeidae, Pycnogonidae and *Pseudopallene* 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). A similar situation occurs with their position with respect to the femora, but a mid-dorsal position seems to be the more general state (characters 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 is 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 every pair of legs but species of *Rhynchothorax* and *Pycnogonum*, 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 on 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 and 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, have no signs of trunk segmentation.

The position of the ocular tubercle on the cephalic segment is explored as a phylogenetic character (character 23). Its posterior position could be 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 where the proboscis is embedded occurs in the ammotheid *Cilunculus* and has been coded as 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 are observed but all were coded as erect if not colinear with the trunk.

Proboscis

The proboscis was once considered homologue of the proboscis in polychaetes (Henry 1953 in Hedgpeth, 1954), but there is no evidence that the proboscis is anything other than the elongated acron (Boudreaux, 1979), a unique specialization within arthropods.

Fry and 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 main types of proboscis shape those authors proposed (character 31), using the geometrical criteria but not the system of coordinates (Appendix 4). 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 a specialization independently attained. A ventral

position of the proboscis is described in the fossil species (Bergström et al., 1980). This position resembles that observed in some 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, so this could not be included as a defined character in this analysis. Morphological adaptations to preferred prey as shown for *Austrodecus*, *Rhynchothorax* and *Pycnogonum* (Fry, 1965) could also be further investigated for evolutionary implications.

3.3.2.2 Characters and characters states

Those characters referred as *ordered* in the list were considered as such only when indicated in the analysis.

0. Chelifores: Present (0); absent (1) (Fig.3.2).

1. When present, number of segments of the chelifore scape: Three-segmented (0); two-segmented (1); one-segmented (2). *Ordered.*

2. Chelae: Present (0); absent (1).

3. Chelifores: (only applicable when chelifores are present): Absence of dorsal spines on scape (0); presence of dorsal spines on scape (1).

4. Palps: Present in males (0); absent in males (1) (Fig. 3.2).

5. Palps: Number of segments in males: 10-segmented (0); 9-segmented (1); 8-segmented (2); 6-segmented (3); 5-segmented (4); 4-segmented (5); 2-segmented (6); 1 segmented (7). *Ordered.*

6. Palps: Present in females (0); absent in females (1).

7. Palps: Number of segments in females: 10-segmented (0); 9-segmented (1); 8-segmented (2); 6-segmented (3); 5-segmented (4); 4-segmented (5); 1-segmented (6). *Ordered.*

8. Ovigers: Number of segments in males: 11-segmented (0); 10-segmented (1); 9-segmented (2); 7-segmented (3); 6-segmented (4); 4-segmented (5). *Ordered.*

9. Ovigers: Present in females (0); absent in females (1).

10. Ovigers: Number of segments in females. 11-segmented (0); 10-segmented (1); 9-segmented (2); 6-segmented (3); 4-segmented (4). *Ordered.*

11. Ovigers: Absence of terminal claw (0); presence of terminal claw (1).

12. Propodi: Absence of auxiliary claws (0); presence of auxiliary claws (1) (Fig. 3.2).

13. Cement gland(s): Presence on femora or other segments of the leg (0); complete absence (1).

14. Cement gland(s): One cement gland on each femur (0); multiple cement glands on each femur (1).

15. Cement gland(s) shape: Pore (s) or slit on the cuticle (0); Tube (s) or protuberances (1) (Fig. 3.2).

16. Cement gland(s) position on the legs: dorsally (0); laterally (1); ventrally (2).

17. Cement gland(s) on femora: Located distally (0); midpoint (1); proximally (2); distributed all along the femora (3).

18. Trunk: Elongate shape, crurigers or lateral processes separated by their own diameter distance or more (0); intermediate shape: crurigers separated by less than their own diameter distance 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 four pairs of legs (2); present on the fourth pair of legs only (3).

21. Genital pores in females: Present on all four pairs of legs (0); present on the fourth pair of legs only (1).

22. Propodi: Heel spines: Present (0); absent (1).

23. Ocular tubercle: anterior on the cephalon (0); equidistant to the anterior and posterior margins (1); posterior on the cephalon (2) (Fig. 3.2).

24. Ovigers: Largest segment: Sixth (0); fifth (1); fourth (2); third (3); second (4).

25. Ovigers: Spines present on the last segments (strigilis) (0); spines absent on the last segments (1).

26. Ovigers: Spines compound or denticulate (0); spines simple (1).

27. Ovigers: Spines arranged in a single row (0); spines arranged in multiple rows (1).

28. Chelae: Teeth present (0); teeth absent (1).

29. Chelae: Oriented opposing to each other (0); pointing downwards, in front of the tip of the proboscis (1).

30. Abdomen: Horizontal in the same direction as the trunk (0); erect diagonally or pointing upwards (1).

31. Proboscis shape: (See Appendix 4) A = straight (0); B = inflated proximally, acute distally (1); C = inflated distally (2); D = tapering or pipette-like (3) (Fig. 3.2, also Fry and Hedgpeth, 1949).

32 Ventral spurs on second coxae of last pairs of legs in males. Absent (0); present (1).

33. Proboscis: Length less than half the length of the trunk (0); length the same (± 1mm) as half the length of the trunk (1); length equal or greater than the trunk length (2).

34. Proboscis: Frontal and fixed (0); positioned in angle and movable (1); ventral and highly movable (2)

35. Cephalic hood: Present (0); Absent (1).

3.3.3 Cladistic analysis

A parsimony analysis under an '*a posteriori* weighting' approach implemented by the implied weights of the program Pee-Wee (Goloboff, 1993b) was used to produce a phylogeny of the Pycnogonida based on morphological characters. Sometimes, weighting of characters has been used to facilitate choice among a set of equally most parsimonious cladograms (Carpenter, 1988). It has been recognized that parsimony analyses require weighting to achieve self-consistent results (Goloboff, 1993a; Kitching et al., 1998). Platnick et al. (1996) further suggested that equally weighted or 'unweighted' analyses as they are commonly called, are only preliminary estimates of the relative value of the data.

Based on Farris' weighting system, Goloboff proposed a non-iterative method that uses evidence on homoplasy to estimate character reliability (Goloboff, 1993a). This method does not depend on initial estimations of weights and produces trees of maximum fit $F=\Sigma f_i$, which imply 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 and 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 four and below were introduced, the analysis resulted in 21 most-parsimonious trees (MPTs) and a decrease of 6-23% in total fitness compared to the results with k=5. When the extreme 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. Fits or weights of the characters were scaled to 10 in order to obtain finely grained scale values of fitting functions and differentiate optimal trees from close suboptimal ones (Goloboff, 1993a).

The heuristic search was run in Pee-Wee using the commands "hold500; hold/20;mult*50" (hold 500 trees in memory; hold 20 starting trees; perform Tree-Bisection-Reconnection (TBR) swapping on 50 random addition replicates). The command "jump" was used for

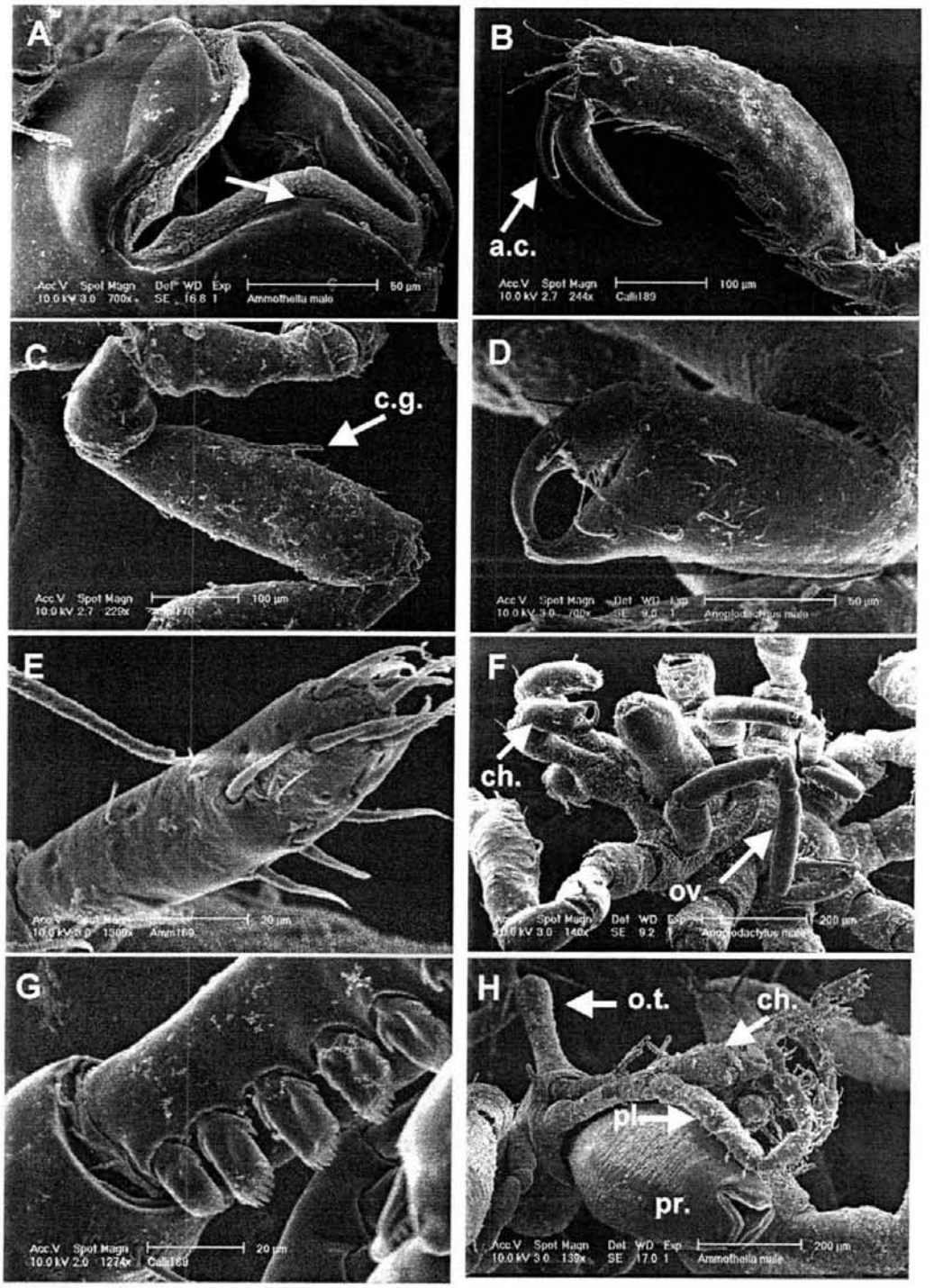

Figure 3.2 Scanning-electron microscopy images of some characters. A) Frontal-ventral view of proboscis and oral surface of *Ammothella* sp 'slender form'. Arrow indicates dorsal portion. B) Lateral view of propodus of *Callipallene* sp., a.c.=auxiliary claws. C) Lateral view of a femur showing the cement gland (c.g.) of *Anoplodactylus* n. sp B. D) Lateral view of a chela of *Anoplodactylus* n. sp B. E) Terminal segment of a palp of *Ammothella* sp 'slender form. F) Ventral view of *Anoplodactylus* n. sp. B, ch.=chelifores, ov=ovigers. G) Terminal segment of oviger with compound spines of *Callipallene* sp. H) Lateral view of the cephalon of *Ammothella* sp. 'slender form', o.t.=ocular tubercle, pr=proboscis, pl=palp, ch.=chelifore.

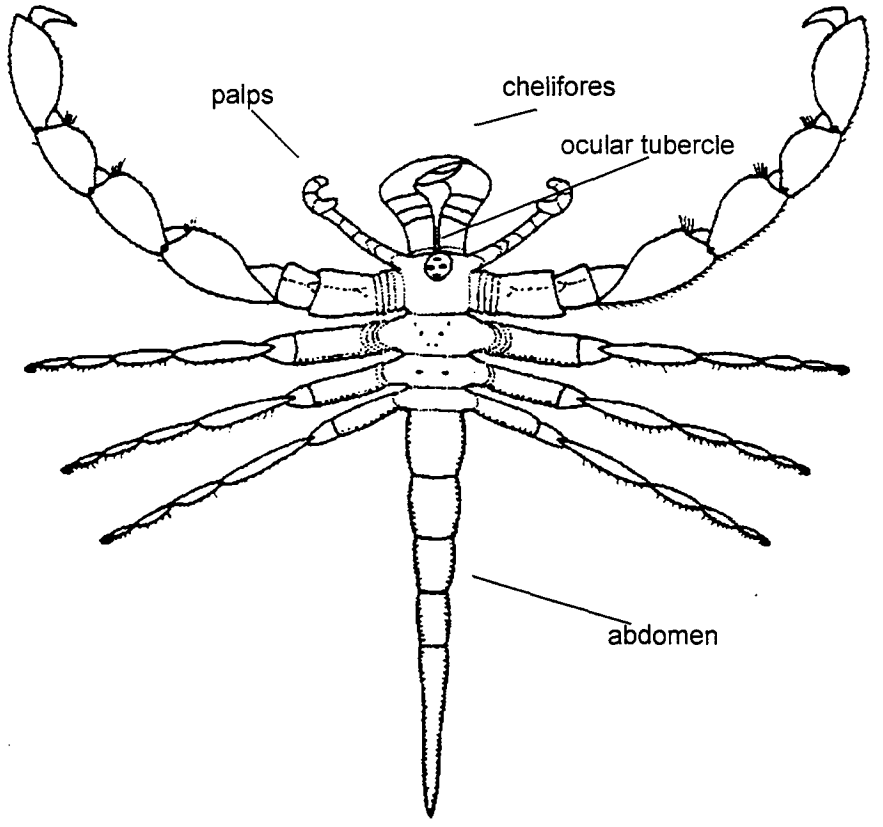

Figure 3.3 Reconstruction of the Devonian pycnogonid fossil *Palaeoisopus problematicus* Broili in dorsal view (Trunk length ≈ 125 mm) from Bergstrom et al. 1980.

additional swapping among multiple 'islands' of trees (Goloboff, 1995). This same analysis was done in PAUP* 4.0 (Swofford, 1996) by running heuristic searches after selecting the unambiguous optimization setting (minimum branch length) under the parsimony menu and entering the 'Goloboff', 'GPeewee' and 'GK' options. Winclada B0.9 (Nixon, 2000) was used as a shell for Pee-Wee, and also for character evaluation and editing of cladograms together with TreeView 1.6.1 (Page, 1996).

The synapomorphies shared by all the dichotomous trees were found using the command "apo" in Pee-Wee (Goloboff, 1997; Szumik, 1996). 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 NONA (Goloboff, 1997) using the command "hold 1000; bsupport10" (hold a maximum of 1000 trees and branch support indices up to 10 extra steps).

With the aim of comparing the results with those obtained using unordered characters, additional heuristic searches were run after converting five multistate characters to ordered (characters 1, 5, 7, 8, 10). They represent the number of segments of chelifores, palps and

ovigers. 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 Pee-Wee (Szumik, 1996; Prendini, 2000).

3.4 Results

A single most-parsimonious polytomous tree with a maximum fit (2391.9 [53%], L=180) was found using non-additive characters and implied weights (Fig. 3.4). The two polytomies on this tree, one for *Achelia* species and 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. 3.4). From now on, characters are referred to as plesiomorphic in the context of this tree.

Two major lineages of pycnogonids are obtained: Ammotheids grouped with Colossendeidae, *Austrodecus* and *Rhynchothorax* (clade A) and nymphonids grouped with callipallenids, *Pycnogonum*, *Anoplodactylus* and *Endeis*, with *Pallenopsis* being basal (Clade B) (Fig. 3.4). The clade A is supported by the absence of chelae in the adults (character 2) and the size of the proboscis relative to the trunk (character 33) as synapomorphies. However, character 33 changes twice from state 1 to state 2 in *Ascorhynchus ramipes* and *Austrodecus*. The 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 modern pycnogonids, A + B is given mainly by the presence of fewer segments of chelifores and ovigers (characters 1, 8 and 10) in the living taxa than in the fossil *Palaeoisopus.*

After converting the subset of five multistates from unordered to ordered, I did the same analysis in Pee-Wee and obtained 100 most-parsimonious dichotomous trees, summarized in the strict consensus tree of Fig. 3.5 showing five collapsed nodes (2361.2 [49%], L=195). Fit decreased for eleven characters and increased for three when ordered characters were introduced (Table 3.2), 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. 3.5). 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 to visualize the possible evolution of these characters according to the resulting topology (Fig. 3.6).

3.5 Discussion

The internal relationships within each of the two major clades are discussed below according to the currently accepted families of pycnogonids, which are used throughout the study while their validity is being tested:

3.5.1 Ammotheidae+Colossendeidae+Rhynchothoracidae+Austrodecidae—

Two main groupings are found in clade A indicating the Ammotheidae is paraphyletic. Large forms of the Ammotheidae such as *Eurycyde* and *Ascorhynchus*, are grouped with the generally gigantic forms of Colossendeidae. The presence of a terminal claw (character11) and multiple rows of spines in the ovigers (character 27), the mid-position of the ocular tubercle (character 23) and the shape of the proboscis (character 31), the two latter reversals, support this clade. Munilla (1999) overlooked the presence of the multiple rows of spines on the ovigers in the ammotheids, presenting this character as an autapomorphy of Colossendeidae. 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 most of the taxa of the clade for ammotheids and colossendeids share preference for deeper waters, with the exception of a few *Eurycyde* and *Rhopalorhynchus* species.

Eurycyde and *Cilunculus* have been considered very primitive forms of living pycnogonids based on comparisons with the fossil *P. problematicus* (Stock, 1994). The present cladogram shows *Eurycyde* and *Cilunculus* both as basal taxa to the rest of the ammotheids (Fig. 3.4). Meanwhile, *Ammothea,* a difficult genus due to an enormous variability of its diagnostic characters, is presented here as a sister taxon of ((*Ammothella+Nymphopsis*) + (*Achelia* + (*Rhynchothorax* + (*Tanystylum* +*Austrodecus*) contrasting with 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 generally accepted as valid taxa (Fry and Hedgpeth, 1969; Child, 1998b). The great morphological diversity within the family has led taxonomists to propose some genera as separate families, such is the case of the Tanystylidae (Schimkewitsch, 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,* based on the fewer number of segments of the palps in *Tanystylum* (Hedgpeth, 1954; Clark, 1977; Munilla, 1999). The results of the present study differ from

that position and agrees with Stock's classification in showing *Achelia* and *Tanystylum* as closely related taxa (Stock, 1954); however, no synapomorphies were found to hold *T. haswelli* and *T. rehderi* in a single clade. A similar case is presented by the *Ammothella* species; *Ammothella* sp. A *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 has been associated to ammotheid forms when it used to be a genus of the family Tanystylidae (Hedgpeth, 1955b), but also was considered to belong to Colossendeidae (see Arnaud and Krapp, 1990). More recently Stock (1994) suggested its affinity to *Austrodecus*. Thompson (1904) conferred it a family status, creating the family Rhynchothoracidae, re-defined and widely accepted more recently (Arnaud and Bamber, 1987; Arnaud and 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 segments of the palps (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 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 a '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 the sharing of simple spines on the ovigers (character 26). The presence of six-segmented palps in the females of *T. rehderi* Child and the species of *Austrodecus* show these taxa as 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 and Hedgpeth, 1969). A revision of the family is a difficult task, mainly because of the great number of species to be examined and also the scarce type material available of rare genera. The relationships presented here are a first impression of what might be the course of the evolution of this lineage.

Figure 3.4 Single most parsimonious polytomous 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. Black circles are those synapomorphies present in nine dichotomous trees. Numbers above the circles are the characters and the numbers below are the states of the characters. Nodes indicated with a solid square show the maximum Bremer support value of 3 found in the tree.

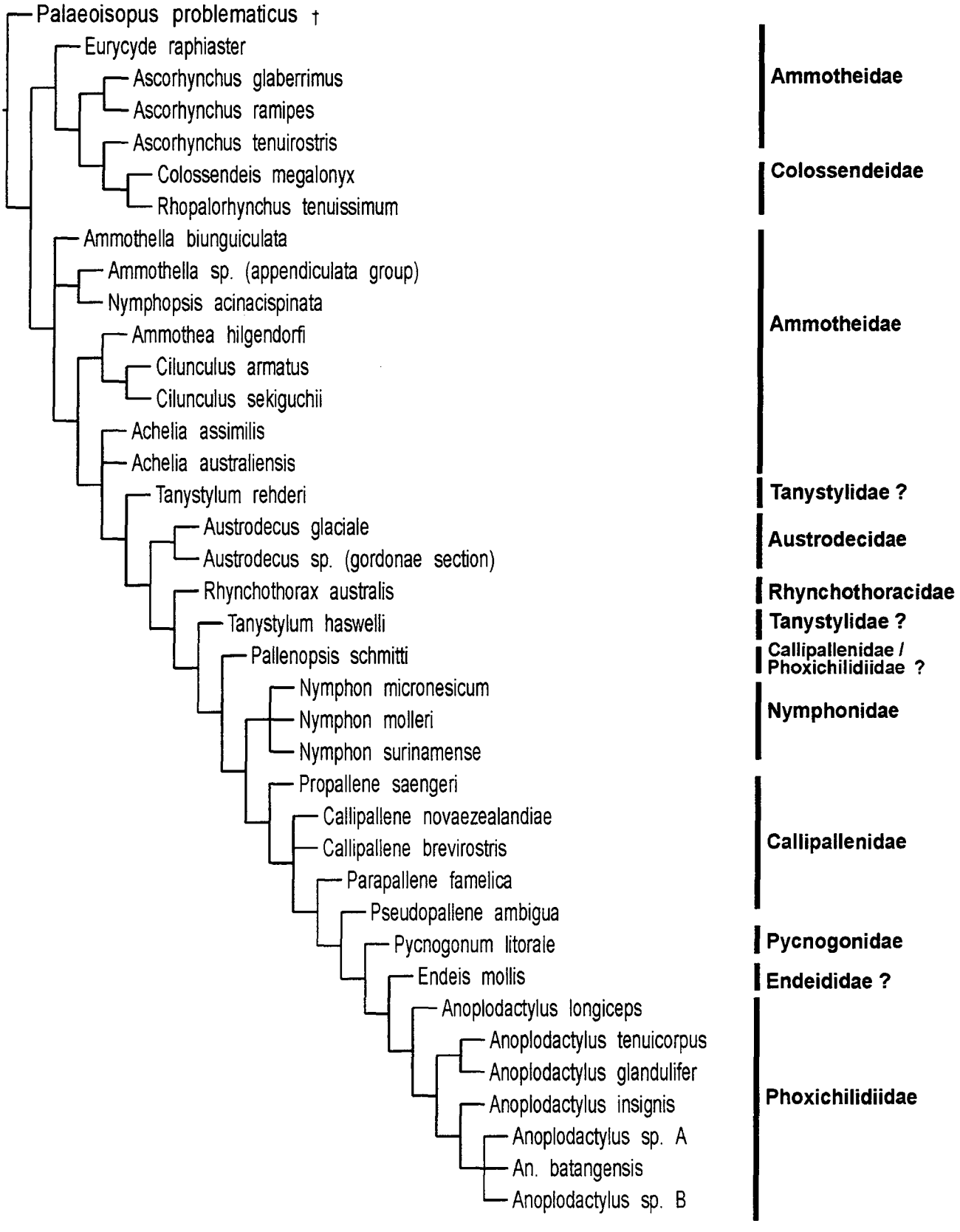

Figure 3.5. Single polytomous tree obtained with five multistate ordered characters (1, 5, 7, 8, 10) (F= 2361.2 [49%]; length= 195; ci= 36; ri= 69).

Table 3.2. Character statistics from both unordered and ordered analyses in Pee-Wee. Multistate characters for which the hypothesis of reduction is being investigated are indicated in bold. Prior fits or weights of all the characters have been scaled to 10. ci= consistency index; ri= retention index.

	Non-additive				Additive multistates (5)			
Character	Length	Fit	ci	ri	Length	Fit	ci	Ri
0	50	55.5	20	33	50	55.5	20	33
1	30	**83.3**	**66**	**66**	30	**83.3**	**66**	**75**
2	10	100.0	100	100	20	83.3	50	92
3	30	71.4	33	33	30	71.4	33	33
4	10	100.0	100	100	10	100.0	100	100
5	70	**100.0**	**100**	**100**	90	**71.4**	**77**	**93**
6	10	100.0	100	100	10	100.0	100	100
7	60	**100.0**	**100**	**100**	70	**83.3**	**85**	**96**
8	60	**83.3**	**83**	**85**	80	**62.5**	**62**	**88**
9	10	100.0	100	100	10	100.0	100	100
10	40	**uninformative**			**50**	**83.3**	50	**66**
11	50	55.5	20	66	50	55.5	20	66
12	80	41.6	12	56	90	38.4	11	50
13	30	71.4	33	33	30	71.4	33	33
14	40	62.5	25	72	40	62.5	25	72
15	60	50.0	16	61	60	50.0	16	61
16	60	55.5	33	63	60	55.5	33	63
17	70	55.5	42	71	90	45.4	33	57
18	110	35.7	18	40	120	33.3	16	33
19	80	45.4	25	33	90	41.6	22	22
20	80	50.0	37	16	80	50.0	37	16
21	20	83.3	50	0	20	83.3	50	0
22	60	50.0	16	61	50	55.5	20	69
23	60	55.5	33	63	60	55.5	33	63
24	110	41.6	36	68	110	41.6	36	68
25	10	100.0	100	100	10	100.0	100	100
26	40	62.5	25	50	50	55.5	20	33
27	10	100.0	100	100	10	100.0	100	100
28	30	71.4	33	71	30	71.4	33	71
29	30	71.4	33	66	30	71.4	33	66
30	90	41.6	22	41	90	41.6	22	41
31	100	41.6	30	63	110	38.4	27	57
32	50	55.5	20	55	50	55.5	20	55
33	80	45.4	25	64	100	38.4	20	52
34	60	55.5	33	50	60	55.5	33	50
35	10	100.0	100	100	10	100.0	100	100

3.5.2 (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 in the ovigers (character 11) relating the three *Nymphon* species. This family has been considered a relatively homogeneous lineage of numerous (ca. 240) closely related species. *Nymphon surinamense* Stock 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* Clark and *N. micronesicum* Child.

The position of the ocular tubercle (character 23) and the presence of teeth on the chelae (character 2) relate Nymphonidae and callipallenid genera. According to Stock (1994), these two lineages are closely related, however, an electrophoretical analysis had shown Nymphonidae as basal clade of the Pycnogonida away from the Callipallenidae (Munilla and De Haro, 1981). This 'ancient' condition has been also 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 Pycnogonida, until more fossil evidence is made available or molecular data allow us to make conclusions on this regard. 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 *Callipallene brevirostris* Johnston 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, Endeididae and Pycnogonidae, all related by the absence of palps in the females (character 6). *Pseudopallene* links the callipallenids to the (*Pycnogonum* + *Endeis* + *Anoplodactylus*) based on the anterior position of the ocular tubercle and the ventral orientation of the chelae (character 23 and 29 respectively). The absence of ovigers in the females (character 9) and the glabrous condition of the ovigers when present, relate *Pycnogonum* to *Anoplodactylus* and *Endeis*. The position of *Pycnogonum*, derived from a callipallenid ancestor, reflects the same relationships proposed by Stock (1994) in his diagram (Fig. 3.1).

The genus *Endeis* has been considered by some specialists a distinct entity that needs to be placed as a separate family [Endeidae (Hedgpeth, 1947; King, 1973); 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 in the ovigers (character 11). *E. mollis* appears as a sister-taxon of *Anoplodactylus longiceps* Stock, 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 many clear autapomorphies (Fig. 3.4) that support it as a separate taxon from *Anoplodactylus*. The inclusion of *Phoxichilidium*, sister taxon of *Anoplodactylus*, in future analyses might be of help to test the proximity of *Anoplodactylus* and *Endeis*. *Endeis* is assumed here as a phoxichilidiid taxon, the family Endeididae suggested not to be valid.

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, just to mention some characters. Remarkably, characters such as the absence of palps, absence of ovigers in females and the number of segments of the male ovigers, are quite stable among the species. 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) and has been varyingly 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. The node is supported by the presence of a single cement gland (character 14), the mid-position of the ocular tubercle (character 23) and the absence of teeth on chelae (character 28). However, *Pallenopsis* shares these characters with taxa from one or the other family and certain variation is also known within the genus (e.g. toothed chelae in *P. mascula* Bamber 2000). The controversy regarding the position of the genus, within one of the established families has been suggested to be better solved by giving *Pallenopsis* its own familial rank (Child, 1992). The present outcome seems to support that argument by not including *Pallenopsis* either in Callipallenidae or Phoxichilidiidae, however, it might also represent weak support and lack of sufficient information. Evidence from molecular data is expected to help solving the conflict on *Pallenopsis* relationships.

3.5.3 Character evolution

Presence or 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 the highest to the lowest number of segments of the appendages should yield the most-parsimonious resolution of the 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. 3.6 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. 3.6A). 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 (Staples and Watson, 1987; King, 1973), but the absence of chelae in adults has not been discussed in functional or ecological terms. A gradual reduction of the chelifores from three- to two- and to one- segmented is not as evident as in the other appendages.

The absence of palps (characters 4 and 5) appears as a derived characteristic relating *Pycnogonum* and Phoxichilidiidae to 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 segments of the palps is also evident within the ammotheid clade (*Achelia* + *Rhynchothorax* + *Tanystylum*), but here there is not a complete loss (Fig. 3.6B). 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 (King, 1973). However, for those taxa in which palps are completely absent, alternative sensory structures in some other part of the body, such as the proboscis, 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 females of callipallenid forms, suggesting also sexual dimorphism might be involved.

A reduced number of segments of the ovigers in the males is common to *Pycnogonum* and phoxichilidiids; in *Austrodecus* (Fig. 3.6D) not only the number of segments are fewer, but also ovigers are extremely reduced in size (Stock, 1957). A complete absence of ovigers has been observed in species of *Pycnogonum* (Child, 1998b) 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 are not as extremely reduced as in *Austrodecus*. The number of segments of the ovigers in the females appears as an uninformative character in the analysis

(character 10). Although it is not possible to identify a common origin for the reduction of the ovigers in the females (Fig. 3.6E), their complete absence (character 9) clearly defines the clade for *Pycnogonum*, *Anoplodactylus* (Phoxichilidiidae) and *Endeis* (Fig. 3.4). 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, 1998b). 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.

3.5.4 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.

According to his diagram (Fig. 3.1a), Stock seemingly proposed a clade for 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 3.3). 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 3.3). 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 3.3).

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 fit was just two units lower than the unconstrained cladogram, however, only one character performed better (Table 3.3). The basal position of *Pallenopsis* in the clade B suggests it could be proposed as a higher taxon, however it might also indicate lack of sufficient informative characters to attach the genus to any of the known taxa.

The clade Rhynchothoracidae + Pycnogonidae proposed by Munilla (1999) was enforced and the overall fit consistently decreased 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 constraint 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 3.3). The close relationship between *Anoplodactylus* and *Endeis* has been

shown here, and I suggest *Endeis* is left within the Phoxichilidiidae until further evidence becomes available to decide whether the two genera are within a single family or the two nominal families should be grouped within a higher taxon.

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

3.6 Conclusions

The phylogeny proposed shows two main clades of extant sea spiders. Ammotheidae is shown as a paraphyletic group including *Rhynchothorax*, *Austrodecus* and Colossendeidae. These are segregated from callipallenids, also a paraphyletic group, together 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 based 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 reduction of the palps and ovigers occurs 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 idea of *Pallenopsis* as a phoxichilidiid genus and Colossendeidae, Austrodecidae and Rhynchothoracidae as sister taxa. Sea spider phylogeny is not yet clear, the little work done has been based on a set of traditional taxonomic characters and there is strong need for additional characters from different sources that either confront the clades proposed or give a more robust support.The lack of appropriate outgroups for comparison and polarization remains as a major problem to relate pycnogonids with other arthropod taxa and to permit recognition of ancestral states for the characters. Although recent comprehensive studies have addressed the issue (Giribet and Ribera, 2000; Edgecombe et al., 2000), further investigation is needed to reveal the sister taxa of the Pycnogonida.

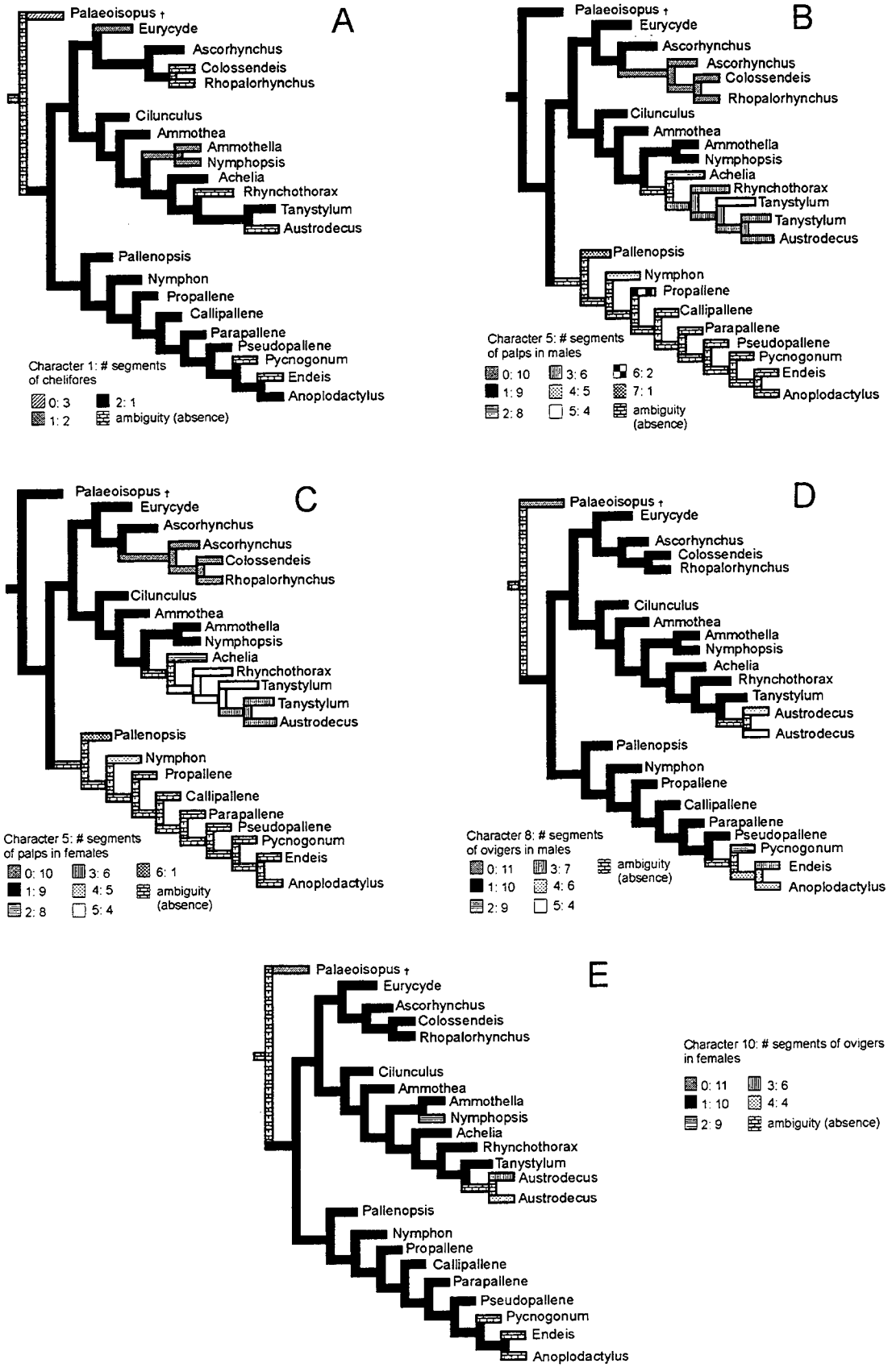

Figure 3.6. Evolution of characters 1, 5, 7, 8 and 10 according to the proposed phylogeny presented in figure 3.4. Species in the same genus have been collapsed unless different states are found. Note that character 10 has been shown as parsimony uninformative.

Table 3.3 Clades enforced according to previous classifications and their fit compared to the phylogeny proposed in this study. Prior fits or weights of all the characters are scaled to 1 for matter of presentation.

Tree	Fit difference	Characters with better fit	Characters with worse fit
Colossendeidae+Rhynchothoracidae+ Austrodecidae (Stock 1994)	-4.6 (2%)	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 (<1%)	0(1/0.7); 20(1/0.5); 22(1/0.5)	7(1/1.7); 26(1/0.7); 31(1/0.3)
Pallenopsis as Phoxichilidid (Stock 1978)	-6.3 (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)
Pallenopsis as Callipallenid (Hedgpeth 1948; Child 1979)	-2.3 (<1%)	22(1/0.5)	14(1/0.7); 23(1/0.5); 28(1/0.9)
Pycnogonidae+Rhynchothoracidae (Munilla 1999)	-8.5 (3.6%)	0(1/07); 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(1/04)
Endeis+Phoxichilidiidae (Stock 1994)	-0.7(<1%)	No better fit for any character	12(1/0.3); 33(1/0.4)

CHAPTER FOUR

First molecular approach to the phylogenetics of sea spiders (Pycnogonida, Arthropoda) using nuclear ribosomal DNA and morphology

4.1 Summary

The Subphylum Pycnogonida comprises at least eight families that are distributed in all the seas over the world. I examined the phylogenetic relationships of six lineages using partial sequences of the units of nuclear ribosomal DNA, 18S and 28S genes. Hypotheses of relationships were obtained from separate and combined analyses of these data sets under both maximum parsimony and maximum likelihood criteria. Trees derived from the molecular data set were compared with those from the set of 36 morphological characters previously analyzed. Estimates of phylogeny were found to be significantly different between the molecular and the morphological data sets, and topological differences between 18S and 28S analyses were also observed. The position of Colossendeidae was a major cause of conflict, being supported as relative of Ammotheidae by morphological characters but appearing closely related to Callipallenidae with 18S or to Nymphonidae with 28S data. Under molecular data, *Austrodecus* is identified as a basal taxon for the Pycnogonida, whilst it is part of the small-sized ammotheids clade according to morphology, although not strongly supported. The tree obtained with a simultaneous analysis of the three data sets available is shown as a final preferred hypothesis of pycnogonid phylogeny. However, significant discrepancies in estimates among different data sets, non-optimal taxon sampling of the molecular data set and problems associated with the analysis of morphological characters due to non-independence of characters by absence coding, might be influencing these results.

4.2 Introduction

Pycnogonids show a rather simple external morphology. Most of the diagnostic characters are from the appendages on the cephalic segment: chelifores, palps and ovigers, which constitute the main source of morphological variation. However, any or all of these pairs of appendages, can be extremely reduced or absent in some of the taxa. The presence-absence of the chelifores, palps and ovigers has been the basis of the classification of the major lineages that is supported on the hypothesis of evolutionary trend in sea spiders towards reduction and loss of appendages.

Difficulties that prevent research into the phylogenetics of the sea spiders using morphological characters arise from the absence of these structures in many of the taxa.

Unlike many other arthropod groups, there are no genital structures or sets of mouthparts that could be used for species diagnosis or as a source of phylogenetic information. This is a situation that complicates the use of cladistic techniques applied to external morphology. Appropriate coding of inapplicable characters has been considered a major problem in cladistics without a completely satisfactory method of analysis to deal with it (Strong and Lipscomb, 1999)

In a first attempt to use cladistic methods to propose phylogenetic relationships among sea spiders, the analysis of 36 morphological characters and 38 taxa presented in the previous chapter addressed the reductive trend hypothesis. Two major clades were found, one for Ammotheidae, Rhynchothoracidae, Colossendeidae and Austrodecidae, and the other includes Nymphonidae, Callipallenidae, Phoxichilidiidae, Endeididae and Pycnogonidae. These two major clades seem to represent the basal divergence of the extant lineages of the Pycnogonida. Ammotheids are characterised by the absence of chelae in adults (although a few chelate species are known as exceptions within Ammotheidae), but the taxon is paraphyletic, including three other nominal taxa lacking not just the chelae but the whole chelifores. Meanwhile, the other major clade includes all the taxa with chelate adults (Nymphonidae, Callipallenidae and Phoxichilidiidae), but also includes two lineages, Pycnogonidae and *Endeis*, in which chelifores are completely absent. The morphological analysis presented suggests the possibility of parallel evolution events of reduction and loss of the appendages in sea spiders. This pattern would need to be further examined using additional sets of characters given the analytical difficulties mentioned and also the low support values obtained for some of the clades (see Fig. 3.4).

The development of molecular systematics in the last three decades has provided a new insight into many phylogenetic problems that might have been considered impossible to approach by morphologists (Hillis, 1987). Some of those problems involve difficulties in resolving phylogenies where few homologous characters are available for comparison, and also the possibility of character convergence due to shared lifestyles across taxa. Also, the inconveniences inherent in the analyses of morphological data sets with too many missing values and lack of information for outgroup comparisons, make the use of molecular data desirable to complement and confront a morphological data set or any other type of information (Hillis, 1987).

No molecular data have been used to explore the evolutionary history of the pycnogonids (but see Lovely, 1999). A few sea spiders have been sequenced for broader arthropod phylogeny studies (Wheeler and Hayashi, 1998; Regier and Schultz, 1997; Colgan et al., 1998; Giribet and Ribera, 2000; Giribet et al., 2001), but internal relationships of the Pycnogonida have not

been examined, and the usefulness of different molecular markers for the study of different levels of evolutionary relationships needs to be investigated.

To study the phylogenetic affinities of six of the eight families of pycnogonids, I examined partial sequences of the small and the large sub units of nuclear ribosomal DNA. The contribution of nuclear ribosomal DNA in phylogenetic inference has been considerable (Hillis and Dixon, 1991) and these particular regions are known to be informative for higher-level phylogenetic analyses in studies of both chelicerates and arthropods in general (Friedrich and Tautz, 1995; Black et al., 1997; Edgecombe et al., 2000; Giribet et al., 1999; Giribet and Ribera, 2000).

The two molecular data sets were examined with phylogenetic methods separately or together and also in conjunction with morphological characters (see Methods Chapter 3: cladistic analysis of morphology). The aim of this work is to examine the relationships among the Pycnogonida, confronting morphological data with patterns derived from sequences of nuclear ribosomal genes. Outgroup relationships are tested using sequences of 18S rDNA, widely used in phylogenetic studies of arthropods, and largely available in the databases. Due to the paucity of the fossil record, the time of divergence of the main lineages of living sea spiders is unknown. Markers such as 18S, that have been widely used for the study of animal phylogenies, might be useful to give approximate estimates of rates of divergence among pycnogonids.

4.3 Materials and Methods

4.3.1 Sampling of the molecular data

Molecular data were obtained for representative genera of the main lineages of pycnogonids. I obtained partial sequences of the 18S for nine pycnogonid taxa either myself or from the Genbank database (accession numbers Table 4.1). Six families were represented and three genera of Ammotheidae were included. Outgroup taxa were selected based on results of recent comprehensive studies of arthropod phylogeny (Giribet and Ribera, 2000) and chelicerate phylogeny (e.g. Wheeler and Hayashi, 1998) suggesting possible sister taxa of pycnogonids.

Partial sequences of the large nuclear ribosomal unit 28S, were obtained from 11 species representing all the families of sea spiders except Pycnogonidae and Rhynchothoracidae. The fragment of 28S from D4-D7 region is not commonly sequenced for chelicerates or closely related groups and unfortunately could not be obtained for outgroup taxa. It was used in this study in order to complement sequences of the D3 region, being sequenced in a 'concurrent'

study (Lovely, 1999). Sequences of D4-D7 available are mostly for Diptera (Friedrich and Tautz, 1997) that proved to be too divergent for my purpose.

Initially, sequences of the 16S subunit of mitochondrial ribosomal DNA were obtained for four taxa, however, they were too variable for a high level phylogenetic analysis (p-distance= 24%-44% among four genera in three families). Sequences obtained were deposited in the Genbank database but no further analyses were carried out to propose high-level phylogenetic affinities.

4.3.2 Sampling of the morphological data

The molecular information complements the phylogenetic analysis of morphological characters presented earlier in this work. Details of the taxon sampling, collection localities and deposited material for the morphological study are described in chapter 3. Morphological data for the 10 genera included in the molecular analyses were extracted from the data set presented in Table 3.1 to be analyzed in a simultaneous analysis with the DNA data in the present chapter. After the exclusion of taxa not to be used in the combined analysis, eight characters appeared as parsimony uninformative. The taxa used in the combined analysis are presented in Table 4.1.

4.3.3 Preparation of molecular samples

DNA samples were obtained from fresh material or 90-95% ethanol-preserved specimens when possible, although fairly old samples preserved in 70% ethanol were tried when fresh specimens were not available. Whole individuals or a piece of tissue from the legs was used for the extractions depending on the size of the individuals, following the protocol for the Chelex® extraction (Walsh et al., 1991).

The V4 and flanking regions of the 18S rDNA has been found useful to propose relationships among tick subfamilies (Acari) (Crampton et al., 1996) and genera of Aphidiinae (Hymenoptera: Braconidae) (Sanchis et al., 2000) among other studies, suggesting its usefulness to explore relationships among supraspecific taxa in different arthropod groups. It was PCR-amplified using the following primers designed for prostigmatid mites (Otto and Wilson, unpublished; Black et al., 1997): Mite18S-F (5'ATATTGGAGGGCAAGTCTGG3') and mite 18S-R (5'TGGCATCGTTTATGGTTAG 3'). Amplifications were carried out in 20µl reactions with 0.5 units of Taq Polymerase (Qiagen), 2uM dNTPs and 10µM of each primer. The PCR program consisted of a 2 min denaturation step at 94°C, and 35 amplification cycles at 50°C (94°C for 30 s, 50°C for 30 s, 72°C for 1 min 30 s). On a few occasions a 'touchdown' PCR program (from 51°C to 49°C) was used (Don et al., 1991).

The 28S fragment from the D4 to the D7 region of the 28S rDNA was amplified using the pair of primers 28SD3N-5'TAGTAGCTGGTTCCTTCCG 3' [the reverse form of 28Sb in

Whiting et al. (1997), and 28SD7C-5'GACTTCCCTTACCTACAT 3', used in a high level phylogeny study of Diptera (Friedrich and Tautz, 1997). Amplifications were carried out in 20-25µl reactions and similar conditions to those used for amplification of 18S sequences. The basic PCR program included a 2 min. denaturation step at 94°C and 40 amplification cycles (92°C for 45 s, 50°C for 1 min, 72°C for 1 min 30 s).

Most of the PCR products were purified using the Qiagen PCR Purification Kit (Qiagen). Alternatively, 70% isopropanol precipitation method was carried out according to 'Applied Biosystems' instructions. The samples were directly sequenced using an automated ABI Prism 377 DNA sequencer using Taq polymerase® and dye-labeled terminators (BDT Big Dye Terminator, Applied Biosystems). Each amplification was carried out in a 20µl reaction: 8µl of BDT mix, 10ng/ml of PCR product, 1µM of primer and dH2O to 20 µl. The products were precipitated with 70% isopropanol, centrifuged for 20 min at 13000 rpm. The pellet was rinsed with 500µl of 70% isopropanol and dried in a speed-vac for 5 min.

PCR products were sequenced in both directions and twice for each sequence. Complementary sequences were read, compared and edited in Genedoc (Nicholas and Nicholas, 1997) and MacClade (Maddison and Maddison, 1992). Primer sequences were excluded from the analyses. Base frequencies and corrected average pairwise sequence divergence were calculated between taxa for each data set under complete deletion of gaps in PAUP*4.0 V4.0b8 (Swofford, 1996) and MEGA 2.1 (Kumar et al., 2001).

4.3.4 Phylogenetic analysis

DNA sequences were aligned using Clustal W (Thompson et al., 1994) followed by manual editing in MacClade (see alignments in Appendix 5). Alignments were analyzed under maximum-parsimony (MP) and maximum likelihood (ML) criteria. Parsimony analyses were run using branch and bound searches in PAUP*; maximum likelihood (ML) analyses were run with parameter settings indicated by MODELTEST (Posada and Crandall K. A., 1998), which compares model goodness-of-fit by using hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC) (Posada and Crandall. K.A., 2001). ML analyses were implemented in PAUP* using heuristic search and tree bisection-reconnection (TBR) as a branch swapping algorithm (Swofford, 1996). Transition-transversion ratios and other parameters were estimated during the ML analysis. Both parsimony and maximum likelihood were applied to complete (aligned in Clustal W) and reduced versions of each of the data sets. The omission of highly variable regions is recommended for analysis (Swofford et al., 1996), but the information contained within these regions was also examined, running the same analyses without excluding the highly variable portions of the sequences.

Table 4.1. Species included in the study and the type of data used for each of them.

	Morphology	*18S*	*28S*
Pycnogonida			
Ammotheidae			
Achelia assimilis Haswell, 1884	X	-	AF448557
Achelia echinata Hodge, 1864		AF005438*	-
Ammothella sp. *appendiculata*-group	X	AF448552	AF448558
Ascorhynchus ramipes Bohm, 1879	X	AF448555	-
Colossendeidae			
Colossendeis megalonyx Hoek, 1881	X	-	AF448562
Colossendeis sp.		AF005440	-
Austrodecidae			
Austrodecus sp. *gordonae*-section	X	AF448554	AF448559
Nymphonidae			
Nymphon micronesicum Child, 1982	X	AF448556	AF448560
Callipallenidae			
Callipallene novaezealandiae Thomson, 1884	X	-	AF448563
Callipallene sp.		AF005439*	-
Callipallenidae or Phoxichilidiidae (?)			
Pallenopsis schmitti Hedgpeth, 1943	X	-	AF448564
Phoxichilidiidae			
Anoplodactylus sp. B (*evansi-digitatus* group)	X	-	AF448565
Anoplodactylus tenuicorpus Child, 1988		AF448553	-

Table 4.1. Continued.

	Morphology	*18S*	*28S*
Endeididae (?)			
Endeis biseriata Stock, 1968	X	-	AF448561
Endeis laevis Grube, 1871		AF005441*	
Outgroup taxa used only for 18S analysis:			
Xiphosura			
Limulus polyphemus Linnaeus 1758		U91490	-
Scorpiones			
Androctonus australis		X74761	-
Araneae			
Hypochilus pococki Platnick 1987		AF062951	-
Ricinulei			
Pseudocellus pearsei Chamberlin & Ivie 1938		U91489	-
Myriapoda			
Polyxenus lagurus L. 1758		X90667	-

For purposes of comparison between genes and for the combined analysis, the 28S sequence of *Colossendeis megalonyx* has been paired with the 18S sequence of *Colossendeis* sp. The 28S sequence of *Callipallene novaezealandiae* has been paired with the 18S sequence of *Callipallene* sp. .The 28S sequence of *Anoplodactylus* sp. B has been paired with the 18S sequence of *Anoplodactylus tenuicorpus*. The 28S sequence of *Achelia assimilis* has been paired with the 18S sequence of *Achelia echinata*. The 28S sequence of *Endeis biseriata* has been paired with the 18S sequence of *Endeis laevis*. *Sequences reported in Giribet and Ribera (2000).

Since the 18S sequences were used to infer internal and also outgroup relationships of the Pycnogonida, similar analyses were run with and without outgroup taxa. Only parsimony-informative characters were used to calculate parsimony indexes. All sites were included in the ML analyses.

I analyzed the 18S and 28S data sets separately, and also combined in a single data matrix. A controversy on combining data sets for phylogenetic analyses has been extensively reviewed (Miyamoto and Fitch, 1995; Bremer, 1996; Huelsenbeck et al., 1996; Page and Holmes, 1998, among others). However, these days it is widely accepted that a combined analysis is an appropriate method for phylogenetic reconstruction and also that there is no conflict in analyzing the data sets separately as well (Bremer, 1996). The Incongruence Length Distance Test (Farris et al., 1995), was performed to examine heterogeneity of the two molecular data sets. It was run in PAUP* (Partition Homogeneity test) using a branch-and-bound search with 10000 replicates for each case. Analyses included the molecular partitions combined in a single matrix in which taxa lacking either 18S or 28S data were coded as missing values or excluded, to see the effects of missing values on the results. Finally, I combined morphological data (in Arango, in press) for the taxa used in the molecular analysis with the sequence data in a single matrix for maximum parsimony analysis. Non-parametric bootstrapping was run in PAUP* with number of replicates set to 1000 for all the analyses performed. The 'Decay Index' command in MacClade in combination with PAUP* produced the Bremer support values.

4.4 Results

4.4.1 Alignment and nucleotide variation of 18S fragment

A total of 488 sites of the 18S were aligned for nine pycnogonids and five outgroup taxa, *Limulus polyphemus* (Xiphosura), *Hypochilus pococki* (Araneae), *Androctonus australis* (Scorpiones), *Pseudocellus pearsei* (Ricinulei) and *Polyxenus lagurus* (Myriapoda) (in Appendix 5). The sequence of *Peripatopsis* sp. (Onychophora) showed a large divergence compared to pycnogonid sequences (>25%) and it was excluded. The 18S fragment selected was alignable with the 561-1165 sites of the 18S rDNA sequences of sea spiders and other arthropod taxa sequenced (Giribet and Ribera, 2000) (Genbank accession numbers in Table 4.1) located between the V3 and V5 domains in Black et al. (1997).

There were a total of 134 (27%) variable characters of which 54 (11%) were parsimony informative. Overall base composition was statistically homogeneous across taxa (χ^2.= 3.72, *df*=39, *P*= 1.0; PAUP* results), the average observed base frequencies being: A= 25.57%, C= 20.66%, G= 27.15%, T=26.66%. Uncorrected sequence pairwise divergence (p-distance)

within pycnogonid taxa calculated with complete deletion of gaps (Swofford et al., 1996) was not large, ranging from 1% to 3% (19 parsimony-informative sites). Mean sequence divergence between outgroups and pycnogonids was 9%.

4.4.2 Phylogenetic analysis of 18S ribosomal DNA

Maximum parsimony searches based on the informative characters of 18S, yielded three most-parsimonious trees (Fig. 4.1A; L=203) differing in the internal relationships among chelicerate outgroups (*Limulus*, *Hypochilus, Androctonus* and *Pseudocellus*). The diplopod *Polyxenus* showed the highest sequence divergence overall (14%-15%) and was placed as the root of the trees. Monophyly of the Pycnogonida was fully supported by 100% bootstrap support. The Nymphonidae appears to be closely related to Colossendeidae +*Callipallene*. Separately, *Anoplodactylus* and *Endeis* joined the clade basally, although both weakly supported. The ammotheids *Ammothella* + *Achelia* were shown as sister taxa, however, the monophyly of the Ammotheidae including *Ascorhynchus* was weakly supported. *Austrodecus* was shown basal to all the pycnogonids.

For 18S, a TrNef model with a discrete approximation of the gamma distribution (TrNef + Γ; Γ=0.27) was preferred under the hLRTs. According to this criterion, the assumption of equal base frequencies (JC vs. F81) was met, there were equal rates for transversions (TrNef vs. TIMef), and no significant proportion of invariant sites, but the assumptions of an equal ratio of transition and transversion rates (JC vs. K80), equal rates for transitions (K80 vs. TrNef), and equal rates among sites (TrNef vs. TrNef+ Γ) were all rejected. These assumptions slightly changed under the AIC, which indicated two unequal rates for transversions (TrNef vs. TIMef), a significant proportion of invariant sites (I=0.36) and slightly higher value for the gamma distribution (Γ=0. 64). The best tree obtained under ML settings using the model preferred by the AIC (TIMef + I) did not differ from the one produced with hLRTs parameters. The ML tree was obtained from a heuristic search using the MP topology as starting tree. Both the ML and the MP trees showed the same pattern of relationships among pycnogonid lineages (Figure 4.1A).

Two small hyper-variable regions were identified in the positions 123-137 and 216-219. These particular regions consist of indels alignable among subsets of taxa. The same analysis was carried out excluding these regions to see the influence of these sections on the topologies obtained. Less resolution was obtained when the variable regions were excluded [total parsimony informative characters = 45 (9.6%)]. Only the clade (*Nymphon* (*Colossendeis* + *Callipallene*) and the basalmost *Austrodecus*, were supported in the strict consensus tree (Figure 4.1B). The first fragment mentioned (positions 123-137) contained a peculiar 15-site insertion only present in Pycnogonida and *Polyxenus*.

A separate set of data excluding the outgroup taxa except *Limulus* was used for subsequent analyses to inspect the interference of outgroups with pycnogonid relationships resulting from the 18S analysis. The MP search with 19 (4%) parsimony informative characters produced a single shortest tree that showed exactly the same pattern of pycnogonid relationships to the one found with outgroups included (Figure 4.2A; L=90). The model and the parameter settings selected by the hLRTs remained the same as those selected for the complete data set, but under the AIC the data rejected the assumption of equal base frequencies (JC vs. F81) and accepted the assumption of equal transversion rates (TrN vs. GTR). Regardless of the criterion followed, the topology was the same under both sets of conditions (Figure 4.2B). This tree differed from the one produced by the complete set of taxa in the basal position of *Endeis* and the inclusion of *Austrodecus* with the ammotheids. The results of all the analyses performed are summarized in Table 4.2.

4.4.3 Alignment and nucleotide variation of 28S fragment

A total of 877 sites of 28S sampled from nine genera of pycnogonids, were included in the analysis. Of these, 177 sites were variable (20%) and 61 were parsimony-informative (7.0%). Average base frequencies A= 19.7%, C=32.15%, G= 25.6% and T=22.4%, were statistically homogeneous across taxa (χ^2 = 7.64, df=24, P = 0.99). Uncorrected pairwise sequence divergence (p-distance) with complete deletion of gaps ranged from 1.4% to 10.9% among pycnogonid taxa, *Colossendeis* showing the highest mean values of divergence (7.12-10.3%).

4.4.4 Phylogenetic analysis of 28S ribosomal DNA

Branch-and-bound parsimony analysis of 877 sites produced a single MP tree (Figure 4.3; L=239). Nymphonidae + Colossendeidae strongly supported clade, constituted the most divergent clade from other pycnogonid taxa. Unlike the 18S analysis, *Callipallene* was a sister taxon to *Anoplodactylus* + *Pallenopsis*, both nodes well supported as well. Unexpectedly, *Endeis* joined the ammotheid clade and *Achelia* did not appear as sister taxon of *Ammothella* as shown by the analysis of 18S.

The model HKY with a discrete approximation of the gamma distribution (HKY + Γ; Γ=0.76) provided the best fit for the data according to the hLRTs and the AIC. Assumptions of equal base frequencies (JC vs. F81), equal ratio of transition and transversion rates (F81 vs. HKY) and equal rates of substitutions among sites (HKY vs. HKY + Γ) were rejected by the data. Transition and transversion rates were assumed equal (HKY vs. TrN and HKY vs. K81 respectively), ti/tv was 0.76, and no significant proportion of invariant sites (I) was found. The resulting topology of the ML analysis was the same as the MP tree (Figure 4.3).

A great part of the variation in the 28S sequences was concentrated in positions 251-280 and 508-544 (alignment in Appendix 5). Although these two regions were difficult to align

unambiguously, there were insertions that could be aligned for subsets of taxa. When these highly variable positions were excluded, the variable sites were 133 (16%) and parsimony-informative sites were 40 (5%). Neither the statistics for homogeneity of base frequencies or the settings indicated by hLRTs and AIC for ML analyses changed after the omission of variable regions (see Table 4.2). The ML topology was the same as that obtained with all the characters included (Figure 4.3), but the two resulting MP trees (not shown; L=171; CI= 0.76; RC=0.66) showed support only for the nodes in (*Callipallene* (*Anoplodactylus* + *Pallenopsis*).

4.4.5 Combined analysis of the 18S and 28S ribosomal DNA

The topology produced by the 28S data set is not the same as that obtained with the 18S sequences. However, the two estimates of phylogeny (Fig. 4.2 and Fig. 4.3 respectively), did not appear significantly different according to the homogeneity test (nreps=10000, p=0.0662, PAUP* results). Disagreement between 18S and 28S was concentrated in the unexpected position of *Endeis* relative to ammotheids according to 28S, and the strong affinity between *Callipallene* and *Colossendeis* shown by 18S. *Callipallene* as sister-taxon of *Pallenopsis* and *Anoplodactylus* in the 28S analysis, is in some agreement with conventional classifications (see Child, 1992). The 28S data showed *Anoplodactylus* closer to *Pallenopsis* than to the ammotheids, as shown by 18S, but *Pallenopsis* sequence was not available for the 18S analysis.

Sequences of 18S and 28S of 11 taxa were combined in a single matrix using *Limulus* as outgroup introducing missing values for its 28S fragment. Missing values were also included for 28S of *Ascorhynchus* and the 18S fragment of *Pallenopsis*. This procedure did not have any effect on the topology and it is preferred over excluding available information (Wiens and Reeder, 1995). The combined data set comprised 1363 sites, of which 486 were 18S and 877 were 28S. Branch-and-bound search under parsimony criterion of the combined data set resulted in a single MP tree (Figure 4.4; L= 334). Nymphonidae + Colossendeidae were related to *Callipallene* with *Anoplodactylus* + *Pallenopsis* as a sister clade. *Endeis* was placed basal to this major clade. The ammotheid genera form a monophyletic group diverging earlier, but not strongly supported, and *Austrodecus* remained at the base of the tree.

4.4.6 Combined Morphological and molecular data

Despite the significant heterogeneity shown by the ILD test between the molecular and the morphological partition (nreps=10000, P=0.0001) a combined analysis was carried out. It has been shown that the ILD test can fail to determine data combinability giving inversely proportional results of incongruence to accuracy of phylogenetic signal (Yoder et al., 2001). Data combination was explored using an assembling of the three data partitions (morphology, 28s and 18S). Table 4.1 shows the data included for each of the partitions and Table 4.2 a

comparison of the analyses performed. A single shortest tree was obtained with the maximum parsimony criterion (Fig. 4.4; L=423; CI=0.67; RC=0.45). The tree remained the same after excluding highly variable regions from the DNA partitions and also under differential weighting of the morphological characters based on total number of characters (1.3:1). The best-supported clades were *Anoplodactylus* + *Pallenopsis; Nymphon* + *Colossendeis* and *Achelia* + *Ammothella. Austrodecus* remained as the basal taxon (Fig. 4.4).

4.5 Discussion

4.5.1 Outgroups

A range of taxa such as chelicerate representatives, the Xiphosuran *Limulus,* the basal millipede *Polyxenus* and a species of Onychophora were included as outgroups in the analysis of 18S sequences. These taxa on one occasion or the other, depending on the parameters used in analyses of arthropod phylogeny, had been shown as relatives of pycnogonids (Giribet and Ribera, 2000). The chelicerate clade remained monophyletic and the myriapod was segregated showing the highest values of sequence divergence. However, the presence of an insertion in the V4 domain of the 18S shared between most pycnogonids and the myriapod *Polyxenus* represents a particular character that might be worth studying under different conditions of analysis, such as the "direct optimization" method (Wheeler, 1996), which assesses the number of DNA sequence transformations required by a phylogenetic topology without the use of multiple sequence alignment (Wheeler, 1996; Giribet and Ribera, 2000). The monophyly of the Pycnogonida was strongly supported as it was expected according to their peculiar morphological autapomorphies (Boudreaux, 1979). Pycnogonids showed 12 molecular autapomorphies compared to chelicerates and *Polyxenus*.

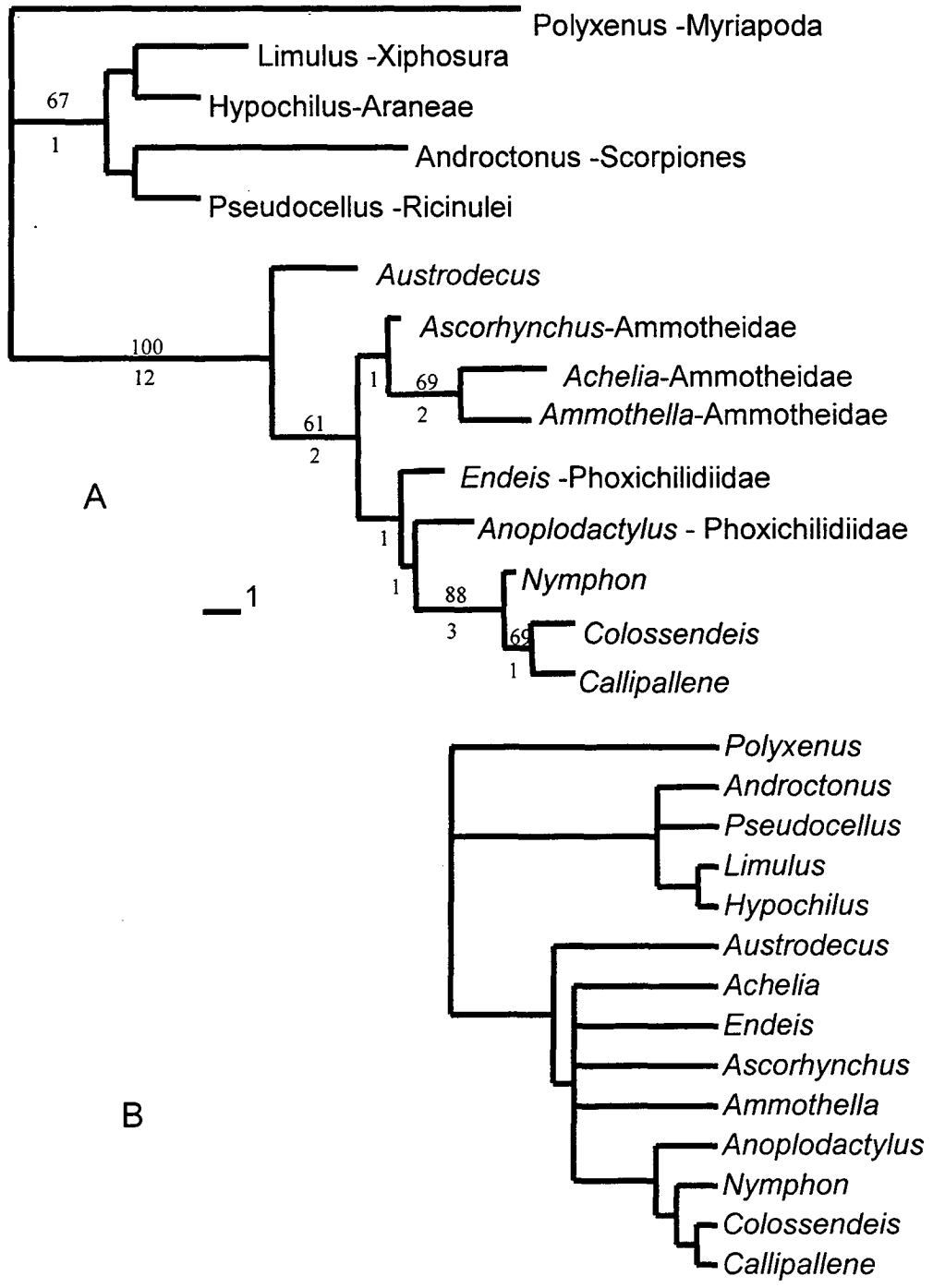

Figure 4.1 Phylogenetic tree of the Pycnogonida based on 18S. A) Same topology obtained under maximum parsimony (1/3 MP trees, L=203, CI=0.62, RC=0.57) and maximum likelihood based on TIMef + I + Γ model, -Ln likelihood= 1697.25. Bootstrap values (above) and Bremer values (above) are based on the MP analysis. Branch lengths and scale bar shown are proportional to character changes using MP (it applies to all figures). B) Consensus tree of seven MP trees (L=172) obtained after excluding hyper-variable regions of the 18S.

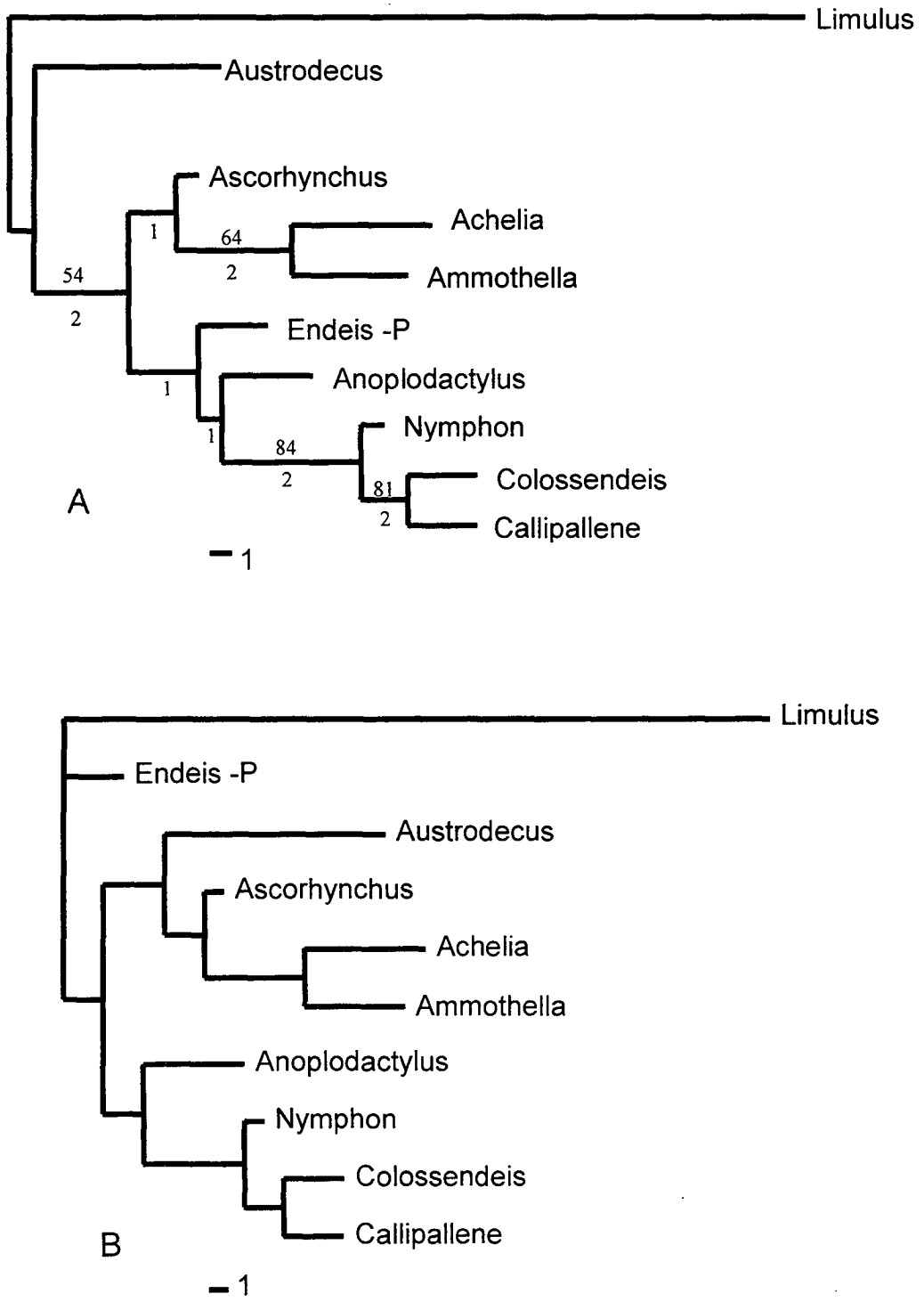

Figure 4.2 Phylogeny based on 18S after exclusion of outgroups. A) Single MP tree obtained under Maximum Parsimony analysis (L=90, CI=0.66, RC=0.55). B) Maximum likelihood analysis based on the TrN + I model, -Ln likelihood=1164.33.

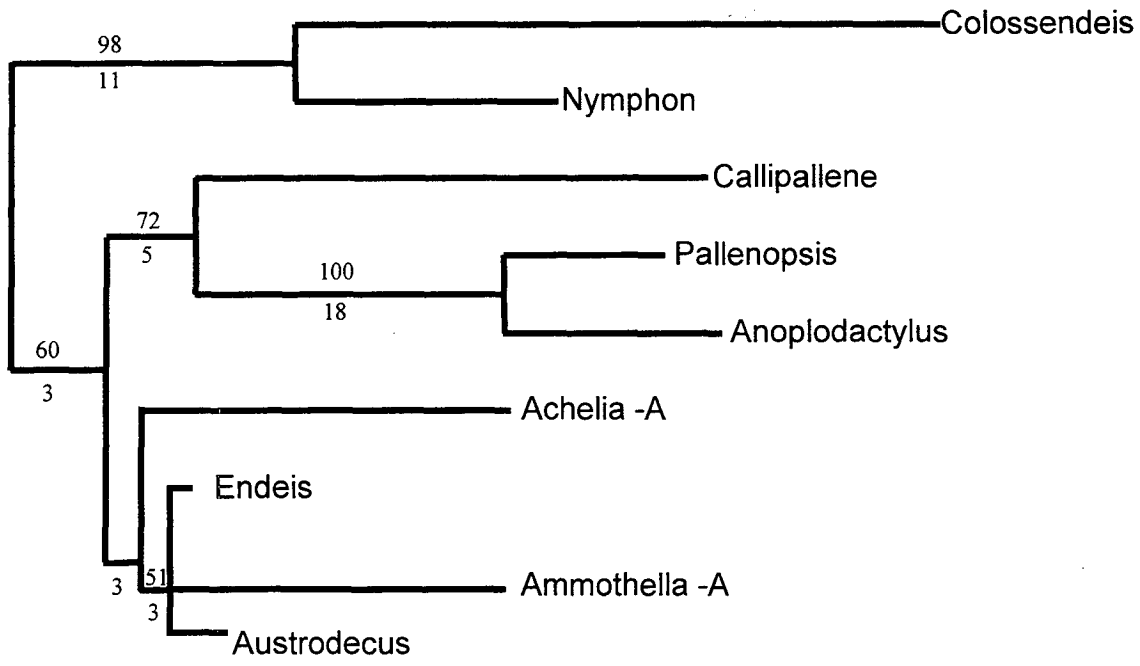

Figure 4.3. Phylogeny based on 28S. Single MP tree rooted at midpoint (L=239, Ci=0.74, RC=0.61). It is the same topology to that obtained with maximum likelihood analysis based on the HKY + Γ model. -Ln likelihood=2473.26. Same topology was obtained after exclusion of hyper-variable regions. Bootstrap and Bremer support values are based on the ML analysis.

4.5.2 Ingroup relationships

4.5.2.1 *Colossendeis* in conflict

According to the molecular data, *Colossendeis* is closely related to *Nymphon* and *Callipallene*. This result contrasts with that of the morphological analysis showing *Colossendeis* + *Ascorhynchus* (Ammotheidae) supported by the number of segments of the palps and the presence of terminal claw and multiple rows of spines in the ovigers (see Chapter 3). Traditional classifications have assumed a close affinity between Colossendeidae and Ammotheidae (Hedgpeth, 1947; Stock, 1994), however, these molecular data do not show the same pattern. A strong affinity between *Colossendeis* and *Nymphon* is suggested by the 28S sequences, while 18S shows *Colossendeis* closely related to *Callipallene*. The combined analysis of the two molecular partitions shows the three lineages grouped in a single clade. The morphological characters yield neither of these patterns of relationships and despite the strong support shown with molecular data, they cannot be explained on morphological grounds. In the morphological analysis, Colossendeidae is a long branch joined to the Ammotheidae by reversals of characters (23 and 31) (Figure 3.4, Chapter 3).

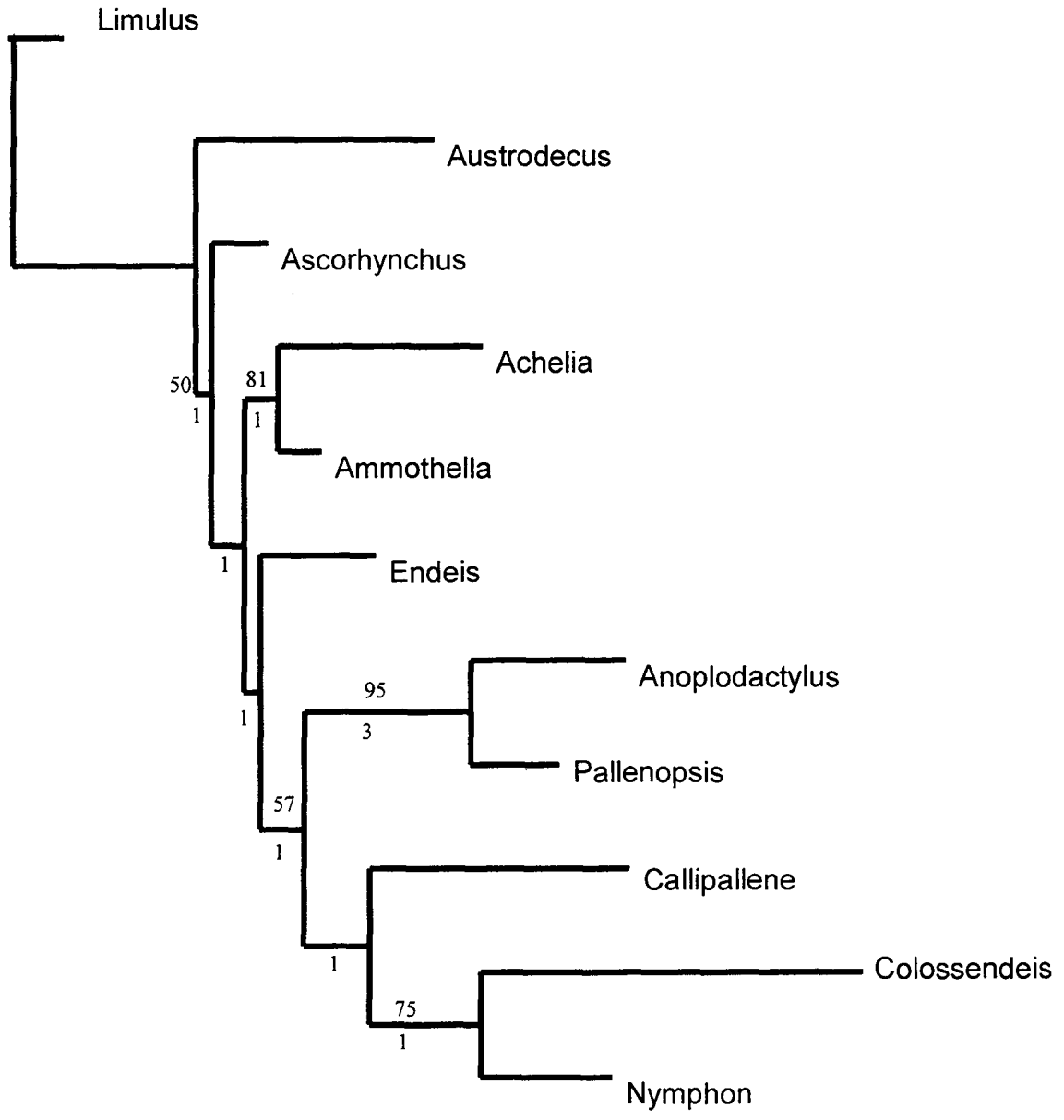

Figure 4.4. Estimate of phylogeny based on 18S + 28S and morphology. Maximum parsimony analysis with *Limulus* as the root (L=423, CI= 0.64, RC= 0.45). Same topology was yielded by the combined DNA partition 18S + 28S (L= 335, CI=0.69, RC=0.54).

4.5.2.2 Austrodecidae: a basalmost lineage

Austrodecus is shown as the most basal taxon in the 18S analysis and the combined analysis of the two molecular data sets. This particular lineage shows a number of molecular apomorphies (18S= 6 autapomorphies and 3 plesiomorphies, 28S=14 autapomorphies) and quite particular morphological features including a pipette-like proboscis and extreme reduction in the size of the ovigers. This taxon has never been mentioned as a possible primitive form of sea spiders, and its basal position here contrasts with the traditional view of nymphonids or ammotheid forms as primitive, while *Austrodecus* has been considered derived because of the reduction and absence of appendages. The morphological analysis in Chapter 3 shows Austrodecidae in a derived position related to the small-sized ammotheids *Tanystylum*. However, it also shows a number of autapomorphies suggesting it might be a divergent lineage within the evolution of sea spiders.

Ammotheid forms have been sometimes considered to be the most basal forms of pycnogonids because of their segmentation pattern and sutures in proboscis and abdomen indicating similarities with the Devonian fossil (Stock, 1994). Munilla also placed Ammotheidae as the basal lineage in his recent discussion of the phylogeny of the Pycnogonida, although in a previous study examining affinities among major lineages in an immunological essay, Nymphonidae appeared as the most primitive lineage of sea spiders (Munilla and De Haro, 1981). In this study, *Nymphon* appears in a derived position related to *Colossendeis+Calllipallene* in the combined analysis of molecular data, however, 28S shows *Nymphon+Colossendeis* as a divergent clade.

The problem of recognising a basal lineage of pycnogonids and the conflict between the morphological and the molecular data needs further examination perhaps within the context of the phylogenetic affinities of the ammotheid genera including *Nymphon* and *Colossendeis* as outgroup taxa.

4.5.2.3 'Transitional' *Pallenopsis*

The problematic genus *Pallenopsis* sometimes regarded as a 'transitional' form between Callipallenidae and Phoxichilidiidae is defined by molecular data as the sister-group of *Anoplodactylus*, the type genus of Phoxichilidiidae. This agrees with Stock's intuitive classification (Stock, 1965) of including *Pallenopsis* within Phoxichilidiidae but opposes other classifications in which *Pallenopsis* is regarded as a callipallenid (Hedgpeth, 1947; Child, 1992). There is morphological resemblance between *Pallenopsis* and *Anoplodactylus*, but the presence of ovigers in females of *Pallenopsis* is in conflict with one of the most characteristic features of the Phoxichilidiidae that is the females lacking ovigers. In the weighted morphological analysis presented in Chapter 3, *Pallenopsis* is basal to the non-

ammotheid taxa: (Nymphonidae + Callipallenidae + Phoxichilidiidae + Pycnogonidae). Although, morphological data have been not decisive regarding the position of *Pallenopsis*, DNA data has given additional evidence of proximity between *Pallenopsis* and *Anoplodactylus* as Stock suggested.

4.5.2.4 Ammotheidae paraphyletic ?

The limited taxon sampling of the molecular study did not allow making any inferences on the monophyly of the taxa based on DNA. However, at least *Achelia* + *Ammothella* were grouped together as expected from the morphological data. The weak support for their affinity with *Ascorhynchus* can be related to the paraphyletic appearance of the family already suggested by morphology. Ammotheidae is a highly diverse family and a more extensive sampling is needed for a complete molecular analysis of the relationships within this lineage. The inclusion of *Endeis* in the Ammotheidae in the 28S analysis cannot be explained; and should be treated with caution. Instead its position in the non-ammotheid clade in the 18S tree is more in agreement with the morphological results but a more defined relationship with *Anoplodactylus* was expected.

4.5.3 Additional data for 28S

Available sequences of the D3 domain of the 28S for six taxa (accession numbers: *Achelia* AF005459, *Callipallene* AF005460, *Colossendeis* AF005461, *Endeis* AF005462 and two species of *Anoplodactylus* AF062971 and AF062972) were explored for the aims of comparison with the 28S results obtained here. A maximum parsimony analysis of 345 aligned characters (not shown) does not agree with the yielded topologies of the D4-D7 region, instead it revealed a pattern for *Colossendeis* +*Callipallene*, similar to the results with 18S here. As mentioned before, this pattern of relationship is not supported from morphological grounds.

4.5.4 Confronting morphology and DNA

These preliminary analyses of nuclear ribosomal DNA and morphological characters show disagreement among the data sets. Most of the incongruence centers on the lineage Colossendeidae, which is related to Nymphonidae and/or Callipallenidae according to DNA but is related to Ammotheidae genera according to morphology. Parsimony and maximum likelihood resulted in very similar topologies in most cases, thus the conflict seems to be more related to the type of data used rather than the method of analysis.

There are many possible explanations for the lack of congruence between molecular and morphological phylogenies and there are extensive reviews in the literature covering the issue of incongruence among data sets. Wiens and Hollingsworth (2000) mentioned a mismatch between the gene phylogeny and species phylogeny as a possible source of incongruence. In

the case of pycnogonids, a robust species phylogeny has not yet been defined and there exist problems related to nonindependence of characters and the lack of appropriate outgroups for analysis (see Chapter 3). A robust morphological phylogeny is still a main task to be approached that could be aided by further studies in search of additional morphological, ecological and palaeontological characters.

Taxon sampling in high-level phylogeny studies is a relevant issue usually limiting a complete phylogenetic analysis of a group of organisms (Swofford, 1996). The analysis of morphological characters for a subset of taxa (selected according to molecular data available), yielded a different topology (not shown) from the tree obtained in the complete analysis of 38 taxa (Chapter 3). This difference suggests that inclusion of Pycnogonidae and Rhynchothoracidae and more representatives of the other taxa might change the pattern observed in a future outcome. Difficulties in finding material of many rare pycnogonid taxa have prevented more comprehensive studies. However, this study encourages more extensive sampling at all taxonomic levels, expanding the collections to deeper habitats and different latitudinal areas. Thus, the data set could be enhanced and more solid conclusions drawn about pycnogonid phylogeny.

4.5.5 Divergence times

The analysis of the V4 region of 18S revealed a low level of sequence divergence (<5%) in pycnogonids. This rather low rate of variation of this portion of 18S within Pycnogonida is similar to results obtained for the phylum Ctenophora, believed to have the lowest level of sequence variability at the level of the 18S rDNA gene in any metazoan phylum (Podar et al., 2001). It was expected that the V4 region would give representative variation, as has been the case of other arthropod groups, however, the result of an absolute average distance value among lineages of pycnogonids of 10 (2.8%), contrasts with a much higher value among genera of the family Ixodidae (Acari) (sequences from Black et al., 1997) (37.5; 8%) and among orders of Chelicerata (20; 6.1%). Pycnogonids have been assumed to be an ancient group of animals and even placed at the base of the arthropod tree (Giribet et al. 2001). The fossil records suggest they existed in the Devonian and probably even in the Cambrian (Walossek and Dunlop, in press). Being such an old group, the fact that 18S sequences are so homogeneous among pycnogonids suggest a possible recent divergence of the extant lineages with a high rate of extinction of taxa preceding them or an extreme slow rate of evolution of the 18S.

Table 4.2 Summary of results obtained with each of the data sets used to infer phylogenetic affinities of the Pycnogonida. (E) represents sets for which hyper-variable regions were excluded. The number of taxa includes pycnogonids (P) and outgroup taxa (O). CI= Consistency indices, RC= rescaled consistency indices (calculated without uninformative characters). Model selected by hierarchical likelihood tests is 'model hLRTs', model selected by the Akaike information criterion is 'model AIC'.

	18S with outgroups	18S (E) with outgroups	18S pycnogonids	18S (E) pycnogonids	28S	28S (E)	Combined 18S+28S	18S+28S +morphology
No taxa	9 P, 5 O	9 P, 5 O	9 P, 1 O	9 P, 1 O	9 P	9 P	10 P, 1 O	10 P, 1 O
Characters included	487	487	486	471	877	810	1363	1399
Characters excluded	-	19	-	15	-	67	-	-
Variable	134	116	68	57	177	133	245	278
Pars.- informative	54	45	24	18	61	40	85	111
Length MPT	203	172	91	75	239	171	334	423
Number MPT	3	7	1	4	1	2	1	1
CI	0.62	0.80	0.66	0.66	0.76	0.76	0.70	0.65
RC	0.57	0.59	0.55	0.56	0.61	0.66	0.57	0.47
Model hLRTs	TrNef + Γ	TrNef + Γ	TrNef + Γ	TrNef + Γ	HKY + Γ	HKY + Γ	-	-
	Γ=0.27	0.30	Γ=0.01	Γ=0.27	Γ=0.22	Γ=0.22		
-LnL	1693.80	1451.36	1169.77	1536.36	2474.80	2085.13		
Model AIC	TIMef + I + Γ	TrN + I + Γ	TrN + I	TrN + I+ Γ	HKY + Γ	HKY + Γ		
	I= 0.36; Γ= 0.64	I= 0.39; Γ= 0.76	I=0.79	I=0.41; Γ=0.75	Γ=0.22	Γ=0.22		
-LnL	1690.96	1445.99	1166.28	1531.63	2474.80	2085.13		

The possibility that the extant lineages of sea spiders are relatively young has not been considered since Hedgpeth (1947) when he argued that metameric instability and 'transitional' forms could be features of a recent group "...still undergoing active evolution". A few years later, describing a Devonian fossil, Hedgpeth retracted and preferred the hypothesis of pycnogonids as a primitive branch of the arthropods (Hedgpeth, 1955a). In a similar situation to that exposed for the comb jellies (Ctenophora) (Podar et al. 2001), the pycnogonids might be so ancient (see Giribet et al., 2001) that it is difficult to establish their position within Arthropoda, but the extant taxa may have evolved from a recent common ancestor. A long, well-supported branch leading to Pycnogonida, but very short branches within the group shows this. A more extensive approach using the complete sequences of 18S and other molecular markers is desired, to evaluate a possible recent divergence of extant lineages of pycnogonids.

The problems in the high level phylogenetics of the controversial pycnogonids have been exposed and explored using different data sets and current phylogenetic methods. The analytical procedure has shown that the main lineages of sea spiders might not be evolutionarily related in the manner shown by intuitive comparisons of presence and absence of head appendages. A trend towards reduction is suggested by morphological data, but this pattern is not congruent with the information contained in DNA sequences. The conflict suggests there could be much more complex evolutionary processes involved in the history of sea spiders. Despite being considered the most basal arthropods, partial sequences of 18S suggest that extant lineages could have diverged more recently than expected. I believe that an increase in the number of characters collected and taxa analysed could have a positive effect in resolving the conflicts among the different data partitions and in providing more solid hypothesis of phylogeny.

GENERAL DISCUSSION

5.1 Overview and implications for future research

The aim of this thesis has been to do a first investigation of the evolutionary relationships of sea spiders using cladistic analysis of morphological features and molecular data of the main families. The two main goals were to examine the phylogenetic relationships among sea spiders using morphological characters (Chapter 3) and to test the hypothesis of gradual reduction and loss of the appendages. Associated with this is the reconstruction of a molecular phylogeny (Chapter 4) for representatives of most of the traditionally accepted families to examine the usefulness of nuclear ribosomal DNA sequences in the study of high-level relationships of pycnogonids.

The results of this pioneering attempt to propose a phylogeny for the pycnogonids based on morphological and molecular data are preliminary. Despite the conflicts between estimates of phylogeny of the different data partitions, and the low support for some of the relationships proposed, this study has provided a testable basis for future work. It is the first time problems of the phylogeny of sea spiders have been exposed in a modern cladistic context, and the study has provided molecular grounds for extended research not only regarding internal pycnogonid phylogenies but also arthropod evolutionary history.

5.1.1. Implications for taxonomic classification

The taxonomy of pycnogonids has been based on the classification of characters rather than animals (Arnaud & Bamber 1987). The current classification has been useful for taxonomic purposes and has been stable for more than fifty years, except for a few problematic genera that 'jump' from one family to another. It has rarely been confronted despite the wide recognition that this traditional classification does not represent phylogenetic affinities. It is known there is a strong need to unravel how close that artificial classification could be to possible patterns of evolutionary history within the group. The few attempts to propose changes have failed due to equivocal results. Initially, I expected this analysis to provide either support for the existing pycnogonid taxonomic classification, or evidence to propose changes to it. However, after exploring and analyzing different data sets using different techniques, I have decided to adopt a conservative approach and not propose changes until more solid and well-supported phylogenies are found. This does not mean it has been an unsuccessful study: on the contrary, for the first time the difficulties and major causes of conflict in the cladistic analysis of Pycnogonida have been explicitly revealed.

The morphological analysis has provided indications that the Pycnogonida has diverged in two main clades, and that Ammotheidae and Callipallenidae are not natural (monophyletic) groups. Although the general results are more or less compatible with previous taxonomic or evolutionary-intuitive works, it has to be said that the external morphology of pycnogonids is not very amenable to cladistic analysis. This is due not only to the difficulties arising from the coding of absent structures; as discussed in Chapter 3, but also because some distinct clades or taxa such as Colossendeidae, Austrodecidae and *Endeis*, are supported by homoplasious synapomorphies and are placed on long branches on the morphological tree. Despite the DNA analysis being limited by taxon sampling, this preliminary molecular analysis gave evidence that the same taxa are also on long branches on the molecular topologies. It remains to be seen what the situation is like for other long-branch taxa such as Rhynchothoracidae and *Nymphopsis*, not included in the molecular analysis; but so far, it is clear that especially Colossendeidae and Austrodecidae are unstable taxa. They are not only morphologically very distinct but also the DNA shows divergence or conflict in regard to their position. Changes in high-level taxonomy of pycnogonids related to the creation of suprafamilial ranks, e.g. Orders, and the split of the families Ammotheidae and Callipallenidae will most likely follow in the next few years.

5.1.2 Evolution of characters: Reduction and loss

Tracing the evolution of characters was a central theme in the phylogenetic analysis and the project was designed to test the hypothesis that the number of segments of the appendages has been gradually reduced in the course of evolution. The reduction and loss of appendages was shown to have occurred as parallel events in divergent clades of pycnogonids and not as a trend of sequential gradual reduction in the whole group. Secondary loss or reduction of the appendages might have occurred independently in each lineage according to ecological and functional constraints, with, in all probability, different mechanisms controlling the functionality of each of the appendages separately. Alternatively, it is possible that the apparent evolution of the appendages is associated with genetically regulated metameric instability. Evidence for this could also be the so-called polymerous forms (they have one or two extra segments, each with a pair of legs) found in Nymphonidae, Colossendeidae and Pycnogonidae (Hedgpeth, 1947). This instability may have caused the morphological differences early in the divergence of lineages; and marked differences in habitat or diet (if any) presently observed are a product of that genetic constraint that became stable within major lineages. It has been indicated here that the loss of chelifores in adults is an event that has occurred independently in divergent taxa of pycnogonids. The last moult in the transformation from juvenile to adult is the key event where the loss of chelifores occurs.

What is (are) the factor(s) involved in the appearance or the non-appearance of chelifores in that last moult? It might be that the transformational process from presence to absence of chelifores during that pre-adult stage should be examined as a phylogenetic character, following the interpretation of Alberch (1985) and Kluge and Strauss (1985) of ontogenetic transformation (see also Kitching et al., 1998).

Regarding the palps, it is possible that feeding preferences are related to the reduction or absence of palps. To see whether ecological expediency rather than phylogenetic history mainly accounts for the reduction pattern among the lineages, it would be necessary to examine differences in feeding traits between taxa with palps and taxa without them.

5.1.3 Nuclear ribosomal DNA, how useful?

Regarding the molecular data (Chapter 4), the fragment of 18S used was shown to provide a rather conserved set of characters containing fewer informative sites than expected, when contrasted with other arthropod studies. It is a remarkable fact that according to this result, extant lineages of pycnogonids might have diverged recently, or, alternatively, the group shows a very slow rate of evolution of the genes concerned. The result obtained with the 28S needs to be confirmed with a longer sequence of the same marker and compared to possible outgroups.

The 'total evidence' approach, that is combining all available data partitions, provided a final cladogram (Fig. 4.4). Remarkably, this cladogram shows Austrodecidae as the basal pycnogonid taxon, divergent from the rest of extant forms. *Callipallene*, Colossendeidae and Nymphonidae form a well-supported clade indicating that the phylogenetic significance of presence-absence of chelifores, palps and ovigers suggested by a traditional view needs to be re-evaluated.

5.1.4 Pycnogonids from North Queensland: A valuable outcome

Finally, the collection of pycnogonid fauna (Chapter 2) not only constituted the basic and primary source of material for phylogenetic analyses but also is in itself an addition to the knowledge of tropical pycnogonid fauna. To date, there is almost no information on life histories, ecology or behaviour of tropical sea spiders. The continuing study of these marine arthropods would be a contribution to the understanding of the marine biodiversity of Australia, not only taxonomic but also of behaviour, lifestyles and associations with different organisms. During the development of this work, observations of feeding behaviour were made for some of the species. *Endeis mollis* and *E. biseriata* were observed feeding on hydrozoan corals and zoanthids, and *Anoplodactylus longiceps* attacked and fed upon the nudibranch *Okenia* sp. both collected from an intertidal site. Other species of the same genus of pycnogonids prey on several nudibranch species (Piel, 1991; Rogers et al., 2000).

Information about other species of *Anoplodactylus* and other taxa might help to clarify the ecological significance of morphological structures and permit the use of ecological characters in future phylogenetic analyses. Furthermore, these observations of feeding activity by sea spiders on chemically defended prey from tropical habitats, might have further ecological implications as has been shown for temperate species (Tomaschko, 1994; Sheerwood et al., 1998; Rogers et al., 2000).

This study of the pycnogonid fauna from North Queensland supported some zoogeographical patterns proposed by Bamber (1998b). I found species new to science and members of groups of species with very similar morphology known from the Indo-West Pacific and the Australian coast. These findings support the notion of a corridor of related species (products of allopatric speciation) occurring from south to north, a distribution determined mainly by oceanic currents (Stock, 1957; Bamber, 1998b) and subject to passive drift. The absence of planktonic larvae severely restricts dispersion in these animals, giving the chance for high rates of speciation (King, 1973; Bamber, 1998b). Studies on genetic flow, morphological differentiation and population structure would help to estimate speciation rates in populations of *Ammothella* and *Anoplodactylus* species that are abundant in shallow waters and very diverse in limited geographical areas as shown in Chapter 2.

Australasian waters were suggested to be the centre of distribution of callipallenid taxa (Clark, 1963); however, northeastern shallow tropical waters do not seem to shelter those genera such as *Anoropallene, Oropallene, Stylopallene* or *Pseudopallene*, known to be abundant in south eastern Australia. A boundary for the north-south distribution of the callipallenid forms is yet to be identified and phylogeographic analyses of the genera might help to redefine the artificial family Callipallenidae. Radiation and speciation in pycnogonids would need to be studied specifically for the taxon, the habitat and a particular geographical location before making generalisations.

5.2 Conclusions

Although no clear pattern of overall relationships among sea spiders is yet defined, several patterns important for future systematic work in the group were identified. Ammotheidae appears as a paraphyletic group, small-sized forms such as *Achelia* and *Ammothella* are related taxa but large *Ascorhynchus* and possibly *Eurycyde* could be joined in a separate clade. *Austrodecus* was shown to be highly divergent from other extant sea spiders and it is proposed as basal for the first time. Whether Colossendeidae is related to ammotheid forms or is more related to the Nymphonidae or the artificial Callipallenidae remains to be solved. *Pallenopsis* shows strong affinity with *Anoplodactylus* according to 28S, but morphological data are neutral to, or inconsistent with, this association. Parallel reduction and loss of body

parts have characterized the evolution of Pycnogonida, metameric instability and a high specialization of the proboscis might be part of the explanation.

In light of the lack of support for the traditional gradual reduction of the appendages as an evolutionary trend, I have proposed alternative hypotheses of phylogeny considering evolutionary processes such as convergence, parallelisms and secondary loss to explain the evolutionary history of Pycnogonida. However, there is a clear need for new morphological characters present in all the species of pycnogonids sampled, thus avoiding the problems associated with inapplicable characters and also the bias of the traditional model of regressive evolution. Ultrastructural characters can be promising, the internal structure of the proboscis, characterization of the cuticle and also sperm morphology have shown discrete characters states among genera of pycnogonids, their phylogenetic value yet to be determined.

Molecular data will be expanded and probably before the new morphological evidence becomes available. This can be predicted based on the rapid progress of molecular systematics and the strong interest of many research teams of arthropod phylogenetics. The mitochondrial genome and protein-coding genes are in the spotlight, and this preliminary phylogeny would be the start point for comparison and for the estimation of better molecular markers. Conflicts between data sets can only be explained when more characters are available.

The importance of the study of phylogenetic relationships of pycnogonids is not merely on the resulting phylogenetic hypothesis, but also in the implied search for new information in the form of characters, life stages, ecological traits and so on. This search for potential phylogenetic signal together with appropriate analytical procedures will lead us to new patterns of relationships that in time will serve to replace the artificial taxonomy of the group. Although useful for identification purposes, the traditional taxonomic classification has been based on the presence-absence of organs under a model of reductive trend and does not reflect tested evolutionary affinities.

The contradictory results produced by the numerous phylogenetic studies of Arthropoda encourage the search for new ecological, anatomical and genetic evidence especially from poorly known taxa. It is important to propose a solid phylogeny of the subphylum Pycnogonida, recently indicated as sister-taxon of all the other extant euarthropods, in order to gain knowledge of their evolution and understand the pycnogonids' place in arthropod evolution.

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Appendix 1

Key for genera of shallow-water pycnogonids *from North Queensland* collected in this study

1. Chelifores present.....2

1'. Chelifores absent.....13

2. Chelae present and functional.....3

2'. Chelae absent or very reduced .....6(**Ammotheidae**)

3. Palps present 4

3'. Palps absent 10

(Phoxichilidiidae, some Callipallenidae)

4. Palps 5-segmented.....*Nymphon* (**Nymphonidae**)

4'. Palps of less than 5 segments.....5

5. Ocular tubercle anterior in cephalic segment.....***Pallenopsis***

5'. Ocular tubercle posterior in cephalic segment.....***Propallene***

6. Body circular, discoid-shaped; scape 1-segmented.....7

6'. Body elongate; scape with 1 or 2 segments.....8

7. Proboscis inflated at midpoint; scape about 40% the length of the proboscis; crurigers spinose, palps usually with more than six segments, mostly eight .....***Achelia***

7'. Proboscis funnel-like or barrel-shaped; scape 30% the length of proboscis or less (not visible), palps always with four or six segments .....***Tanystylum***

8. Proboscis almost as long as trunk length, bent ventrally, with sutures.....***Ascorhynchus***

8'. Proboscis frontal, without sutures, inflated at midpoint or all.....9

9. Tubular, long spines in body and legs; tall ocular tubercle.....***Ammothella***

9'. Spinose tubercles in dorsum and legs in arborescent shape, body excessively ornamented; chelifores trumpet-shaped .....***Nymphopsis***

10. Chelae pointing downwards, fingers small and delicate.....***Anoplodactylus***

10'. Chelae oppose at each other, robust, fingers denticulate.....**11**

(some Callipallenidae)

11. Large specimens of more than 3-4 mm with long neck; general slender appearance.....***Parapallene***

11'. Small forms of less than 3 mm, robust; short neck, consticted.....**12**

12. Terminal claw present on ovigers, denticulate.....***Seguapallene***

12'. Terminal claw on ovigers absent .....***Callipallene***

13. Proboscis pipette-like, long, thin, with annulations.....***Austrodecus***

13'. Proboscis short or long, straight or diagonal.....**14**

14. Very long proboscis, stalked, thin anterior portion and inflated terminal portion with a dorsal hook (hook lacking in *R. dampieri* from WA) .....***Rhopalorhynchus***

14'. Proboscis straight or barrel-shaped.....**15**

15. Long proboscis placed at 45° angle, with distal setae; elongate body .....***Endeis***

(Endeidae)

15'. Short proboscis, straight, barrel-shaped; body, robust.....***Pycnogonum*** *

(Pycnogonidae)

* *Rhynchothorax* (Rhynchototacidae) not included in this collection would fit this description, the main difference between *Pycnogonum* and *Rhynchothorax* is the presence of palps in the latter.

Appendix 2

Key for species of Anoplodactylus from North Queensland collected in this study:

1. Trunk length 1 mm or less 2

1'. Body size larger, trunk length more than 1 mm.....5

2. Largest heel spine in propodus pectinate .....***A. pectinus***

2'. Heel spines smooth, without armature 3

3. Proboscis pipette-like, upturned, tapering distally .....***A. batangensis***

3'. Proboscis straight, robust, slightly inflated in the middle **4**

4. Male cement gland a mid-dorsal tube in femur; males with ventral genital spurs in the second coxae of last pair of legs; legs, short, robust (*Halosoma*-group); sides of neck concave; extremely small species .....***Anoplodactylys* sp. A**

4'. Male cement glands 2-5 dorsal cups in femur; trunk not distinctly segmented; propodus with five heel spines .....***A. glandulifer***

5. Ocular tubercle extremely tall, half the length of the trunk; crurigers widely separated (by twice their diameter). The species occurs in soft bottoms .....***A. tubiferus***

5'. Ocular tubercle low or of medium size, not as tall as above 6

6. Body extremely slender and tenuous; crurigers separated almost six times their own diameter .....***A. tenuicorpus***

6'. Body not that elongate, crurigers separated by twice their diameter or less 7

7. Cement glands of males two low cups dorsally in femur; ocular tubercle erect, pointed; long tubercle distally in femur; no genital spurs in male.....**A. *longiceps***

7'. Cement gland a dorsal duct or tube, short or long; long genital spurs ventrally in second coxae of third and fourth legs 8

8. Proboscis of female with two ventral protuberances; ocular tubercle pointing anteriorly; individuals of small size (1.0-1.3mm trunk length) ..... ***Anoplodactylys* n. sp. B**

8'. Proboscis of females with four ventral protuberances; individuals of larger size (1.6 mm to 2 mm trunk length) **9**

9. Legs smooth, no conspicuous spines or dorsal tubercles; cement gland a long and straight mid dorsal tube .....***A. digitatus***

9'. Legs with spines on coxae; distal spinose tubercles on femur and first tibia; cement gland a short duct dorsal on femur.....***A. versluysi***

Appendix 3

Species examined for the cladistic analysis of morphology (Chapter 3)

Species used for the analysis (*) and other material examined. The following acronyms for institutions are used: AM, Australian Museum, Sydney; ICN, Instituto de Ciencias Naturales, Bogotá; MNT, Museum of Northern Territory, Darwin; MTQ, Museum of Tropical Queensland, Townsville; MWA, Museum of Western Australia, Perth; NMNH, National Museum of Natural History of the Smithsonian Institution, Washington; QM, Queensland Museum; Brisbane; SMBL, Seto Marine Biological Lab, Kyoto University; UAB, Universitat Autónoma de Barcelona; ZMA, Zoölogisch Museum Amsterdam. Those species, for which no material was available, were scored based on published descriptions.

Ammotheidae

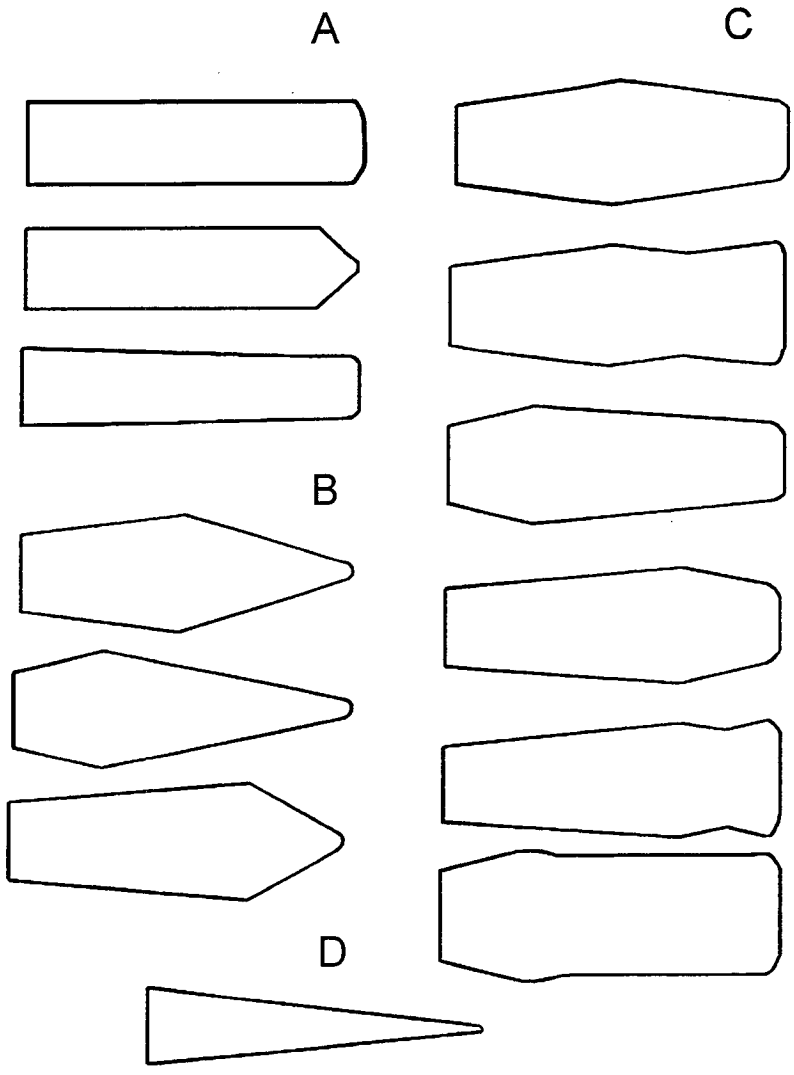
**Eurycyde raphiaster* Loman 1912	Curaçao; ZMA
Eurycyde gorda Child 1979	Specimens not seen
**Achelia assimilis* Haswell 1884	Queensland, Australia, AM
**Achelia australiensis* Stock 1954	South Australia, AM
Achelia bullosa Child 1996	Specimens not seen
Achelia dorhni Thomson 1884	Specimens not seen
Achelia echinata Hodge 1864	Japan, SMBL
Achelia mixta Stock 1994	Specimens not seen
Achelia nana Loman 1908	Great Barrier Reef, Australia; MTQ
Achelia shepherdi Stock 1973	Specimens not seen
Achelia transfuga Stock 1954	Specimens not seen
Achelia variabilis Stock 1954	Specimens not seen
**Ammothella biunguiculata* Williams 1940	Australia; AM
Ammothella elegantula Stock 1968	Specimens not seen
Ammothella indica Stock 1954	Specimens not seen
Ammothella prolixa Child 1990	Queensland, Australia; MTQ
Ammothella stauromata Child 1982	Specimens not seen
Ammothella theitidis Clark 1963	New South Wales Australia, AM
Ammothella tippula Child 1983	Specimens not seen
**Ammothella* n. sp. A (*appendiculata*-group)	Queensland, Australia; MTQ
**Ammothea hilgendorfi* (Böhm 1879)	Japan; SMBL
Tanystylum bredini Child 1970	Specimens not seen
Tanystylum excuratum Stock 1954	Specimens not seen
**Tanystylum haswelli* Child 1990	Queensland, Australia; MTQ
Tanystylum hooperi Clark 1977	New South Wales, Australia; AM
Tanystylum nesiotes Child 1970	Specimens not seen
Tanystylum philippinensis Child 1988	Specimens not seen
**Tanystylum rehderi* Child 1970	Queensland, Australia; MTQ
Tanystylum scrutator Stock 1954	East Australia; AM
**Nymphopsis acinacispinata* Williams 1933	Queensland, Australia; MTQ
Nymphopsis armatus Haswell 1884	South Australia, AM
Nymphopsis korotnewi Child 1975	Specimens not seen

Ascorhynchus compactum Clark 1963	New South Wales, Australia; AM
**Ascorhynchus glaberrimum* Schimkewitsch 1913	Japan; SMBL
Ascorhynchus melwardi Flynn 1929	Queensland, Australia; AM
**Ascorhynchus ramipes* (Böhm 1879)	Japan; SMBL
**Ascorhynchus tenuirostris* Carpenter 1892	Queensland, Australia; MTQ
Ascorhynchus unidentified sp	Northern Territory, NTM
**Cilunculus armatus* (Böhm 1879)	Japan; SMBL
Cilunculus australiensis Clark 1963	New South Wales, Australia; AM
**Cilunculus sekiguchii* Nakamura and Child 1983	Japan; SMBL
Nymphonidae	
Nymphon draconum Child 1990	Specimens not seen
**Nymphon micronesicum* Child 1982	Queensland, Australia; MTQ
**Nymphon molleri* Clark 1963	Queensland, Australia; MTQ
**Nymphon surinamense* Stock 1975	Colombia Caribbean; ICN
Nymphon charcoti Bouvier 1911	Antarctica (Courtesy T. Munilla, UAB)
Nymphon striatum Losina-Losinsky 1929	Japan; SMBL
Colossendeidae	
**Colossendeis megalonyx* Hoek, 1881	*Antarctica (Courtesy T. Munilla, UAB)*
Colossendeis sp.	Northern Territory, NTM
**Rhopalorhynchus tenuissimum* (Haswell, 1884)	Queensland, Australia; MTQ
Austrodecidae	
**Austrodecus glaciale* Hodgson 1907	Specimens not seen
Austrodecus gordonae Stock 1975	Specimens not seen
**Austrodecus* n. sp. (*gordonae*-section)	Queensland, Australia; MTQ
Austrodecus staplesi Stock 1990	New South Wales, AM
Rhynchothoracidae	
**Rhynchothorax australis* Hodgson 1907	Antarctica (Courtesy T. Munilla, UAB)
Callipallenidae	
**Callipallene novazealandiae* Stock, 1954	Queensland, Australia; MTQ
**Callipallene brevirostris* Johnston 1837	Queensland, Australia; MTQ
Callipallene n. sp. A	Queensland, Australia; MTQ
Parapallene australiensis Hoek 1881	New South Wales, Australia; AM
**Parapallene famelica* Flynn 1929	Queensland, Australia; MTQ
Parapallene haddoni Carpenter, 1892	Northern Territory, NTM
Propallene cyathus Staples 1979	New South Wales, Australia; AM
Propallene kempi Calman, 1923	Northern Territory, NTM
**Propallene saengeri* Staples 1979	Queensland, Australia; QM
**Pseudopallene ambigua* Stock 1956	Tasmania, Australia; MTQ
Pseudopallene unidentified sp.	Northern Territory, NTM
Pallenopsis denticulata Hedgpeth, 1944	Northern Territory, NTM
Pallenopsis hoeki Miers 1884	Queensland, Australia; MTQ
**Pallenopsis schmitti* Hedgpeth 1943	Colombia Caribbean; ICN
Phoxichilidiidae	
**Anoplodactylus batangensis* (Helfer 1938)	Queensland, Australia; MTQ
**Anoplodactylus evansi* Clark 1963	Queensland, Australia; MTQ
**Anplodactylus glandulifer* Stock 1954	Queensland, Australia; MTQ
**Anoplodactylus insignis* Hoek 1881	Colombia Caribbean, ICN
**Anoplodactylus longiceps* Stock 1951	Queensland, Australia;MTQ
Anoplodactylus simplex Clark 1970	Queensland, Australia; MTQ
**Anoplodactylus tenuicorpus* Child 1988	Queensland, Australia; MTQ
Anoplodactylus tubiferus (Haswell 1884)	Queensland, Australia; MTQ
Anoplodactylus unidentified sp.	Northern Territory, NTM

**Anoplodactylus* n. sp. A	Queensland, Australia; MTQ
**Endeis mollis* Carpenter 1903	Queensland, Australia; MTQ
Endeis biseriata Stock 1968	Queensland, Australia; MTQ
Endeis meridionalis Bohm 1879	Queensland, AM
Endeis flaccida Calman 1923	Queensland, Australia; MTQ
Endeis nodosa Hilton 1942	Antarctica (Courtesy T. Munilla, UAB)
Pycnogonidae	
**Pycnogonum litorale* Ström 1762	Reared by K.H. Tomaschko, Univ. Ulm
Pycnogonum gaini Bouvier 1910	Antarctica (Courtesy T. Munilla, UAB)
Pycnogonum n. sp. A	Queensland, Australia; MTQ

Diagrams of geometrical representation of the shapes of the proboscis in the Pycnogonida.

This is a simplified form of the classification by Fry and Hedgpeth (1969). The shapes were grouped in A, B, C or D according to the similarities in the distal part (pointing to the right-hand side of the page), a criterion believed to have some relevance in relation to feeding habits.



Appendix 5

i) Alignment of 506 sites of the V4 domain of 18S rDNA.

```
                          10         20         30         40         50
Polyxenus          TTCCAGCTCCAATAGCGTATACTAAAGTTGTTGCGGCTAAAAAGCTCGTA
Limulus            ..........D........T........C.....T.............
Androctonus        .A.................T..........T..T.............
Hypochilus         ...................T..........T..T.............
Pseudocellus       ...................T.............T.............
Achelia            ...................T.............T.............
Ammothella         ...................T.............T.............
Ascorhynchus       ...................T.............T.............
Austrodecus        ...................T.............T.............
Colossendeis       ...................T.............T.............
Callipallene       ...................T.............T.............
Nymphon            ...................T.............T.............
Anoplodactylus     ...................T.............T.............
Endeis             ...................T.............T.............

                          60         70         80         90        100
Polyxenus          GTTGGATTTCAGTCGTAGGCCGGTGGTCCACCGC-CCGGTGGCTACTGCC
Limulus            .......C.....TC...A.T..C.....G.TT--....C..T.......
Androctonus        .......C....ATCCG.AG.T.C.........-.......T.....TA
Hypochilus         .......C.....TCC..A.G..C.....G..TAA.-.....T.......
Pseudocellus       .......C.....TCC..A.T..C.........-.............
Achelia            ..........T..TCC..A.T.AC.......TCA-T.......G....T.
Ammothella         ..........T..TCC..A.T.AC.........-TT......G....T.
Ascorhynchus       ..........T..TCC..A.T.AC.........-T.......G....T.
Austrodecus        ..........T...TC..A.T.AC.........-TT......G....T.
Colossendeis       ..........T..TCC..A.T..C.........AA-.......G....T.
Callipallene       ..C.......T..TCC..A.T..C.........AA-.......G....T.
Nymphon            ..........T..TCC..A.T..C.........AA-.......G....T.
Anoplodactylus     .......C..T..TCC..A.T.AC.........AAT.......G....T.
Endeis             ..........T..TCC..A.T.AC.........-T.......G....T.

                         110        120        130        140        150
Polyxenus          TGGTCTGGACACCTTGCCAAGCTCTCCGGCAATGCTCTCCGGCAATGCTC
Limulus            ...C..AA...T.C....GGTT.--------------...CT.GG...C.
Androctonus        GC.A.C....GTT.G...GGAT----------------...TTTG.....
Hypochilus         ...C...A...A.CA...GGTT----------------...CTAG...A..
Pseudocellus       ...A...A...TT..AT.GGTT.----------------...CT.GG.....
Achelia            A..C...A...TTCG.T.GGTTCG..TCCGG.CTTCG...C..GG.....
Ammothella         A..C...A...TTCG.T.GGTT....TT--GG.-TTCG...C..GG.....
Ascorhynchus       A..C...A...TTCC.T.GGTT.--..GACAG.-TT.T...C..GG.....
Austrodecus        A..C.......T.CC.T.GGTT-A.---CA------.T...C..G......
Colossendeis       A..C...A...T.CG.T.GGTT-...GACAG.---.T...C..GG.....
Callipallene       A..C...A...T.CG.T.GGTT-...GACAG.A---T...C..GG.....
Nymphon            A..C...A...T.CG.T.GGTT-...GACAG.--T.T...C..GG.....
Anoplodactylus     A..C...A...T.CC.T.GGTTCT.TT-CA------T...C..GG.....
Endeis             A..C...A...T.CC.T.GGTTC.C.TAC-----T.T...C..GG.....
```

```
                          160        170        180        190        200
Polyxenus          TTGACCGGGTGTCGTTGGTGGCTGGAACGTTTACTTTAAAAAAATTAGAG
Limulus            ....TT.A.....T.G......C..C..........G............
Androctonus        ..TG...A.....T.G....T.C..C..........G............
Hypochilus         ..C.TT.AT....T.G....A.C..C..........G............
Pseudocellus       ..C.TT.A.....T.G....A.C..T..........G............
Achelia            ..CGGT.A......CG..A...C.AC.A........G............
Ammothella         ..C.GT.A......CG..A...C.AC.A........G............
Ascorhynchus       ..C.GT.A......CG..A...C.AC.A........G............
Austrodecus        ..C.GT.A......CG..A...C..C.A........G............
Colossendeis       ..TGTT.A......CG..A...C.AC.A........G............
Callipallene       ..TGTT.A......CG..A...C.AC.A........G............
Nymphon            ..CGTT.A......CG..A...C.AC.A........G............
Anoplodactylus     ..CGTT.A......CG..A...C.AC.A........G............
Endeis             ..CGTT.A......CG..A...C.AC.A........G............

                          210        220        230        240        250
Polyxenus          TGCTCTAAGCAGGTGCTATCGGCTTGAATAACACAGCATGGAATAATGGA
Limulus            .....A.......C..A.-..-..C.......TGGT..............
Androctonus        .....A.......C.T--C.-..C.......TGGT..............
Hypochilus         .....A.......C.TG.-.-..C.......TGGT..............
Pseudocellus       .....A.......C.TG.-.-..C.......TGGT..............
Achelia            .....A.......C.-G-..A..C.......TGGT.........G.....
Ammothella         .....A.......C.-G-..A..C.......TGGT.........G.....
Ascorhynchus       .....A.......C.-G-..A..C.......TGGT.........G.....
Austrodecus        .....A.G.....C.-G-..A..C.......TGGT.........G.....
Colossendeis       .....A.......C.T.-.....C.......TGGT.........G.....
Callipallene       .....A.......C.T.-.....C.......TGGT.........G.....
Nymphon            .....A.......C...-.....C.......TGGT.........G.....
Anoplodactylus     .....A.......C.-G-..A..C.......TGGT.........G.....
Endeis             .....A.......C.----..A.TC.......TGGT.........G.....

                          260        270        280        290        300
Polyxenus          ACACGACCTTGGTTCTGTTCTGTTGGTCTTTGGAAGCCAAGGTAATGATT
Limulus            .T.G.....C......A..T.......T..C....CA.G...........
Androctonus        .T.A.....C.TC...A..T.......T..C....AA.G...A.......
Hypochilus         .T.G.....C......A..T.......T..C....CA.G...........
Pseudocellus       .T.G.....C......A..TG......T..C....CA.G...........
Achelia            .T.G.....C......A..T.......T..C.C..CT.G...........
Ammothella         .T.A.....C......A..T.......T..C.C..CT.G...........
Ascorhynchus       .T.G.....C......A..T.......T..C.C..CT.G...........
Austrodecus        .T.G.....C......A..T.......T..C.C..CT.G...........
Colossendeis       .T.G.....C......A..T.......T..C.C..CT.G...........
Callipallene       .T.G.....C......A..T.......T..C.C..CT.G...........
Nymphon            .T.G.....C......A..T.......T..C.C..CT.G...........
Anoplodactylus     .T.G.....C......A..T.......T..C.C..CT.G...........
Endeis             .T.G.....C......A..T.......T..C.C..CT.G...........

                          310        320        330        340        350
Polyxenus          AATAGGGACGGACGGGGGCATTCGTATTGCGACGCTAGAGGTGAAATTCT
Limulus            ..G......A.....................................
Androctonus        ..G......A.....................................
Hypochilus         ..G......A.....................................
Pseudocellus       ..G......A.....................................
Achelia            ..G......A.....................................
Ammothella         ..G......A.....................................
Ascorhynchus       ..G......A.....................................
Austrodecus        ..A......A.....................................
Colossendeis       ..G......A.....................................
Callipallene       ..G......A.....................................
Nymphon            ..G......A.....................................G.....
Anoplodactylus     ..G......A.....................................
Endeis             ..G......A.....................................
```

```
                          360        370        380        390        400
Polyxenus         TGGACCGTCGCAAGACGAACTACTGCGAACGCATTTGCCAAGAACGTTTT
Limulus           ............................A.............T.....
Androctonus       ............................A.............T.....
Hypochilus        ............................A.............T.....
Pseudocellus      ............................A.............T.....
Achelia           ....................T......A.............T.....
Ammothella        ....................T......A.............T.....
Ascorhynchus      ....................T......A.............T.....
Austrodecus       ............................A.............T.....
Colossendeis      ......CG....................A.....C.......T.....
Callipallene      ............................A.............T.....
Nymphon           ............................A.............T.....
Anoplodactylus    ....................T......A.............T.....
Endeis            ....................T......A.............T.....

                          410        420        430        440        450
Polyxenus         CATTAATCAAGAACGAAAGTCAGAGGTTCGAAGGCGATCAGATACCGCCC
Limulus           ...................T............................
Androctonus       ...................T............................
Hypochilus        ...................T............................
Pseudocellus      ...................T............................
Achelia           ...................T............................
Ammothella        ...................T............................
Ascorhynchus      ...................T............................
Austrodecus       ...................T............................
Colossendeis      ...................T............................
Callipallene      ....G..............T............................
Nymphon           ...................T............................
Anoplodactylus    ...................T............................
Endeis            ...................T............................

                          460        470        480        490        500
Polyxenus         TAGTTCTGACCATAAACAATGCCAACCAGCGATCCGCCGGAGTTACTCCC
Limulus           .......A.........G...................T.....C...AA
Androctonus       .......A.........G............A.......T.....C...AA
Hypochilus        .......A.........G...................T.....C...AA
Pseudocellus      .......A.........G...................T.....C.A.AA
Achelia           .......A.........G.........................C..T.T
Ammothella        .......A.........G.........................C..TT.
Ascorhynchus      .......A.........G.........................C..T.T
Austrodecus       .......A.........G....G..............T...C....T
Colossendeis      .......A.........G.........................C..T.T
Callipallene      .......A.........G.........................C..TTT
Nymphon           .......A.........G.........................C..T.T
Anoplodactylus    .......A.........G.........................C..T..
Endeis            .......A.........G.........................C..T.T

Polyxenus         ATGACT [505]
Limulus           .C.... [488]
Androctonus       ...... [488]
Hypochilus        ...... [488]
Pseudocellus      ...... [489]
Achelia           ...... [503]
Ammothella        ...... [500]
Ascorhynchus      ...... [500]
Austrodecus       ...... [494]
Colossendeis      ...... [500]
Callipallene      ...... [500]
Nymphon           ...... [501]
Anoplodactylus    ...... [497]
Endeis            ...... [498]
```

ii) Alignment of 877 sites of the D4-D7 region of 28S rDNA.

```
                          10         20         30         40         50
Achelia                   ACGGGCCGAGCTATGTGCACCGGACACCGGAAGAGCCGC-GGTGCTTCAC
Ammothella                .A.......CAGGG..............C.........-.......T..
Endeis                    .A.......CAGGG..............C.........-.......T..
Tanystylum                .A.......CTGGG..............C.........-.......T..
Austrodecus               .A.......CAGGG..............C.C..C....-.......T..
Colossendeis              .A...A...CAGGG..............C.........-.......T..
Callipallene              .A.......CAAGG..............C.........-.......T..
Nymphon                   .A...A...CAGGG..............C.........T.......T..
Pallenopsis               .A.......CGGGG..............C.........-.......T..
Anoplodactylus            .A.......CGGGG..............C.........-.......T..

                          60         70         80         90         100
Achelia                   CGTAA-CAAACCT-TGTCCCTATCTCCGGGCAAGCCGATTTCAGGGAGTC
Ammothella                G.G..-.......-..............................C.........
Endeis                    G.G..-.......-..............................C.........
Tanystylum                G.G..-.......-..............................C.........
Austrodecus               G.G..-.......-..............................C.........
Colossendeis              G.G.TG-......A.-.......A..T.............C..C.....G
Callipallene              G.G..TTT.....-C.............................C.........
Nymphon                   G.G..G-......A..............................C.........
Pallenopsis               G.G.TA.GT..G.-C.............................C.........
Anoplodactylus            G.G.TGA.T..G.-C.............................C.........

                          110        120        130        140        150
Achelia                   AGTCCCTTACAAAGAAAAGACAACTCTTTCCGGGACCCCTGCCGACGCCT
Ammothella                ..........G.................C.................T..
Endeis                    ..........G.................C.................T..
Tanystylum                ..........G.................C.-...............T..
Austrodecus               ..C.......C................CC.................T..
Colossendeis              ..A.......G..........C..................T....ATG.
Callipallene              ..........G.................C.................T..
Nymphon                   ..A.......C.....................................T..
Pallenopsis               ..........G.................C.........TC.......T..
Anoplodactylus            ..........G.................C.........TC.......T..

                          160        170        180        190        200
Achelia                   CCGAGTACGGTT-GCAGTCTCCGCACGAGACCCCGAAGGGTCGGTCTACG
Ammothella                ......-.....T..-...G.............................C..
Endeis                    ......-.....T..-...G.............................C..
Tanystylum                T.....-.....T..-...G.............................C..
Austrodecus               ...C-.-.....T..-...G.....................G...C.AG..
Colossendeis              .T...-......T.T-..G.............................C..
Callipallene              ..C....-....T..-...G......T.....................C..
Nymphon                   ....-C......T..-...G.............................C..
Pallenopsis               ......-.....T..-...G......TT............C......C..
Anoplodactylus            ......-.....T..-...G......TT....................C..

                          210        220        230        240        250
Achelia                   CATACGGGTTCGGGAATGTGAACCCGATTCCCTTTTGGTCGTCGGCGGGC
Ammothella                ...C..............T..............C..............
Endeis                    ...C..............T..............C..............
Tanystylum                ...C..............T..............C..............
Austrodecus               ...C.....G........C..............C.....C........
Colossendeis              ..........T..............C.......................
Callipallene              ...C..............T..............C...........A..
Nymphon                   ...C..............C..............C.....C........
Pallenopsis               ...C..............T..............C.....AT.......
Anoplodactylus            ...C..............T..............C.....AT.......
```

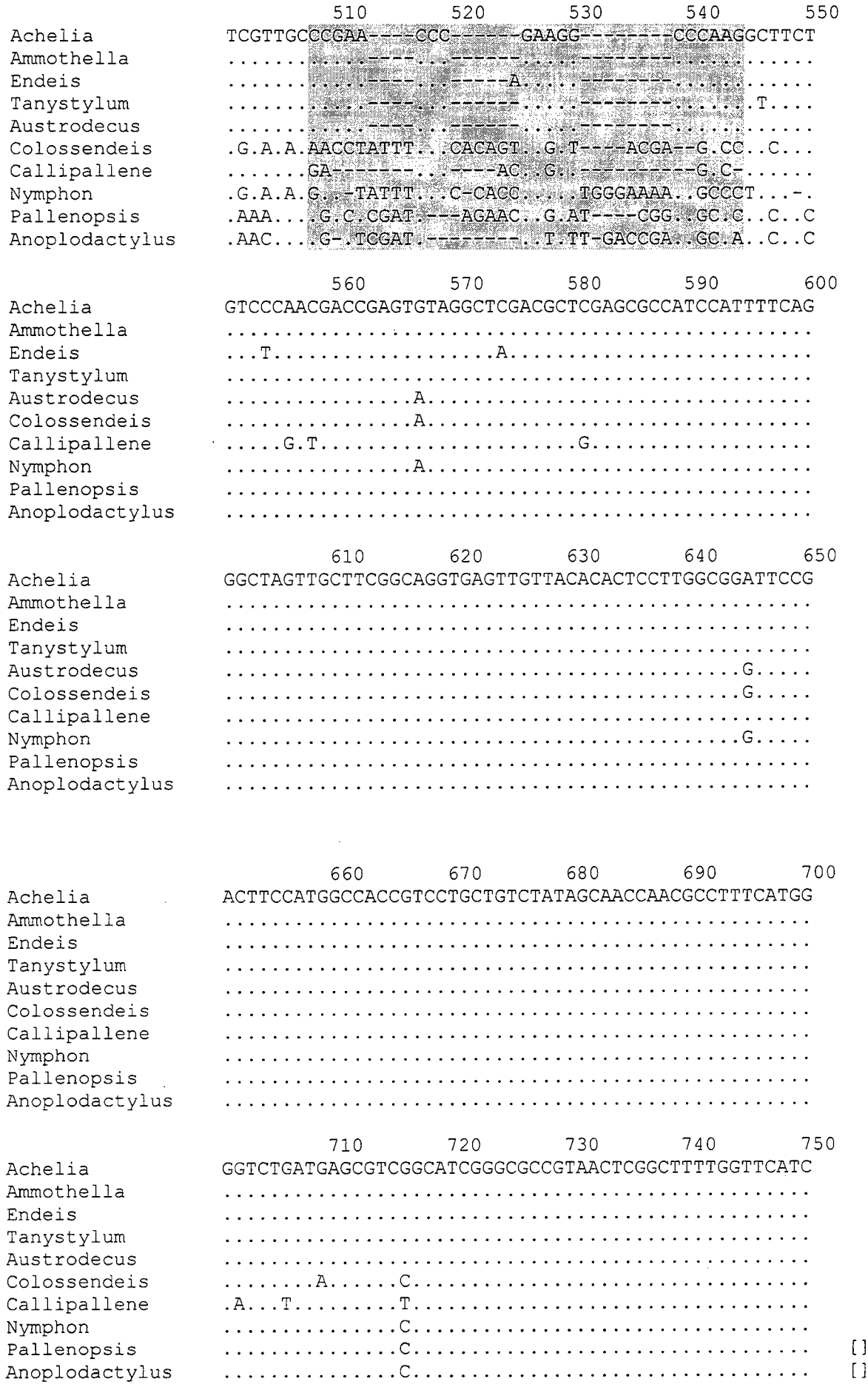
```
                    260        270        280        290        300
Achelia             -TAT-GTTT-ACAA-GACAT----------ACGCCCTGCTTTACGAACGG
Ammothella          -.T.-.C..-...-.-..-----------.....------.-.......
Endeis              -.T.-.C..-...-.-..-----------.....------.-.......
Tanystylum          -.T.-.C..-...-.-..-----------.....------.-.......
Austrodecus         -.T.-.C..-...-.-..-----------.....------.-.......
Colossendeis        AATAC.--.AGT..A...G.A---------.....A.....-.......
Callipallene        -.C.---AAA....CAGAG.ATTGAAAAG-....T.-.....-.......
Nymphon             ACTAC.--.A.T..A...G.A---------.....A.....-.......
Pallenopsis         A.T.C.G..CGT..C..AT.----------.A....A.....-.......
Anoplodactylus      A.T-CA...CGTCTG..A.GATGCAAGAAA.A....G.....-.......

                    310        320        330        340        350
Achelia             ACTTCTCCTATA-CTTATGACCGACTGACCCATGTTCAACTGCTGTTCAC
Ammothella          ...........CC....G..............................
Endeis              ...........CC....G..............................
Tanystylum          ...........CC....G..............................
Austrodecus         ...........CC....C..............G...............
Colossendeis        ....A.......CT...G..............CT.....A.......
Callipallene        ...........CC....G..............................
Nymphon             ....A.......C....G..............................
Pallenopsis         .A..A......TC....G..............................
Anoplodactylus      .A..A......CC....G..............................

                    360        370        380        390        400
Achelia             ATTGATACCCTTCTCCACTTCGGCCCTCAAGATTCTCACTCG-AGTATTT
Ammothella          ..G..A-.....................................-.......
Endeis              ..G..A-.....................................C.......
Tanystylum          ..G..A-........-............................-.......
Austrodecus         ..G..A-............TC.G.....................-.......
Colossendeis        ..G..A-.....TGGA.-...T.....A.T..........A.-.......
Callipallene        ..G..A-.......................G..........-.......
Nymphon             ..G..A-.......AG....T..G.....................-.......
Pallenopsis         ..G..A-.....................................-.......
Anoplodactylus      ..G..A-.....................................-.......

                    410        420        430        440        450
Achelia             GCTACTACCACCAAGATCTGCACCAGCGGCGGCTCGGGACGGGCTCGCGC
Ammothella          ..............................................
Endeis              ..............................................
Tanystylum          ..............................................
Austrodecus         ..............................................
Colossendeis        .TC.....T.....C..A..A.....A..T..........A.......
Callipallene        ...................................A.........A...
Nymphon             ..............................T...............
Pallenopsis         ...................................A...........
Anoplodactylus      ...................................A...........

                    460        470        480        490        500
Achelia             CCGGCACCTTCCGCGCACACCG-CTGCGACCTCCCTACTTAGTTGGGACC
Ammothella          ......................-..............-C.........
Endeis              ......................-..............-C.........
Tanystylum          ......................-..............-C.........
Austrodecus         ....................GT..............C.........
Colossendeis        .-..-T.......G..T.....-...G......G.....-C-......A.
Callipallene        ...A..................-........CT......-CA.C.....
Nymphon             .G.........-.-..T.....-..............-C........-
Pallenopsis         ...A..................-..............C........A
Anoplodactylus      ...A..................-..............-C.........
```


510 520 530 540 550
Achelia TCGTTGCCCGAA---CCC------GAAGG--------CCCAAGGCTTCT
Ammothella
Endeis
Tanystylum
Austrodecus
Colossendeis .G.A.A.AACCTATTT..CACAGT..G.T----ACGA--G.CC..C...
Callipallene
Nymphon .G.A.A.G..-TATTT...C-CACC.....TGGGAAAA..GCCCT...-.
Pallenopsis .AAA...G.C.CGAT.---AGAAC..G.AT----CGG..GC.C..C..C
Anoplodactylus .AAC...G-.TCGAT.--------..T.TT-GACCGA..GC.A..C..C
560 570 580 590 600
Achelia GTCCCAACGACCGAGTGTAGGCTCGACGCTCGAGCGCCATCCATTTTCAG
Ammothella
Endeis ...T.....A.....
Tanystylum
Austrodecus .....A.....
Colossendeis .....A.....
Callipallene .....G.T.....G.....
Nymphon .....A.....
Pallenopsis
Anoplodactylus
610 620 630 640 650
Achelia GGCTAGTTGCTTCGGCAGGTGAGTTGTTACACACTCCTTGGCGGATTCCG
Ammothella
Endeis
Tanystylum
Austrodecus .....G.....
Colossendeis .....G.....
Callipallene
Nymphon .....G.....
Pallenopsis
Anoplodactylus
660 670 680 690 700
Achelia ACTTCCATGGCCACCGTCCTGCTGTCTATAGCAACCAACGCCTTTCATGG
Ammothella
Endeis
Tanystylum
Austrodecus
Colossendeis
Callipallene
Nymphon
Pallenopsis
Anoplodactylus
710 720 730 740 750
Achelia GGTCTGATGAGCGTCGGCATCGGGCGCCGTAACTCGGCTTTTGGTTCATC
Ammothella
Endeis
Tanystylum
Austrodecus
Colossendeis .....A.....C.....
Callipallene .A...T.....T.....
Nymphon .....C.....
Pallenopsis .....C..... []
Anoplodactylus .....C..... []

```
                          760        770        780        790        800
Achelia           CCACAGCGCCAGTTCTGCTTACCAAAAGTGGCCCACTAGGCACTCGCATC
Ammothella        ...................................................
Endeis            ...................................................
Tanystylum        ...................................................
Austrodecus       ...................................................
Colossendeis      ...................................................
Callipallene      ...................................................
Nymphon           ...................................................
Pallenopsis       ...................................................
Anoplodactylus    ...................................................

                          810        820        830        840        850
Achelia           CTTCGTCCGAGCTTCAGTCGAGCAAGCCGGACTTCTCACCCATTGAAAGT
Ammothella        ...................................................
Endeis            ...................................................
Tanystylum        ...................................................
Austrodecus       ..............................................A.....
Colossendeis      ..G................................................
Callipallene      ..................A...............G..............
Nymphon           ..G......G.........................................
Pallenopsis       ...................................................
Anoplodactylus    ..A................................................

                          860        870
Achelia           TTGAGAATAGGTTGAGGTCGTTTCGAC    [838]
Ammothella        ...........................    [834]
Endeis            ........................G...    [836]
Tanystylum        ........................G...    [832]
Austrodecus       ..............G.........-..    [834]
Colossendeis      ...........................    [847]
Callipallene      ...........................    [840]
Nymphon           ...........................    [853]
Pallenopsis       ...........................    [852]
Anoplodactylus    ...........................    [857]
```

iii) Alignment of 533 sites of 16S mtDNA.

```
                          10         20         30         40         50
Achelia           ---------------------------------------------ATAA
Ammothella        TCAGATCGCGGTAAATTTTTAAAAGTCGAACANGACTTTATTCATAT...
Nymphon           TCAGATCACG-TAAGATTTTAATAGTCGAACAGA---CTACTTAAAT...
Endeis            TCAGATCACG-TAGGACTTTAATCGTTGAACAAA--CGAACCTTTA...G

                          60         70         80         90         100
Achelia           CTTCTTCATTATG-AGGAAAAATAATCCAACATCGAGGTCGCAATCTCTT
Ammothella        ..C........AT-.AAT..T.....................T.....TC.
Nymphon           TA.T...T...ATT.A.TTTCT..................AT..G.AT..
Endeis            .GG..G..CC..CGG.ATGTCC.G................T..A.C..A

                          110        120        130        140        150
Achelia           TCATTAATATGAACTCTCCGAAAGAATAACGCTG---TTACCCCCATAGT
Ammothella        .TT.A...T......TA.TA..GA..........---.........C...
Nymphon           .TG.CG..T..GG....TAA...AT..T.T....CTG...T...T.AG..
Endeis            .TG..G.....G.....AGA.T..G..TG.....---...T...T.GG..

                          160        170        180        190        200
Achelia           AATTT----------------TATTTTTTAAATTTTA-ATAAAAGTTCAA
Ammothella        .....------------AA--.CG.----.....AA.-......---TC.
Nymphon           .....---------------T.C...----....--.-.....TAAGTTC
Endeis            ..C..GTTCCGTTGGTCAAGT....GGA.C....GAGT...GT.....GC
```

```
                    210        220        230        240        250
Achelia         TTAATAAATTCAAATAATTATTAACACTAATAAAAGAT--AATTTTATTA
Ammothella      ..........T.T-.......A..T.-...A.......--.-..C....T
Nymphon         ..T.......ATT----..C..TTTTT...A..T....--TTA......T
Endeis          ..TGACTGG.G..G.CT.AGCATGT...GC.CGG..G.TGGG..C.GC.C

                    260        270        280        290        300
Achelia         TTT--CCGCCCCA---GAAAAACAATATTCAAACAATAAAT----AGATT
Ammothella      ...--..T.....---.....---...A..TATT..T.C----.AGAC
Nymphon         AA.--.-T.....---ATT...T.--..AG..TTTT.TGT.----TTGAA
Endeis          CGAGGT.......ACC....TTTTTA..G..GGTTTGGT.GTTTAG..CC

                    310        320        330        340        350
Achelia         TTTACAATT-----------------TAACTATAATAAAACTTAATGGGG
Ammothella      .A..AGT..-----------------.C.T----G........G......
Nymphon         .A..A..AA-----------------.TTT.T.T..T.....CT..A...
Endeis          .G.GGGT..GTTAGGTACTGTTTGCA.T.A..A.T....G..CC..A...

                    360        370        380        390        400
Achelia         TCTTCTCGTCCCTCAATGTAATTATATCAGCCTTTTTACTTTAAAGTGAA
Ammothella      ....T.....TT.T...AA....TAT.A.A.T....C.TA.A...A.A..
Nymphon         ....A......T.TTG.TGT...T.T---..T.......AAA...A.A..
Endeis          ..........TTG.TG...C..GCCC---....C..C..GGGC.G..C..

                    410        420        430        440        450
Achelia         CTTC-AAATATAATAATAACAGACAGTTATTCTTACGTCAAACCATTCAT
Ammothella      A...-.............G-.A.....A......C...G.....T.....
Nymphon         T...T....T.T..TT...-...A....-..T..T....T.G..T.....
Endeis          T...ACTGGT...A.G...G......C.GAA.CCT...GG.G........

                    460        470        480        490        500
Achelia         TCCAGTCTCCAATTAAAAGACAAATTATTATGCTACCTTTGCACAGTCAA
Ammothella      .G-...T.T.........A.........................GA.T.T
Nymphon         ..A.....TT.....G.......T...............C.-----...
Endeis          A.AG...C.T.T....GGA....G.G.................G..T.G


                    510        520        530
Achelia         TTTA-CTGCGGCCATTCAATTAA-TCATCGTGG
Ammothella      AA..-TC.....T...T...A..T.T..ATCAT
Nymphon         AA.--T.......G..T.....TTCAC.G.GCA
Endeis          GG..C.C......CG.T..AC.TG.GTCAC...
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(Shaded fragments correspond to hypervariable regions as indicated in Chapter 4).
