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Evolution of pycnogonid life history traits

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EVOLUTION OF PYCNOGONID LIFE HISTORY TRAITS

BY

Eric Carl Lovely
B. A., Bloomsburg University, 1992
M. S., University of New Hampshire, 1995

DISSERTATION

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ABSTRACT

EVOLUTION OF PYCNOGONID LIFE HISTORY TRAITS

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The Pycnogonida is a class of arthropods with interesting life histories. Pycnogonids prey on hydroids and some invade hydranths while larvae. Males brood the eggs and larvae hatch as protonymphons. Questions relating to the evolution of life history characteristics were addressed. Evolutionary relationships were poorly understood. It was necessary to determine the relationships within the Pycnogonida and compared to other arthropods.

Twenty-four morphological characters were coded for twenty-three pycnogonid genera and one fossil ancestor, *Palaeoisopus problematicus*. A branch and bound analysis resulted in fifteen most parsimonious trees. The Nymphonidae were found to be basal. The Ammotheidae were paraphyletic and led to two clades. The first contained the Callipallenidae, and Phoxichilidiidae. The second contained the remaining pycnogonids.

A phylogeny was also compiled using sequences of the D3 expansion segments of 28S rDNA. This resolved relationships of

sampled families as follows (Ammotheidae + ((Nymphonidae + Colossendeidae) + (Endeididae + (Pycnogonidae + Phoxichilidiidae)))). The Ammotheidae was found to be paraphyletic and basal. The results from the D3 region yielded perplexing relationships when compared with morphology.

Phoxichilidium tubulariae Lebour 1947 is a valid species. It appeared to be specialists on the hydroid *Tubularia larynx*. Annual population dynamics of *P. tubulariae* were seasonal. Density of adult animals was highest in mid to late summer with reproduction being greatest in July and August. The abundance of pycnogonids peaked as the hydroid population declined. Some populations were shown to have two generations. Adult migration may play a larger role in the distribution of this species than larval dispersal.

Phoxichilidium tubulariae had an atypical protonymphon type developmental mode that reduced the typical number of molts, and developed rapidly in the gastrovascular cavities of the host. It decreased developmental time from 35-40 days to 15-20 days. This was adapted to exploit the seasonal abundance of *Tubularia larynx*. The male looped the egg mass over his oviger. The larvae hatched, infected the hydroid, and developed inside the gastrovascular cavity of *T. larynx*. The larvae developed for several molts and then hatched, destroying the hydranth. The ancestral pycnogonid stock were external parasites. The internalization of the larval stages appeared to have happened at least twice.

General Introduction

The evolution of the Metazoa is an intriguing topic. Historically, morphology was the most important source of information for determining evolutionary relationships between metazoan taxa. More recently, life history, biochemical, and molecular data have also been used. Compiling these relationships can be a daunting task. McHugh and Halanych (1998) estimated there are $1 \times 10^{6,000,000}$ possible unrooted phylogenetic trees for the 1,033,614 estimated species of animals. The task of evaluating these relationships is intimidating, but nevertheless a popular pursuit. The increase in studies using molecular sequence information since 1988 has both helped and confused the issues of these evolutionary relationships (Field *et al.* 1988; Lake 1990; Turbeville *et al.* 1991; Wainright *et al.* 1993; Winnepenninckx *et al.* 1995; Kim *et al.* 1996; Winnepenninckx *et al.* 1998).

The evolution of developmental patterns is a popular and growing area of biological research, aptly named "evo-devo". Studies of gene expression and regulation have added interesting results. Clearly, animals have conserved genes and altered their uses through evolutionary time. It is change in some of these transcriptional regions that can result in major morphological change in relatively short periods of time. These changes have led to adaptive radiations and convergences. It is the task of identifying these homologies and convergences that can be one of the most challenging problems for

modern zoologists. These homologies and convergences exist not only in morphology but also in life history characteristics and behavior (Wray 1995a).

The Pycnogonida is a special and enigmatic class of arthropods with interesting life histories worth exploring. Pycnogonids prey on hydroids and some species invade hydranths as larvae. Males brood the eggs and larvae hatch as protonymphons. The purpose of this dissertation was to use this group as a model for addressing questions relating to the evolution of life history characteristics. Since the evolutionary relationships of this group were poorly understood, it was first necessary to determine these relationships both within the group and compared to other arthropods. Chapter 1 is a discussion of the evolutionary relationships within the Pycnogonida and compared to fossil chelicerates using morphological analyses. Chapter 2 continues the discussion using molecular data from the D3 region of 28S rDNA. The most common pycnogonid in the Gulf of Maine is in the genus *Phoxichilidium* and yet the species name was unclear until now. Chapter 3 discusses the species name of this animal and whether *Phoxichilidium tubulariae* is a synonym of *Phoxichilidium femoratum*. Chapter 4 describes the life history of *Phoxichilidium tubulariae* in detail. Chapter 5 summarizes what is known about the life history strategies of other pycnogonid species. This summary chapter then uses the evolutionary trees from the first two chapters to put life history strategies into an evolutionary framework to address the phylogenetic relationships within the Pycnogonida.

The Pycnogonida

Pycnogonids, commonly called "sea spiders", superficially resemble true spiders, but are given class status (Hedgpeth 1947). More than 1200 species have been described, but many of the genera are based on a single species. They have historically been called Pantopoda or Podosomata due to the length of their legs. They have an appendage complement similar to chelicerates. It includes a pair of chelicerae called chelifores, a pair of pedipalps or simply palps, and usually four pairs of walking legs. They also have an extra pair of appendages called ovigerous legs, or ovigers. Figure i represents a generalized pycnogonid showing these characters.

Pycnogonids are found from the intertidal zone to the depths of the abyssal trenches in polar, temperate, and tropical seas. For example, *Pycnogonum littorale* occurs from the intertidal to depths of 1262m (Bamber 1985). Other species have been recorded to a depth of 6800m (Hedgpeth 1982). In the deep sea and polar waters some *Colossendeis* species can reach forty to seventy cm leg spans (Arnaud and Bamber 1987). The majority of species are epibenthic, but a few are interstitial or bathypelagic (Arnaud and Bamber 1987).

Information concerning food of pycnogonids is not very abundant. It is usually assumed that animal species with pycnogonids found on them serve as a food source, however this is not necessarily true (King 1974). Adult pycnogonids are mostly external parasites or succivorous predators on cnidarians, poriferans, molluscs, or echinoderms. Pycnogonids are typically so sluggish they can only feed on sessile or slow moving prey (Arnaud and Bamber 1987). The adaptive radiation evident in the varying morphologies

of the group are also seen in food preference (Wyer and King 1974). The Pycnogonidae are actinian feeders. The Endeidae feed on detritus. The Nymphonidae feed on actinians and hydrozoans. The Phoxichilidiidae feed on polyps of hydrozoans or medusae in the plankton. The Ammotheidae feed on bryozoans, hydrozoans, or algae.

Pycnogonids are capable of detecting food using chemosensory receptors that surprisingly are most likely not located on the palps, chelifores, or ovigers. The chemosensory structures may be located on the body, legs, or proboscis (Stock 1978). Pycnogonids typically have four simple eyes arranged on a protrusion to provide 360° vision, but a few species lack eyes. They have a basic arthropod nervous system with a circumesophageal ring and paired ventral ganglia for each leg segment (Hedgpeth 1982).

Pycnogonids are understudied. Most of the scattered and fragmented published work on these animals has concentrated on their taxonomy and zoogeography including new species descriptions. Some natural history information was collected around the turn of the century, but it is only recently that the biology and ecology of pycnogonids has been addressed (Arnaud and Bamber 1987).

Pycnogonid phylogenetics

Morphology has long been used as a criterion for determining evolutionary relationships. However, it was not until the middle of this century that methods for analyzing morphological data were evaluated. Hennig, a German entomologist, began using cladistic methods. He called these methods "phylogenetic systematics"

(Hennig 1950, 1965, 1966). This work began the use of Darwin's ideas to study the modification of morphology through the process of evolution. Hennig's contributions to the study of evolution clearly defined biological relationships and suggested methods for discovering these relationships. His methods were designed to establish sister groups through the analysis of discrete characters. These characters can be from a variety of sources including: morphology, physiology, and molecular biology (Kitching *et al.* 1998).

Characters can be described as plesiomorphic, similar to the ancestral state, or apomorphic which is derived from the ancestral state. Synapomorphies are derived characters that are shared by sister groups. Cladistics attempts to organize taxa so the greatest number of characters can be explained in the simplest way. Parsimony can then be used to choose between alternate hypotheses of character distribution. Monophyletic groups are identified using synapomorphies. Patterson (1982) synonymized synapomorphy with homology.

Phylogeny represents a proposed history of genetic connections through evolutionary time (Maddison 1996). These evolutionary relationships are typically presented as phylogenetic trees. Tree diagrams model genetic decent and have a root at the base. They can be used to visualize character change based on a hypothesized phylogeny. The branches represent populations of organisms that once lived, reproduced and died. Selection and drift lead to changes in characters and, after generations, speciation events lead to separation of the branches.

Since the late 1980's, there have been a plethora of phylogenetic studies using DNA sequence characters to determine evolutionary relationships. DNA, which is the molecular code for structural and enzymatic protein, contains valuable phylogenetic information. Sequences can be informative from coding or non-coding regions of nuclear, mitochondrial, and chloroplast genes. Although phylogenetic information of a morphological or biochemical structure is greater than that of a single locus, molecular sequencing techniques allow for the simultaneous gathering of hundreds of characters. Molecular sequence is surely an important tool in the study of phylogenetics.

The evolutionary relationships within pycnogonids and with other arthropods were poorly understood until recently. Pycnogonids have rarely been included in arthropod molecular phylogenies and until now, the relationships between pycnogonid families have never been studied using molecular techniques.

Double-stranded amplifications were made from genomic DNA with flanking primers. Primers were selected for the D3 expansion segment of 26/28S rDNA. Sequencing was conducted by automated sequencer (ABI 373A), edited using the SeqEd program (ver. 1.0.3; ABI), and aligned with MegAlign (version 3.13: DNASTAR Inc. 1997). Phylogenies were constructed with both distance and parsimony methods. Parsimony analyses were made using PAUP (versions 3.1.1 and 4.0.0d58-64: Swofford, 1993).

Life history details of a pycnogonid with a parasitic larva

Another area of important research is in describing pycnogonid life histories in detail at ecological, and developmental levels. Pycnogonids are a special and enigmatic group that can serve as a model system. The evolution of development and life history strategies is currently a popular area of study. Many authors use embryological characters to construct phylogenies, yet the embryology of groups like the Pycnogonida and the Tardigrada remain so poorly understood that it is difficult to include them in such studies (Grupta 1979). Most pycnogonid species possess a larval stage called a protonymphon. It has three pairs of appendages with characteristic spines, probably used to retain larvae on the adult, attach to a host, or for dispersal (Arnaud and Bamber 1987). There have been a few studies describing the developmental details of pycnogonid species (Okuda 1940; Jarvis and King 1972; Nakamura 1981; Russel and Hedgpeth 1990). However, a complete set of life history data at both ecological and developmental levels is needed to put life histories of pycnogonids into an evolutionary framework. A major goal of this research was to fill these gaps in the body of pycnogonid biological knowledge.

The life history of a symbiont is often a critical element linked to that of the host species. The hydroid *Tubularia larynx* is not only the dominant food for adult *Phoxichilidium* sp., but also the larval host. The life history of hydroids and their nudibranch predators have been studied, but little information is available for their pycnogonid predators. *Tubularia* spp. have been investigated in detail (McDougall 1943; Institution 1952; Miller 1976; Hughes 1983;

Calder 1990). Cooper (1979, 1980) studied the effects of nudibranch predation and environmental factors on the growth and persistence of the hydroid *Tubularia crocea*. In most cases, the hydroids were shown to regenerate polyps lost to predation. Environmental factors were believed to be of greater impact than predation by nudibranchs.

This study adds developmental details of *Phoxichilidium* sp. to the literature. These details were compared with life history information in the literature for other pycnogonids, with and without parasitic larvae.

Evolution of the parasitic larva

The next section addressed ways in which complex life histories evolved in pycnogonids. Many pycnogonids use cnidarians as hosts. Adult and larval pycnogonids feed on cnidarian tissue. The larvae of some species in addition to *Phoxichilidium* develop inside the gastrovascular cavities of hydroids. Have pycnogonids used cnidarians as larval hosts since their early evolution? Is this semi-parasitic life history a monophyletic trait, or has this evolved multiple times within the Pycnogonida indicating it is a polyphyletic trait? This study determined that brooding is a monophyletic trait within the Pycnogonida. Questions regarding evolution of these life history traits can not be answered independent of a phylogenetic framework. Therefore, a better understanding of the relationships of both pycnogonids within the Arthropoda, and within the Pycnogonida is needed. This can only be accomplished through additional morphological and molecular analysis.

Life history information for species from personal studies and the literature was collected. An extensive list of characters from the literature was compiled to be used for morphological analysis. These results were compared with molecular results to hypothesize an accurate phylogeny. Basic life history information and observations were overlaid onto this phylogeny to examine the possibility that parasitism in the Pycnogonida is polyphyletic. Finally, the morphological, molecular, and life history information is synthesized to evaluate the phylogeny of the Pycnogonida.

CHAPTER I

Morphological phylogenetics of the Pycnogonida

INTRODUCTION

Pycnogonids and arthropod phylogeny

The Pycnogonida have been linked with crustaceans, arachnids, or separated into a unique subphylum (Arnaud and Bamber 1987; King 1973, 1974).

Affinities to crustaceans include similarities among larval forms, in vitellogenesis, in gastrulation, in adult molting, and in development type. There are a few hermaphroditic pycnogonids. Brooding of eggs is common in both groups. These similarities could be due to convergence rather than a shared evolutionary history. Hedgpeth (1947) rejected the possibility of a close relationship with the Crustacea since pycnogonids never posses biramous appendages and the protonymphon stage is distinct from the nauplius.

Hedgpeth (1947) placed the Pycnogonida as a separate class of the Chelicerata due to the uniqueness of the ovigers, proboscis, and genital openings. They also lack any defined excretory or respiratory structures. He supported a remote common ancestor of all chelicerates including the Pycnogonida (Hedgpeth 1978). Schram and Hedgpeth (1978) placed the Pycnogonida as a sister group to the

chelicerates in the "Cheliceriformes". However, Stormer (1944) argued against placing pycnogonids within the Chelicerates and placed them outside the non-chelicerate trilobites (from (Wheeler and Hayashi 1998)). Many authors have argued for placing the Pycnogonida as a basal chelicerate, sister taxon to a xiphosuran and arachnid clade (euchelicerata) (Snodgrass 1938; Firstman 1973; Grasshoff 1978; Weygoldt and Paulus 1979; Weygoldt 1986; Wheeler and Hayashi 1998).

Some characters are used to argue a primitive position of the Pycnogonida. The cuticle is similar to that of tardigrades and annelids (King 1973). The gut diverticula resemble those of polychaetes with unique intracellular digestion, and cleavage was described as rudimentary spiral similar to annelids (King 1973). They feed on cnidarians and sponges, and that has also pointed toward their ancient origins. Another character often used to place pycnogonids as a primitive group is metameric instability. Most forms have four pairs of legs but some have five pairs and two groups even have six pairs. The Ammotheidae and Pycnogonidae have members with five pairs. The Nymphonidae and Colossendeidae have members with five and six pairs. This appears to be a result of reduplication of somites by unstable telogonic growth or chromosome polyploidy (Hedgpeth 1982). This trait appears to have existed since the origin of the group because it is found in the fossil form *Pentapaleopycnon* (Hedgpeth 1978). Fry (1978) used the characters of metameric instability and poor fossil record to support that pycnogonids are a very young group currently undergoing a rapid radiation.

Pycnogonids have some similarities with arachnids. Morgan (1890) argued for a close relationship on embryological grounds and similarities between pycnogonid eyes and arachnid median eyes. For example, pycnogonids and arachnids form ectoderm by a process of multipolar delamination. Borner (1904) placed pycnogonids as a sister taxon to the Xiphosura and arachnids (from (Wheeler and Hayashi 1998)). Manton (1978) linked pycnogonids and arachnids due to similarities of leg morphology with Silurian aquatic scorpions. She argued the coxa-body joint in arachnids and pycnogonids were unique in the Arthropoda. She stated that the pycnogonid body showed modified arachnid morphology because caecae of the midgut enter limb bases, embryogenesis in *Callipallene* sp. is similar to arachnids, eyes are similar, pre-oral appendages are similar (Cheliformes), and both lack a deutocerebrum. The chitinous cuticle over the pycnogonid epithelium is perforated by many pores (figures 1.1a and 1.1b). The cuticle is never calcified with linkages similar to arachnids (Arnaud and Bamber 1987). Manton (1978) believed arachnids had more than a single terrestrialization event, millions of years apart. Pycnogonids may have evolved from an aquatic arachnid line that never became terrestrial. Pycnogonids possess neither a cephalothorax nor a prominent abdomen. Parts of the pycnogonid legs are homologous to parts of an arachnid leg (Dencker 1974). However, Hedgpeth doubted a close relationship between terrestrial arachnids and pycnogonids and suggested that the two groups diverged long ago (Hedgpeth 1978, 1982).

The fossil record may give clues to reconstructing pycnogonid evolution. Bergstrom (1979) had no doubt that the Pantopoda was a

monophyletic group. He described three fossil pycnogonids from the lower Devonian Hunsruck shale: *Palaeopantopus maucheri*, *Palaeoisopus problematicus*, and an undescribed form similar to modern pycnogonids (Grupta 1979). *P. problematicus* was the most curious of these with a segmented abdomen. It may have been a swimming beast due to its leg morphology. Over fifty specimens have been found and most were at least 125mm in length (Hedgpeth 1978). Devonian forms have an articulated abdomen and are considered Paleopantopoda. Krapp (personal communication) is currently describing relationships between recent and Paleozoic forms. One form has a proboscis similar to *Ascorhynchus*. This relationship between Paleopantopoda and the extant pycnogonids is critical for determining the common ancestral stock of the Pantopoda.

Pycnogonid phylogenetics

Relationships within the Pycnogonida are more confusing and poorly understood than relationships between pycnogonids and other arthropods. Even the most prolific of pycnogonid biologists are troubled with family trees. There are several genera whose morphology clouds the boundaries between families (Hedgpeth 1947). Fry (1978) stated that using morphological characters "leads to phylogenies which are almost automatically inverting and overlapping sets of genera...". Hedgpeth (1982) agreed, "There is no easily discernible evolutionary progression; attempts to construct such family trees inevitably produce interlocking and anastomosing shrubbery rather than neatly branching trees."

Fry (1978) used multivariate analysis for numerical taxonomy including forty five characters from all the published descriptions of seventy-three genera and classified the Pycnogonida into five orders containing thirty families. However, this is not the traditional organization of the group. His analysis used redundant characters such as palp segments in females and palp segments in males where this character is mostly consistent regardless of sex. Most authors use a scheme based on Hedgpeth (1947) with all living pycnogonids belonging to a single order, Pantopoda, with eight families. Molecular data may help to solve this phylogenetic puzzle and may be used to test Fry's versus Hedgpeth's views of pycnogonid taxonomy. The purpose of this chapter was to hypothesize a pycnogonid phylogeny using a new morphological data set and compare the results with Fry's and Hedgpeth's phylogenetics.

METHODS

One hundred and sixty six pycnogonid species from twenty three genera were used in this morphological analysis. These species exhibited the full range of pycnogonid morphological variation. A list of twenty four distinct morphological characters were selected and coded from the literature and personal observations of museum specimens for a morphological cladistic analysis to compare to the molecular results (see chapter 2). A summary of pycnogonid classification is presented in Table 1.1. A key to pycnogonid families is presented in Table 1.2. The organisms coded from the literature are listed in Table 1.3. The selected characters concerned palps,

chelifores, oviger, trunk, and foot characteristics and possible states are listed in Table 1.4. Figure i represents the H. A. P. (Hypothetical Ancestral Pycnogonid). It has a full complement of chelicerate appendages including; chelate chelifores, palps, and ovigerous appendages on both sexes.

Figures 1.2 through 1.12 illustrate most of the characters used in this analysis. Characters 1 through 3 relate to the pycnogonid palp. Figure 1.2 shows character states for palp origin. Characters 4 through 8 relate to the pycnogonid chelifore. Figure 1.3 shows several chelifore character states. Figure 1.4 shows the proboscis shape represented by character 9. Figure 1.5 shows the eye position states for character 11. Figures 1.6 and 1.7 show trunk segmentation and ornamentation represented in characters 10, 12, 13, and 14. Characters 17 through 21 relate to oviger states, and figure 1.8 shows character states for the oviger spines and claws. Characters 22 through 24 represent walking leg states and are shown on figure 1.9. Figure 1.10 shows the opithosoma states from character 15, and figure 1.11 shows the eye tubercle states from character 16. Figure 1.12 shows a comparison of pycnogonid leg segments vs. arachnid leg segments.

Since the vast majority of species within genera coded identically, the matrix was condensed to genera for analysis. The trees were rooted with the fossil *Palaeoisopus problematicus*. Twenty-four taxa were used. Differences observed within genera are discussed later in this chapter. The coded character matrix is presented in Table 1.5. The matrix was then used to construct phylogenetic trees using PAUP (versions 3.1.1 and 4.0.0d58-64:

Swofford, 1993) assuming parsimony. All characters were unordered and analyzed with equal weight. They were all parsimony informative. Branches were collapsed if the maximum branch length was zero. A branch and bound analysis was used and ran for two hours and fifty minutes.

RESULTS

Observations concerning the evolution of the pycnogonid palp

The genus *Nymphon* has a five segmented palp that is longer than the proboscis and originates on the neck. Krapp (personal communication) precludes the Nymphonidae from being the most primitive pycnogonid family for this reason. The genus *Colossendeis* has palps of ten segments except for one species with nine (Child 1995). They are longer than the proboscis and originate near the ovigers. The palps present in *Rhynchothorax* have four to six segments, the longest with a tall dorso-distal tubercle (Child 1995). Both *Austrodecus* and *Pantopipetta* in the Austrodecidae show five to seven segmented palps. They also show evidence of distal tubercles. *Oropallene* has four segmented palps, *Pallenopsis* has a single segmented palp, and the remaining Callipallenidae lack palps. The following families have no palps: Phoxichilidiidae, Endeididae, and Pycnogonidae. The greatest variation in palp number is present in the Ammotheidae that have from five to ten segmented palps. Some genera such as *Achelia* have a fixed palp segment number, while others, such as the genus *Austroraptus*, has species with five, six, or eight segmented palps. *Tanystylum* shows five basic segments,

however, some species have six or seven. Most *Ammothea* have nine but some have as few as six. *Pigrogromitus* from the Suez Canal, placed in the Callipallenidae, lacks palps. Palps are present in the Paleopantopoda, but it is difficult to count segments in most of these fossil forms. Figure 1.2 shows the variation present in the origin of the pycnogonid palp.

Evolution of the pycnogonid chelifore, proboscis, and trunk.

The Colossendeidae, Endeididae, Austrodecidae, Rhynchothoraxidae, and Pycnogonidae all lack chelifores. Although juvenile endeids have long thin chelifores that are shed at the eight leg stage (King 1974). Nymphonidae, Phoxichilidiidae, Callipallenidae, and some Ammotheidae have chelate chelifores. Most ammotheids have achelate chelifores. *Pallenopsis* spp. have a lateral chelate angle and two scape segments. *Nymphon* spp. and Callipallenidae have teeth on their chelae. The Nymphonidae have a modified two segment chelifore. Several pycnogonid chelifores that show the range of morphological variation are presented on figure 1.3.

The pycnogonid proboscis is typically about the thickness of the body (figure 1.4). Austrodecidae has a derived pipette shaped proboscis (figure 1.4a). Many ammotheids have a stout shaped proboscis (figure 1.4b). Figure 1.5 shows the range of morphological variation in eye position. Most pycnogonids have four trunk segments that have lateral separations except *Tanystylum* sp. that has fused separations (figure 1.6). Most genera have an elongated, segmented trunk without ornamentation (figure 1.7).

Evolution of the oviger and foot

Ovigers are used by males to brood eggs. They may be derived from an "extra" walking leg (Dencker 1974), perhaps from a homeotic mutation (Bain 1992). Pycnogonids generally have ovigers with nine or ten segments including the fossils *Palaeoisopus*, and *Pigrogromitus*, as well as the living colosendids and Nymphonidae (Hedgpeth 1982). Several groups have reduced numbers of segments. Ammotheids and the Phoxichiliidae have reduced ovigers with no compound spines (figure 1.8). Most families exhibit ovigers in both sexes, but the Phoxichilidiidae, Endeididae, and Pycnogonidae are dimorphic with ovigers absent from females. Some species in the family Pycnogonidae even lack ovigers but males still brood eggs in a cake-like mass on their ventral surface (Arnaud and Bamber 1987). It is only in *Colossendeis* that the males don't appear to carry eggs although so little is known about their life history this may not have been observed yet. The last few oviger segments are sometimes modified into a shepherds crook. There is no shepherds crook in phoxichilids, tanystylids, and austrodecids. This structure indicates the primitive function of ovigers may have been cleaning, and grooming has been observed (Arnaud and Bamber 1987). Compound oviger spines and claws appear a derived trait found in *Nymphon* and *Colossendeis* along with the elongated tarsal shape. Presence of accessory claws and heterogeneous sole spination are common in many families (figure 1.9).

Sexual pores are generally ventral on the second coxae of all legs in the females and third and fourth of the males. The male

orifice is typically larger with cement glands present on the femurs in some species (Arnaud and Bamber 1987). *Pycnogonum* and *Rhynchothorax* have a single pair of pores on the last pair of legs. *Nymphon* and the Phoxichilidiidae have them on the last three pairs of legs. A few species including *Decolopoda* have them on all legs. The single pair of gonopores is likely primitive (Arnaud and Bamber 1987). This is a piece of evidence supporting the early divergence of the *Pycnogonum* line.

Gamete morphology

Vitellogenesis tells us little about phylogeny in the Pycnogonida. It is similar in the Pycnogonidae, Nymphonidae, and *Limulus polyphemus* (Jarvis and King 1972). The yolk is produced inside the oocyte with little external contribution. This pattern is also found in some annelids but not in insects. Insects have a much more derived pattern that develops very rapidly. Pycnogonid yolk formation also shares similarities with that of Crustacea. Based on this evidence Jarvis and King (1978) stated pycnogonids may be an early off-shoot from the basic arthropod stock.

Hilton (1916) reviewed what was currently known about egg size in pycnogonids. *Anoplodactylus erectus* had eggs of 0.03 mm. *A. californicus* had eggs of 0.035 mm. *A. spinosissima* had eggs of 0.04 mm. One *Anoplodactylus* spp. had eggs of 0.065 mm. *Palene californiensis* has large eggs of 0.175 mm. *Phoxichilidium femoratum* and *Pycnogonum littorale* make large numbers of small eggs with small amounts of yolk. *Phoxichilidium* being about 0.05 mm (Morgan 1891). *Pallene brevirostris* (0.25mm), *Chaetonymphon*

spinosum, the Endeidae, the Nymphonidae, and the Ammotheidae have few eggs with large amounts of yolk (Jarvis and King 1978), of 0.5 to 0.7 mm (Hilton 1916). *Tanystylum* has eggs of 0.08 mm in diameter (Morgan 1891).

Pycnogonid sperm is varied. It is mostly a 9+0 arrangement. Some *Nymphon* sp. have an increase to a 12+0 or 18+0 arrangement (EL-Hawawi and King 1978). Several species in other families have bi- and triflagellated sperm (El-Hawawi 1978). *Pycnogonum littorale* spermatozoa have been described as aberrant. They are non-flagellated and nonmotile (Arnaud and Bamber 1987). They are full of only longitudinal, isolated microtubules, and are devoid of other organelles (Grupta 1979). Many arachnids also have encysted sperm including the pseudoscorpion, *Chthonius ischnocheles* (Grupta 1979). Arachnid sperm are typically a 9+3 arrangement of microtubules and the flagellum rolls around the nucleus (Foelix 1996).

Ovary structure for many species has been described (Jarvis and King 1972, 1978). The structure ranges from a complete sheet within the trunk as seen in Phoxichilidiidae, to the U-shaped ovary of the Nymphonidae. Intermediate conditions can be seen in *Endeis* and *Pycnogonum* (Jarvis and King 1972, 1978).

Morphological phylogenetics

The morphological analysis resulted in 15 most parsimonious trees (Tree length: 79 steps; CI=0.443, RI=0.727). A strict consensus of these trees is shown in figure 1.13. The trees were rooted with the fossil form *Palaeoisopus problematicus*. The Nymphonidae appear as the basal pycnogonid family based on this morphological

matrix. The Ammotheidae appear to be a paraphyletic group that led to two clades. The first clade contains the Callipallenidae and Phoxichilidiidae. The second clade contains the remaining ammotheids, the Colossendeidae, and the following unresolved groups: Austrodecidae, Rhynchothoraxidae, Pycnogonidae, and Endeididae.

DISCUSSION

Pycnogonid phylogenetics

In 1947, Hedgpeth published "On the evolutionary significance of the Pycnogonida". He divided the class into eight families in a single order containing all the extant forms (Hedgpeth 1947). This is still basically the scheme used today except that Austrodecidae and Rhynchothoraxidae have been raised to family level and Tanystylidae (Schimkewitsch, 1913) is included with the Ammotheidae yielding nine families.

Fry (1978) applied methods of numerical taxonomy to the Pycnogonida. He suggested modifying the taxonomy to five orders containing thirty families. However, Fry's work could not address phylogeny. It is no surprise he split the class to such a degree. He used Gower's Generalized Coefficient and subjected the resulting similarity indices to principle co-ordinate analysis (Fry 1978). This method is designed to accentuate differences in characters rather than hypothesize a phylogeny. There were also problems with the morphological matrix. He used redundant characters such as palp segments in females and palp segments in males where this

character is mostly consistent regardless of sex. His results also implied several strange relationships. He separated *Phoxichilidium* and *Anoplodactylus*, putting *Anoplodactylus* amongst the ammotheid genera. This is a very difficult relationship to accept considering the extreme similarities in morphology. Lebour (1947) stated the division between *Anoplodactylus* and *Phoxichilidium* is most likely not a natural one. Although this work is problematic it is worth mentioning that it was conducted in the early days of phylogenetics and the methods of numerical taxonomy were very popular at the time.

Bain (1992) used cladistic methods to elucidate pycnogonid evolutionary relationships. She coded 57 characters for 86 pycnogonid genera. The results indicated placing the Nymphonidae and most of the Callipallenidae in an order she called Nymphoniformes, and the remaining pycnogonids in an order called Ammotheiformes containing six families and 8 subfamilies. She found no support for the families Endeidae, Rhynchothoraxidae, and Austrodecidae. Support for the Pycnogonidae was inconclusive, and the Ammotheidae, Tanystylidae, and Phoxichilidiidae were all combined in the new order called Ammotheiformes. However, this matrix produced 743 most parsimonious trees. She presented a Nelson consensus tree of all 743 trees. Unfortunately due to the large number of equally parsimonious trees, this study did not yield satisfactory results.

Morphology places the Nymphonidae as the basal pycnogonid family (figure 1.13). The Nymphonidae are often thought to resemble the H. A. P. (Hypothetical Ancestral Pycnogonid) because

they have a full complement of chelicerate appendages including: chelate chelifores, palps, and ovigerous appendages in both sexes. The Ammotheidae appear to be a paraphyletic group. They also resemble the H. A. P. because they have chelate or achelate chelifores, palps, and ovigerous appendages in both sexes. The Callipallenidae, and Phoxichilidiidae are linked with many morphological characters including a reduction of palps. As in Bain (1992), this study provides little support for the following families: Austrodecidae, Rhynchothoraxidae, Pycnogonidae, and Endeididae. These four families share losses of appendages that should cluster them in a single family.

This study supports the following organization: (1) The Nymphonidae and Colossendeidae appear to be valid monophyletic families. (2) The Callipallenidae and Phoxichilidiidae are related taxa but show enough morphological differences to be considered separate families. (3) The Austrodecidae, Rhynchothoraxidae, Pycnogonidae, and Endeididae should be lumped in a single family, the Pycnogonidae. (4) The Ammotheidae are paraphyletic and the taxonomy of this group should be analyzed in future work.

The debate as to the systematic position of pycnogonids continues, but pycnogonids are most likely Chelicerates associated with xiphosurans (horseshoe crabs), scorpionoids, or a unique subphylum (Arnaud and Bamber 1987).

CONCLUSIONS

The Nymphonidae appear as the basal pycnogonid family based on this analysis. The Ammotheidae are paraphyletic. There are two major pycnogonid clades. The first contains the Callipallenidae and Phoxichilidiidae. The second clade contains the remaining ammotheids, the Colossendeidae, and the following unresolved groups: Austrodecidae, Rhynchothoraxidae, Pycnogonidae, and Endeididae (figure 1.13).

Pigrogromitus sp. from the Suez Canal has a body type resembling the Pycnogonidae, but with chelate chelifores and ten jointed ovigers in both sexes. It is placed in Callipallenidae and shows some morphological similarities with fossil pycnogonids. The body and ocular neck morphology characters in this matrix also link the fossil *Palaeoisopus* with the living *Pigrogromitus* and the Pycnogonidae.

This morphological study determined some aspects of the pycnogonid Bauplan. The H. A. P. (Hypothetical Ancestral Pycnogonid) had a full complement of chelicerate appendages including chelate chelifores, palps, and ovigerous appendages in both sexes.

CHAPTER II

Molecular phylogenetics of the Pycnogonida

INTRODUCTION

Several authors have addressed arthropod phylogeny without including the Pycnogonida (Briggs and Fortey 1989; Turbeville *et al.* 1991; Eernisse *et al.* 1992; Boore *et al.* 1995; Friedrich and Tautz 1995; Regier and Shultz 1997; Thomas and Fortey 1998). Wheeler *et al.* (1993) used a total evidence approach to reconstruct arthropod phylogeny including morphological characters, 18S rDNA, and ubiquitin (a protein coding gene) sequences. They found Pycnogonida within Chelicerata, grouping between trilobites and other chelicerates. Horseshoe crabs and arachnids were found to be sister groups, with Pycnogonida outside this clade. Pycnogonids were most likely chelicerates associated with xiphosurans (horseshoe crabs) and scorpionoids. Wheeler and Hayashi (1998) agreed and placed the Pycnogonida as a basal chelicerate, sister taxon to a xiphosuran and arachnid clade (eucheliceratа). Regier and Shultz (1998) used the amino acid sequence of elongation factor 1α to determine evolutionary relationships of arthropod groups. They found a pycnogonid clade represented by (*Tanystylum* + (*Endeis* + *Colossendeis*)) as a polytomy with malacostracan crustaceans and a

clade containing the remaining arthropods including: euchelicerates, hexapods, myriapods, and the remaining crustaceans.

A wide variety of studies have used rDNA genes to assess phylogenetic relationships. Regions of 28S rDNA are ideal for creating phylogenies because different regions evolve at different rates and it can be used at different taxonomic levels (Hillis and Dixon 1991; Litvaitis *et al.* 1994; Litvaitis *et al.* 1996; Nunn *et al.* 1996; Litvaitis and Rohde 1999). The purpose of this chapter was to compare phylogenetic trees using partial 28S rDNA sequences with the morphological results of the previous chapter.

METHODS

Samples of thirteen pycnogonid species from six families were used for the molecular study (Table 2.1). This was not an ideal subset of pycnogonids, but it was the most complete series of representatives possible to obtain during the course of this dissertation. Pycnogonids collected from subtidal habitats near Mediterranean, Antarctic, Atlantic, and Pacific coasts were stored in 95% ethanol at room temperature. They were identified to species level. DNA was extracted according to Litvaitis *et al.* (1994). Briefly, samples were vacuum-evaporated to remove all the ethanol. Tissue was digested using 5µl proteinase K (1% by volume in extraction buffer) at 37°C overnight. The solution was then extracted using equal volumes of phenol, phenol:chloroform, and chloroform. The salt concentration was adjusted to 0.2 M. Nucleic acids were then

precipitated using isopropanol, and washed in 70% ethanol. Total genomic DNA was resuspended in 150 µl TE-buffer (pH 8.0).

Double-stranded amplifications were performed using primers for the D3 expansion segments of 28S rDNA (Litvaitis *et al.* 1996). The sequence of the primers (D3A and D3B) were based on the rDNA of *Caenorhabditis elegans*. The thermal cycling pattern consisted of 94°C for 30 seconds, 45°C for 60 seconds, and 72°C for 120 seconds. Amplified products were electrophoresed on 1% SeaKem agarose gel with a DNA molecular weight standard. The product was excised from the agarose and purified by centrifugation using a Spin-X column (Costar). Alternatively, double-stranded DNA was electrophoresed on a 1% SeaPlaque agarose gel. The correct band was cut from the SeaPlaque, melted at 65°C, cooled to 37°C, and the agarose digested using 1.5 µl agarase overnight at 37°C. Four to five µl of amplified DNA was used in a cycle sequencing reaction (protocol according to ABI Corp.) and products were again purified. The samples were electrophoresed on a 6% polyacrylamide gel in 1X TBE buffer. The nucleotide sequence was determined using an automated sequencer (ABI 373A) at the University of New Hampshire's Sequencing Facility. Both strands were sequenced for each sample.

Sequence results were analyzed and aligned using SeqEd (ver. 1.0.3; ABI) and MegAlign (version 3.13: DNASTAR Inc. 1997). Additional alignment was completed by eye. Distance and parsimony methods were used to construct phylogenetic trees using PAUP (versions 3.1.1 and 4.0.0d58-64: Swofford, 1993). Entire sequences were used and transition to transversion ratios were weighted 3:1 (Litvaitis *et al.* 1996). Gaps were treated as missing. Heuristic

searches were conducted using a random addition sequence. Final DNA sequences were aligned and analyzed using chelicerate outgroups, as well as within the Pycnogonida assigning pycnogonid outgroups. Various phylogenetic hypotheses were tested by constraining monophyly of taxa and comparing these trees with unconstrained trees using nonparametric Templeton tests (PAUP 4.0). Distance options were set at Log/Det for neighbor-joining analysis.

The following were chosen as outgroups: *Phalangium opilio*, *Limulus polyphemus*, *Latrodectus mactans* (Table 2.1). The crustaceans proved difficult to align with pycnogonid sequences, and were removed for this reason. The Acari, *Omartacarus* sp. and *Dermaceutor variablis*, sequences were very similar to *Nymphon* and *Colossendeis* sequences. The Acari extracted DNA may have been contaminated with pycnogonid DNA. The crustaceans and Acari were removed from the analysis and sequences were realigned.

Sequencing was also attempted for 18S rDNA from the nuclear genome and mitochondrial genes (12S, 16S, and Cytocrome Oxidase subunits I and II) to resolve the pycnogonid family relationships.

RESULTS

Fragments of 270-379 base pairs were amplified using the D3A and D3B primers. Crustaceans included an insert of 60-69 base pairs. This made alignment difficult so crustaceans were removed from the analysis and sequences of chelicerates aligned easily. Alignment using all of the outgroup taxa at once was also problematic. Analysis

of this alignment could not keep the Pycnogonida as a monophyletic group. *Omartacarus* sp. and *Dermaceutor variablis* sequences were very similar to *Nymphon* and *Colossendeis* sequences. This could indicate close relationships; however, it could also indicate the Acari extracted DNA may have been contaminated with pycnogonid DNA. The two Acari species need to be extracted and sequenced again to evaluate a possible contamination. Due to these alignment and possible contamination issues, sequences were realigned using *Phalangium opilio*, *Limulus polyphemus*, and *Latrodectus mactans* as outgroups. The mean nucleotide difference between sequences was 16.7% determined using pairwise comparisons.

A distance-based phylogeny using *Latrodectus mactans*, *Phalangium opilio*, and *Limulus polyphemus* as outgroups, is presented in figure 2.1. Genera were monophyletic. The Ammotheidae was found to be paraphyletic. A heuristic search using maximum parsimony with 98 parsimony informative characters, found a single most parsimonious tree, and only one island was present. Parsimony bootstrap values with the same outgroups are shown in figure 2.2.

Regardless of the algorithm employed, *Achelia* appears as the most basal pycnogonid. It was possible to clearly determine the most basal pycnogonid and relationships between pycnogonid families using the D3 region of 28S rDNA. The Ammotheidae is paraphyletic, and represents the most basal pycnogonid family. When the Ammotheidae was constrained to be monophyletic, significantly longer trees resulted in Templeton tests. The Nymphonidae and Colossendeidae are related families. The

Endeididae, Pycnogonidae, and Phoxichilidiidae are also members of the same clade.

The primers used to sequence 18S rDNA from the nuclear genome and mitochondrial genes (12S, 16S, and Cytocrome Oxidase subunits I and II) to resolve pycnogonid familial relationships did not yield sequences and it was determined necessary to rely on the 28S results for this study.

DISCUSSION

Pycnogonids and arthropod phylogeny

Pycnogonids are not close relatives of crustaceans. All crustaceans sequenced contain a large insert of 60 to 69 base pairs. Partial pycnogonid 28S rDNA sequences aligned easily with arachnid and xiphosuran sequences. The similarity of 28S sequence, appendage complement, and evidence in the fossil record such as the Devonian *Palaeoisopus problematicus* place the Pycnogonida within the Chelicerata along with arachnids, xiphosurans, and eurypterids (Manton 1977, 1978). Histone H3 and U2 snRNA sequence analyses also provide support for a relationship between pycnogonids and euchelicerates (Colgan *et al.* 1998).

Hedgpeth (1947) suggested placing the Pycnogonida somewhere between the Annelida and Arachnida. However, recent molecular evidence indicates arthropods are closer to the Nematoda than the Annelida (Ghiselin 1988; Aguinaldo *et al.* 1997). These relationships are also hypothesized based on morphology (Andrassy 1976; Eernisse *et al.* 1992; Schmidt-Rhaesa *et al.* 1998). Similarities

with polychaete gut diverticula are convergent. The ovigers, proboscis, and genital openings are unique in pycnogonids and seem to be derived. The pycnogonid proboscis does not retract like that of an annelid and may be homologous to the rostrum of *Limulus* (King 1973). The evidence to place the Pycnogonida as a separate sub-phylum is hardly convincing. They may be an early branch of chelicerates or derived from arachnids. The chelifores and palps are most likely homologous to the chelicerae and pedipalps of the chelicerates. The proboscis is a specialized sucking structure that could have evolved from less specialized preoral structures similar to those of mites. Pycnogonids are not even as different from Arachnids as caprellids are from some entomostracan crustaceans (Grupta 1979).

Wheeler *et al.* (1993) found Pycnogonida within Chelicerata, grouping between trilobites and other chelicerates. Horseshoe crabs and arachnids were found to be sister groups, with Pycnogonida outside this complex. More studies are needed to clearly determine the evolutionary relationships between pycnogonids and other chelicerates. However, my preliminary 28S data indicate pycnogonids may be more closely related to arachnids than xiphosurans. It is likely that the Arachnida, Xiphosura, and Pycnogonida were all derived from eurypterid stock.

The largest genus, *Nymphon* (Nymphonidae), is the presumed primitive form (Thompson 1909; Arnaud and Bamber 1987), and has no obvious dimorphism with ovigers found on both sexes. This pattern is also seen in the families Callipallenidae, Ammotheidae, Tanystylidae, and Colossendeidae. In the Phoxichilidiidae, Endeidae,

and Pycnogonidae, ovigers are found only on males (Hedgpeth 1982). Munilla and de Haro (1981) used electrophoretic and immunological techniques to study pycnogonid phylogeny. The Nymphonidae had the fewest protein fractions of the families studied which did not include the Colossendeidae. They concluded that the Pycnogonidae and Callipallenidae with the most protein fractions were the most derived pycnogonid families. Additional evidence that has been used to place the Nymphonidae as the most primitive family is associated with the structure of the ovary and sperm morphology. The ovary of most pycnogonids, including *Nymphon gracile,* is U-shaped with open ends pointing anteriorly. *Pycnogonum littorale* has an additional junction between the lateral ovarian arms and was termed intermediate. Jarvis and King (1978) believed *Nymphon* sperm was the primitive pycnogonid sperm type. *Phoxichilidium femoratum* has a complete sheet of tissue with diverticula branching into the legs making up the ovary (Jarvis and King 1972). The morphological analysis presented in the previous chapter also supports the Nymphonidae as a basal group (figure 1.13). However, the 28S sequence results support the Nymphonidae as a derived, morphologically uniform group (figures 2.1 and 2.2). Krapp (personal communication) precludes the Nymphonidae as the most primitive family on the basis of the number of palp segments. He believes the Ammotheidae to be nearest to the ancestral stock which is in agreement with the data presented in this study. These results are also in agreement with Regier and Shultz (1998), who used amino acid sequences of elongation factor 1α and found a pycnogonid clade represented by (*Tanystylum* + (*Endeis* + *Colossendeis*)). The 28S

sequence results identify the most basal pycnogonid family as the Ammotheidae (figures 2.1 and 2.2). The ancestor of modern pycnogonids was not like *Pigrogromitus* from the Suez Canal as originally hypothesized.

The D3 region of 28S rDNA does resolve family relationships (figures 2.1 and 2.2). Regions of 28S rDNA is ideal for creating phylogenies because different regions evolve at different rates, and it can be used at different taxonomic levels (Litvaitis *et al.* 1996; Nunn *et al.* 1996). However, the results from the D3 region yielded perplexing relationships when compared with morphology. To define pycnogonid evolutionary relationships using molecular data, more studies must be done to compare molecular and morphological results.

CONCLUSIONS

Molecular and morphological family trees were basically consistent with Hedgpeth's view of familiar organization. The current analysis identified the basal living pycnogonid family as the Ammotheidae.

This study began with the hypothesis that the Arachnida, Xiphosura, and Pycnogonida were all derived from eurypterid stock (see chapter 1). Although molecular phylogenies did not include the Eurypterida the resulting phylogenies were consistent with this hypothesis. There is an abundance of evidence to indicate pycnogonids are chelicerates, a sister taxon to the living arachnids,

and xiphosurans. The evolutionary relationships between the extant chelicerates and the eurypterids are still unclear.

CHAPTER III

Is *Phoxichilidium tubulariae* (Lebour 1947) a synonym of *Phoxichilidium femoratum* (Rathke 1799)?

INTRODUCTION

A common pycnogonid of fouling communities in the Gulf of Maine is in the genus *Phoxichilidium*. The exact species name for this beast has been somewhat unclear. Traditional as well as recent keys would call it *P. femoratum* (Gosner 1978). However, in the United Kingdom, Lebour (1947) described a similar species, *P. tubulariae*. Lebour identified slight morphological differences and pointed out that *P. tubulariae* was a specialist on the hydroid *Tubularia larynx* both as a parasitic larva and as an adult predator. Lebour reported that *P. femoratum* larvae cause the hydroid host to form cysts while *P. tubulariae* cause no cysts in *Tubularia*. It is not known whether *P. femoratum* is a generalist that can use a variety of hydroids as larval hosts or if populations of this species are specialists on specific hydroids such as *Tubularia larynx*. If the latter case is correct, *P. tubulariae* may be a valid species.

The pycnogonid Lebour (1947) described as *Phoxichilidium tubulariae* is very similar to and often confused with *P. femoratum*. Both supposed species are found on east and west sides of the northern Atlantic (King 1973). *P. femoratum* is reported to be

slightly larger. King (1974) states that the abdomen of *P. femoratum* is about the same length as its lateral processes on the last trunk segment, and the heel of the propodus is armed with three or four large single teeth. The abdomen of *P. tubulariae* is about twice as long as the lateral processes of the last trunk segment, and the heel of the propodus is armed with two large single teeth and a third smaller tooth. Lebour (1947) described *P. tubulariae* saying "...two species hitherto included under the name *P. femoratum*, one of which must be given a new name, and for this *P. tubulariae* is proposed from its invariable habit of breeding inside the polyps of *Tubularia larynx*." (pp. 145). *P. tubulariae* is colorless or pale straw in color while *P. femoratum* is red and feeds on *Syncoryne eximia*. The oviger is divided into five segments. The cephalon and ocular process are short. The auxiliary claws are well developed. Pores of the cement glands are inconspicuous and placed dorsally on the femur. These attributes clearly identify *P. tubulariae* as a separate species (Lebour 1947). *P. tubulariae* is also supposedly smaller and more slender than *P. femoratum*. Its proboscis is wider anteriorly while *P. femoratum* has a cylindrical proboscis. The lateral processes have narrower spaces in *P. tubulariae* than *P. femoratum*.

The purpose of this chapter was to determine the validity of the species *Phoxichilidium tubulariae*. The characters discussed by Lebour (1947) were compared with the local pycnogonid population in the Gulf of Maine. Host specificity is also related to this discussion since *P. tubulariae* was believed to be a specialist.

METHODS

Morphological characters of local *Phoxichilidium* sp. were compared with the drawings of Lebour (1947). More than fifty specimens were collected from various fouling and subtidal communities in the Gulf of Maine, and preserved in 70% ethanol. The following observations were made for each animal: color, trunk length, abdomen length, length of lateral processes on the last trunk segment, number of spines on the propodus, shape of proboscis, and spaces between lateral processes.

Hydroids other than *Tubularia* were exposed to pycnogonid larvae in the laboratory to address the question of host specificity. Colonies of the following hydroids: *Tubularia larynx, T. indivisa, Obelia* spp., *Sarsia tubulosa, Clava leptostyla,* and *Eudendrium* sp. were collected from floating docks in the Gulf of Maine. Healthy colonies often lost their hydranths when brought into the Coastal Marine Laboratory of the University of New Hampshire, but after a few days these colonies usually regenerated their hydranths. Thirty ovigerous males were then placed in a sea table containing samples of hydroid species. After all the eggs hatched, hydranths from each hydroid species were observed under a compound microscope.

RESULTS

There appears to be little difference between the morphological characters of local *Phoxichilidium* sp. with the drawings of *Phoxichilidium tubulariae* by Lebour (1947). Most of the young

pycnogonids collected were feeding on fresh *Tubularia larynx* and were reddish in color while larger animals were often pale. Lebour described *Phoxichilidium femoratum* as red and *P. tubulariae* as pale straw in color. Lebour listed body lengths for *P. femoratum* as about 1.9 to 2.0 mm, and 1.4 to 1.5 mm for *P. tubulariae*. Body lengths of this local species were well within the ranges described for both species. The size frequency distributions are presented in the following chapter (see chapter 4; figure 4.8). The abdomen of these local animals was found to be almost twice as long as the posterior abdominal processes described for *P. tubulariae*. The propodus was found to also be similar to the propodus description by Lebour and her figure 2. Figure 3.1 is a scanning electron micrograph of the propodus and claws of a *Phoxichilidium* sp. specimen from Portsmouth, New Hampshire. It shows two large single and a smaller pair of teeth as described for *P. tubulariae*. The spaces between lateral processes also resemble the description for *P. tubulariae*. However, the proboscis of local specimens was cylindrical as described for *P. femoratum*. Figure 3.2 shows the chelifores and proboscis of this animal.

Each of the attributes that "clearly identify *P. tubulariae* as a separate species" (Lebour 1947) were examined and more variation was found within the local population in some of these characters, such as size, than was described by Lebour. However, in many cases, characters of the local specimens were more like the description for *P. tubulariae* than *P. femoratum*. This indicates *Phoxichilidium tubulariae* Lebour 1947 is not a junior synonym of *Phoxichilidium femoratum* (Rathke 1799) and *Phoxichilidium* spp. are specialist

parasites. This would suggest pycnogonid larvae are host specific. The results of the host specificity study showed that only *Tubularia larynx* contained *Phoxichilidium* spp. larvae. It is also important to note that pycnogonid larvae have never been found in *T. indivisa.* Even after hundreds of *T. indivisa* hydranths have been squashed and observed under the compound microscope. Variation in characters within the local populations of animals were observed when compared to differences between Lebour's descriptions, but they were not extreme enough to cancel the similarities. This is evidence that the local animal is indeed *Phoxichilidium tubulariae.*

DISCUSSION

Phoxichilidium tubulariae Lebour 1947 is not a junior synonym of *Phoxichilidium femoratum* (Rathke 1799). There is evidence to support that it as a valid species. *P. tubulariae* found in the Gulf of Maine relies on *Tubularia larynx* as an adult food and a larval host. It does not appear to parasitise other common hydroids in this area. *Phoxichilidium* spp. have been shown to use other hydroids as a larval host in other parts of the world including *Syncoryne eximia* in Europe (Lebour 1947).

Populations in Europe are specialists on *Tubularia larynx*, and show morphological similarities with local animals specializing on *T. larynx.* It is likely that *Phoxichilidium tubulariae* is a valid species. However, without really addressing the species question with interbreeding studies or population genetics techniques, it is not possible to clearly determine if these two species are in reproductive

isolation. Unfortunately most studies concerning the species question describe morphological variation, and never address propagation. Although this study does not clearly answer this question it does support *P. tubulariae* as a valid species.

CHAPTER IV

Describing life history details in a model system: *Phoxichilidium tubulariae*

INTRODUCTION

A more complete understanding of the basic biology and diversity of life histories present in the Pycnogonida is needed. There have been a few studies that described developmental details of pycnogonids beginning with Morgan (1891). Many of these descriptions are of parasitic species. Okuda (1940) described the development of *Ammothea alaskensis*, a species parasitic on the hydromedusa, *Polyorchis karafutoensis*, but it is still unknown how the pycnogonid larvae reach the jellyfish. Russel and Hedgpeth (1990) described the development of two hydroid parasites, *Tanystylum duospinum* and *Ammothea hilgendorfi*.

Jarvis and King (1972) described the development of *Pycnogonum littorale* from vitellogenesis through larval and juvenile molts. The larvae, juveniles, and adults are all ecto-parasites on anemones. Jarvis and King (1978) reviewed what was known concerning oogenesis and development of pycnogonids and also included information on breeding seasons. Nakamura (1981) described the development of *Propallene longicepts,* a non-parasitic

species. It underwent nine molts from hatching to adult in five months.

Reproductive seasons for a few species have been described in detail. Breeding periods are limited toward polar regions and extended toward the tropics (Jarvis and King 1978). Littoral species tend toward a seasonal release of eggs, while eggs may be released all year long offshore (King 1974). This pattern has been clearly shown for *Pycnogonum littorale* (Jarvis and King 1978). In general, pycnogonids breed in the spring with certain species having a second breeding season in the autumn or winter (Jarvis and King 1978). Cavanna (1877) first determined that the males carry the eggs (Hilton 1916). Lebour (1947) found males of *Anoplodactylus* and *Phoxichilidium* bearing eggs in the autumn. *Endeis spinosus* were found with eggs in January. *Nymphon rubrum* brooded eggs in February and March (Jarvis and King 1978). Larval *Ammothea* were found among *Obelia* in the summer (Jarvis and King 1978). Most European pycnogonids develop eggs from November and throughout the following spring and summer (Jarvis and King 1978).

The pycnogonid *Phoxichilidium* sp. is a common hydroid predator resident in *Tubularia larynx* colonies. King (1973) described *Phoxichilidium femoratum* feeding by tearing pieces of hydranth with the chelifores and transferring them to the mouth. Loman (1907) described *Phoxichilidium* feeding on *Tubularia larynx* especially on the gonophores. It grasps them with its claws and sucks out the contents (Thompson 1909; Stock 1978).

Lovely (1995) began to explain the effects of *Phoxichilidium tubulariae* on *Tubularia*. Adults were feeding on *Tubularia larynx*

from the outside and larvae were found inside *T. larynx* tissues. Larvae were found inside *T. larynx* tissues from July 22 through September 2, 1994, although densities of these larvae were not high enough to explain the high densities of adults. Perhaps these pycnogonids do disperse over greater distances as larvae than Jarvis and King (1978) indicated, or the adults migrate as shown for *Nymphon gracile* (Morgan 1978). More research was needed to understand the life history of *P. tubulariae* and the effect it has on *T. larynx* during each of its life history stages.

The life history of the pycnogonid parallels that of the host *Tubularia larynx*. Both have short dispersal of larvae and winter dormancy. Pyefinch and Downing (1949) demonstrated that *T. larynx* actinulae do not distribute far from the parent colony. Protonymphons with long sticky filaments are also likely to have a short dispersal. The pycnogonid *Phoxichilidium tubulariae* may impact *T. larynx* populations during its larval life as well as during its adult phase so it was necessary to determine when larval pycnogonids were present in *T. larynx* tissues.

Intense field sampling on floats was conducted from May 1993 to May 1999 using a variety of sampling methods to describe the annual population dynamics of *Phoxichilidium tubulariae*, and to answer the following questions relating to the life history of *Phoxichilidium tubulariae*: (1) When does reproduction occur? (2) When do larvae show up in gastrovascular cavities of *Tubularia*? (3) What is the relationship between abundance of hydroid and pycnogonid? (4) What are the annual patterns of density, sex ratios, size, reproductive status, and micro-habitat selection?

METHODS

Quadrat sampling was used to quantify abundances of hydroids and associated predators since the spring of 1993. Weekly samples of *Phoxichilidium tubulariae* were taken on floats near Portsmouth Harbor from May to December of 1997 and 1998. Ten small round quadrats (31.67 cm^2) were collected from each site for each date for quantification of hydroid and pycnogonid density. Monthly samples were continued throughout the remainder of the year. The Portsmouth Fishing Pier (70°44'N, 42°05'W), and the floats at the Coast Guard Station near the Coastal Marine Lab in New Castle, NH were used as the primary study sites. The floats at Prescott Park in Portsmouth, NH were also sampled until they were removed from the water during each winter. Other sites in the local area were occasionally sampled including subtidal sites.

Data collected from samples included density of pycnogonids and *Tubularia* spp., sex, size, reproductive status (gametogenic, ovigerous, larval), and micro-habitat (on hydroids, between mussels, on bare substrate) for the pycnogonids in each sample date. Since sampling continued through the winter, this design also determined what the pycnogonids do during the winter. A subsample of *Tubularia larynx* colonies from each sample date was maintained in running sea water at the Coastal Marine Laboratory.

Intraspecific distribution was calculated for each date at each location from sampling data using variance to mean ratio (Krebs 1989). A Students t value was then calculated for each species on

each date for each location (Sokal and Rolf 1981). Interspecific distribution was determined using the sampling data from all dates to calculate contingency tables of *Tubularia* frequency and predator presence or absence to determine if locations of predators are influenced by the location of *Tubularia* colonies, or the locations of other predator species. These methods were described by Strong (1982). A chi-squared test using the methods of Zar (1984) was used to determine significance.

It was important to determine if pycnogonid larvae are present in the hydroids' tissues and to determine when larvae show up in gastrovascular cavities of *Tubularia larynx.* One hundred hydranths of *Tubularia larynx* were examined with light microscopy from each sample when hydranths were present. A squashed *T. larynx* hydranth containing a *Phoxichilidium tubulariae* larva is shown in figures 4.1a and 4.1b.

Phoxichilidium tubulariae were maintained in running sea water at the Coastal Marine Laboratory and starved for twenty-four hours. Then several were placed in finger bowls (10 cm in diameter) with fresh *Tubularia larynx.* Feeding behaviors were observed and described.

On August 30, 1998, the header tanks at the Coastal Marine Lab were scraped. Before this scraping event there was a population of both *Tubularia larynx* and *Phoxichilidium tubulariae.* The scraping reduced the tanks to only the fiberglass substrate. However, by September 28, 1998, *Tubularia larynx* had returned as well as *P. tubulariae.* Several hydranths of the new *Tubularia larynx* colonies contained larval pycnogonids. Since protonymphon larvae

are rarely associated with actinulae, it is likely both organisms recruited independently. This indicates pycnogonid protonymphons may disperse farther from the brooding male than previously indicated by the literature. This phenomenon was studied in more detail. On September 31, 1998, twenty separate eight foot sections of rope were tied to floats ten feet apart with a brick tied to the bottom end. Ropes were made of natural fibers and two thicknesses were tried. These collectors were in the water for a month and were observed weekly. I expected to find *Tubularia larynx* colonies growing on the ropes with abundance directly related to distance from the float. This was clearly shown by (Pyefinch and Downing 1949). The purpose was to check in these colonies of *Tubularia larynx* for pycnogonid larvae.

The reproductive cycle of *Phoxichilidium tubulariae*

Scanning electron microscopy (S E M) was used to describe the development of *Phoxichilidium tubulariae* on the ovigers of males. Infected *Tubularia* hydranths were fixed for S E M and cracked to observe larvae in the hydroid. Hydranths were embedded in paraffin, sectioned, and then the paraffin was dissolved before preparing for S E M. Also, larvae were dissected out of infected hydroids and observed with S E M. Larval stages were then arranged into a continuous developmental series as in (Russel and Hedgpeth 1990). These methods were used to describe the developmental sequence of *P. tubulariae*, and answer the questions: (1) What is the intimate association between embryos and ovigers? (2) What is the association between the larval pycnogonid and

hydroid tissues?. The emergence of larvae from hydranths was also observed.

Organisms were fixed using 2% osmium tetroxide in 1.25% $NaHCO_3$ for one hour. Some of the samples were prefixed in 2.5% glutaraldehyde but this was deemed not necessary and was therefore discontinued in later protocols. Specimens were rinsed in distilled water and dehydrated in the following concentrations of ethanol for ten minutes each: 20%, 50%, 70%, 95%, 100% and 100%. Specimens were dried using critical point drying. They were sputter coated with 250A Au/Pd using a Hummer V sputtercoater. They were viewed using a Amray 3300 field emission scanning electron microscope at the Instrumentation Center (U. N. H.).

RESULTS

The life cycle of *Phoxichilidium tubulariae* is shown in figure 4.2. Males brood the eggs that hatch as protonymphon larvae. The larvae are consumed by the hydroids and develop in the gastrovascular cavities of the host. The juveniles then breakout and grow until sexual maturity.

As predicted, populations of the pycnogonid tended to peak as the population of *Tubularia larynx* declined. Pycnogonids were usually most abundant during September. Figures 4.3 through 4.6 represent the abundance of *P. tubulariae* on the upper graph (figures 4.3a to 4.6a), and the seasonal abundance of *Tubularia* spp. for the Coast Guard floats near the Coastal Marine Laboratory in 1997 (figure 4.3b), 1998 (figure 4.4b), and the Portsmouth commercial

fishing pier in 1997 (figure 4.5b), and 1998 (figure 4.6b). Figure 4.7 is a diagram representing the timing of the life history patterns observed in 1997 and 1998. This figure illustrates the reproductive status of pycnogonids present in samples including: gametogenically ripe females, brooding males, larvae in hydroid tissues, and newly hatched juveniles. The size frequency distributions for 1997 and 1998 are presented on figure 4.8. Sex ratios were not significantly different from 1:1. Microhabitat data indicated that pycnogonids were aggregated around *Tubularia larynx* colonies, and occasionally found on bare substrate or on mussels. Pycnogonid size was not significantly correlated with the number of egg masses carried by males.

The pycnogonid *Phoxichilidium tubulariae* may impact *Tubularia larynx* populations during its larval life as well as during its adult phase; therefore, it was necessary to determine when larval pycnogonids were present in *T. larynx* tissues. By squashing polyps during 1994, the presence of *Phoxichilidium* sp. larvae was indicated. On July 22, one larva was found from the fishing pier. More larvae were found from the Coast Guard float on August 18. One larva was found from each fishing pier site on August 20, and another was found from the Coast Guard float on September 2. At this point, the numbers of larvae observed did not completely explain the large numbers of adults found in these colonies. The infection rates during the years 1997 and 1998 are presented in figure 4.9. Larvae were present in *Tubularia* polyps from June to September in 1997, and May to October in 1998. Larval development appears to take less than twenty days. High densities of larvae were found in June of

1998, low densities in July, high again in August, low in September, and high densities again in October. The pattern of infection rates is not as clear for 1997. Infection rates (figure 4.9), pycnogonid abundance (figure 4.6), presence of larvae and juveniles (figure 4.7), and size frequency distributions indicate that in 1998 the local pycnogonid population had at least two generations. Animals migrating into shallow fouling communities in the Gulf of Maine to exploit the summer blooms of *Tubularia* spp., or pycnogonids surviving in fouling communities through the winter, are reproductive in late May and early June. The resulting larvae develop rapidly and with abundant food reach sexual maturity by August. It is the hatching and subsequent growth of these larvae that led to the high populations by the late summer. This generation then reproduces yielding larvae in October.

Pycnogonids were significantly aggregated around *T. larynx* on ninety percent of sample dates. Eighty eight percent of quadrats containing *Phoxichilidium tubulariae* also contained *T. larynx*. There were no dates when *P. tubulariae* was found to be intraspecifically segregated.

On a few occasions, *Phoxichilidium* was observed while feeding. One animal was found with its proboscis buried in a detached gonophore and it remained in this position for more than three hours. Others have been found with their proboscis buried in a *Tubularia larynx* hydranth.

A subset of the sampled *Tubularia larynx* colonies and associated pycnogonids were maintained in running sea water at the Coastal Marine Laboratory. These colonies lost their hydranths

within one week of being placed in the sea tables, but pycnogonids were still present in low densities on the clumps of dead hydroid colonies in the following spring.

The rope collectors were in the water for a month and were observed weekly. I expected to find *Tubularia larynx* colonies growing on the ropes with abundances directly related to distance from the float as shown by Pyefinch and Downing (1949). I was interested in looking in these colonies for pycnogonid larvae. Pycnogonid protonymphons may disperse farther than previously indicated. Most of the ropes were unfortunately not recovered. One thin and four thick ropes remained intact at the end of the month. All recovered ropes had developed a diatom film, and had a community containing a portion of the following: Obelioid hydroid, *Botrylloides, Lacuna*, Amphiopods, Isopods, mussels, and large mussels sometimes on the brick weight. Only one rope contained colonies of *T. larynx* and pycnogonid larvae were found in hydranths on both ends of the rope. Although this is an extremely low sample size, it indicates that protonymphons do disperse at least eight feet vertically. The benthos in this area is soft, and the closest *T. larynx* colonies with adult pycnogonids were on at the top of the rope eight feet from this small colony with larval pycnogonids at the bottom of the rope. It is unlikely the hydroids or pycnogonids came from closer than eight feet since the closest hard substrate was the floating dock at the top of the rope. This study was also interesting since in the month the rope was in the water not only did the *T. larynx* develop, but the pycnogonid larvae reached the fourth larval

stage. This indicates the quick rate of *Phoxichilidium tubulariae* development.

Life history stages

The life history of *Phoxichilidium* sp. includes copulation with external fertilization. The male climbs on the back of the female then crawls over her head so that the ventral surfaces are opposed. The hooked ovigerous legs of the male fasten to the extruding egg masses and using rotational movements, they form the egg mass into a ball as fertilization occurs externally. The whole egg masses on the ovigerous legs of *Phoxichilidium tubulariae* are approximately 0.7 mm in diameter. The average diameter of fertilized eggs in these masses is 0.05 mm. Scanning electron micrographs of males brooding eggs are shown on figures 4.10a-4.10b and 4.11a-4.11b. The association between embryos and ovigers is shown. The male loops the egg mass around the oviger and carries it much like a purse. The eggs hatch as protonymphon larvae.

There were five larval stages found in the gastrovascular cavities of *Tubularia larynx* beginning with the first stage, the protonymphon. Stage one is similar to the typical pycnogonid protonymphon (figure 4.12), and is similar to stage one of (Morgan 1891) and (Okuda 1940) only with four long tendrils or larval filaments (figure 4.13). It differs significantly from stage one described by (Nakamura 1981). The attaching larva of (Nakamura 1981) lacks limb buds entirely, and only has complete chelifores.

Stage two shows a loss of tendrils, and an overall body elongation. It is otherwise similar to the previous stage. This stage

was observed under light microscopy, but was not found using S E M. It is similar to the stage two described by (Okuda 1940) and (Nakamura 1981).

Stage three is also found inside the gastrovascular cavities of the hydroid *Tubularia larynx.* This stage has limb buds of the first three pairs of walking legs (figure 4.14a). These three pairs are incomplete legs (figure 4.14b) and correspond to the adult thoracic segments. Its forth pair of walking legs are tiny bumps (figure 4.14c). The animal then molts while still inside the hydroid. A molted cuticle of stage three is shown in figure 4.14d. *Phoxichilidium tubulariae* then skips stages three and four as described by Morgan (1891), Okuda (1940), and Nakamura (1981) which all show a gradual addition of limbs one at a time.

Stage four shows more developed walking legs that are folded (figure 4.15a). This stage can be found still inside the hydroid (figure 4.15b), or if cohorts hatch and destroy their host this stage can continue to develop outside the hydroid. Additional views of this stage found outside *Tubularia* hydranths are shown on figures 4.16a, 4.16b, and 4.16c.

Stage five typically involves the period when *Phoxichilidium tubulariae* hatch from the hydroid. A juvenile that was dissected out of a gastrovascular cavity is shown in figure 4.17. Hatching was observed by a juvenile of stage five (figure 4.18). Notice the most posterior pair of walking legs protruding from the top of the hydranth and an anterior walking leg sticking out the bottom of this hydranth. This animal was caught while emerging from the hydranth. Animals of this and later stages live the remainder of

their lives outside the hydroid tissues, but still associated with *Tubularia* spp. (figures 4.19, and 4.20). In this stage the anus is found open, forming the adult complete gut. (figure 4.21a, and figure 4.21b). This stage is similar to stage five of Okuda (1940) and Nakamura (1981) as well as stage seven of Morgan (1891).

The next stage were adult animals. Adults can be distinguished from juveniles (stage 5) because the adults have a more developed fourth pair of walking legs and possessed gonopores on their coxa. These gonopores are shown for the male in figures 4.22a, 4.22b, 4.22c, and 4.22d. Female gonopores are shown in figures 4.23a, 4.23b, and 4.23c.

The larval development of *P. tubulariae* could be explained using the terms described by Bain (1992) as an atypical protonymphon in which the protonymphon stage (figure 4.12) with four larval filaments (figure 4.13) molts into a stage with limb buds of the first three pairs of walking legs (figure 4.14b). This type of development may be faster than the typical development in which one pair of walking legs are added with each molt, and perfect for an organism that has a limited time to develop inside a host species. The association between the larval pycnogonid and hydroid tissues is also shown to lack a cyst. Instead the larva bathes freely in the liquid of the gastrovascular cavity.

This data may not completely describe the earliest life history stages. The series is likely complete with regards to stages three and later since the sample sizes and abundance of observed animals in these stages were very large, it is unlikely that any stages were missed. However, the earliest stages may be incomplete.

DISCUSSION

The pycnogonid's life history appears to be well adapted to exploit their prey. *Phoxichilidium tubulariae* survives in shallow fouling communities through the winter, or disperses from deeper water in the spring as adults to exploit the summer bloom of *Tubularia* spp. These ideal environments have abundant food and allow the pycnogonids to grow rapidly and reproduce. The larvae produced from these surviving or colonizing adults grow extremely rapidly and hatch from the gastrovascular cavities of the hydroid in fifteen to twenty days. In many shallow fouling communities, food remains abundant for the next several months which allows these juveniles to grow to adulthood and reproduce before the *Tubularia larynx* populations crash in the fall. This seasonal dispersal strategy allows *P. tubulariae* to exponentially increase its population during the season when food and larval hosts are plentiful. This population increase allows numbers to be high enough so the species can survive the winter when food is scarce. These over-wintering organisms move to deeper water both with the sloughing of dead *Tubularia* uprights as well as with adult migration. It appears that the subtidal populations "seed" these ephemeral float islands, and the float islands in turn "seed" the more stable subtidal populations.

Adult *Phoxichilidium* sp. may subsist on alternative food such as detritus when fresh *Tubularia* is unavailable. On several occasions Harris (personal communication) observed *Phoxichilidium* sp. in dense aggregations surrounding and feeding on unhealthy

appearing *Metridium senile*. This observation was made in the field in the autumn after the crash in *Tubularia* abundance and when no hydroids were left in this area. *M. senile* is common in fouling communities throughout the year and could potentially, along with a tolerance for starvation conditions, get the pycnogonids through the winter season.

Pycnogonid species commonly fold legs dorsally into a "basket" posture to allow rapid sinking (Arnaud and Bamber 1987). This behavior will aid in retaining adults close to their area of birth. However, *Nymphon gracile* has been shown to seasonally migrate between the littoral zone and deeper waters using a passive process of riding tidal currents (Fage 1932; King and Jarvis 1970; Morgan 1978). They are weak swimmers and depend on currents for dispersal both as larva and adults. *N. gracile* has endogenous swimming behavior especially active just after high tide (Isaac and Jarvis 1973). Fage (1932) showed this off-shore swimming was seasonal with adult animals abundant in the plankton from January to April. Morgan (1978) showed littoral populations in Swansea were greatest from September to November, and almost no animals were observed between December and March. King and Jarvis (1970) discussed a similar pattern for this species. They concluded that young animals move offshore in the winter and sexually mature animals return to the littoral zone in the spring. This pattern may also be present for *Phoxichilidium tubulariae*.

Munilla (1980) studied the life-cycles of several ammotheid species on the Spanish coast and found annual life cycles in *Ammothella uniunguiculata* (Munilla 1980a), *Tanystylum orbiculare*

(Munilla 1980b), *Ammothella longipes* (Munilla 1980c), and *Achelia echinata* (Munilla 1980d). Jarvis and King (1978) discussed breeding seasons of European pycnogonids. Most species reproduce in the spring and summer, however some have been reported as reproducing in autumn (Lebour 1947). It is possible that this autumn cohort represents the second generation in a season. Wilson and Parker (1996) described the life cycle of the amphipod, *Corophium volutator*. Some populations have a single generation each year. Other populations have two generations per year, with the first generation born in May to mid-June and these young become reproductive in August. *Phoxichilidium tubulariae* also has two generations per year in the southwestern portion of the Gulf of Maine. This is an ideal strategy for this pycnogonid. *Tubularia larynx*, the hydroid needed both as larval host and as adult food is extremely abundant in shallow water in the Gulf of Maine during the summer and abundance is low or non-existent for the remainder of the year in shallow fouling communities (Lovely 1995). Off-shore in locations like Cedar Island Ledge, Isles of Shoals *T. larynx* colonies are patchy and not as seasonally fluctuating (Harris personal communication). Jarvis and King (1978) indicated that some European species have a spring breeding season with a second "smaller" season in the autumn. They did not believe their evidence indicated two generations, but that eggs not released in the spring were spawned in the fall. The evidence presented here, including eggs in ovaries, males brooding, and size frequency, indicates *Phoxichilidium tubulariae* does have two generations in the Gulf of

Maine. This evidence is especially strong for 1998 (figures 4.4, 4.6, and 4.7).

Hydroid community ecology

Few studies have focused on the roles pycnogonids play in hydroid communities. Mercier and Hamel (1994) showed that the pycnogonid *Pigrogromitus timsanus* negatively affected populations of the sea anemone *Bartholomea annulata* in the laboratory. Pycnogonids were unaffected by the anemone's defenses and predation eventually led to retraction of tentacles, difficulty attaching, and death. Piel (1991) discussed the pycnogonid *Anoplodactylus carvalhoi* feeding on sabellid polychetes and nudibranch cerata. Lovely (1995) found pycnogonids aggregated around *T. larynx*, with peak populations of the pycnogonid *Phoxichilidium tubulariae* when populations of *Tubularia larynx* were declining. There is not much known about predators of pycnogonids. Isopods, anemones, and some fishes have been shown to eat small quantities of pycnogonids, but it is unlikely they are a major part of any predator's food supply except maybe in the deep sea (King 1973; Arnaud and Bamber 1987).

Two species of *Tubularia* were found during this study, *T. larynx* Elis and Solander, 1786 and *T. indivisa* (Linnaeus, 1767). Pyefinch and Downing (1949) described the liberation and settlement of *Tubularia larynx* actinulae. The actinulae sink slowly (1 mm/sec); therefore, the heaviest settlement is in the immediate vicinity of the parent colony. *T. indivisa* actinulae develop into a single hydrocaulus (upright), which bears a single large hydranth

(Hughes 1983). Winter growth is slow and maximum growth occurs in July. *T. indivisa* first breeds when six to eight weeks old and will breed two or three times in their lifetime. Hughes (1983) found six annual cohorts of *T. indivisa*. Autonomy of hydranths occurs regularly, mostly as a stress response that may aid in dispersal. Severed polyps can continue to shed actinulae for up to thirty days. This study showed *T. larynx* responds similarly to stress.

The decline in pycnogonid abundance observed as the *Tubularia* population was crashing is unlikely to be due to pycnogonids running out of food leading to mortality due to starvation because pycnogonids have been shown to be resistant to starvation. Pycnogonids are not extremely mobile and it is unlikely they move to other resources by walking. A more likely explanation is that the sloughing of "dead" *Tubularia* material due to sedimentation (McDougall 1943), wave action, effects of predators like *Catriona aurantia* and *Phoxichilidium tubulariae*, and recruitment of later successional stages like mussels removes large numbers of pycnogonids along with the dead colonies. Although most of the *Tubularia* colonies disappear from these floats in October, this is not the end of the story. The *Tubularia larynx* colonies collected and the associated predators were maintained in running sea water at the Coastal Marine Laboratory. The *Tubularia* colonies lost their hydranths within one week of being placed in the sea tables, but pycnogonids were still present in low densities in the following spring. This evidence shows that some pycnogonids can remain in the fouling communities even after their primary prey is

gone and patiently wait for the return of *Tubularia* the following spring.

Life history stages

Pycnogonids have external brooding of eggs similar to many crustaceans such as peracarids except that the male pycnogonid broods the eggs. It can be termed aparental benthic development (McEdward 1995) since the larva must first find the host, followed by an endoparasitic phase. *Phoxichilidium tubulariae* protonymphons feed after hatching. They have an incomplete gut until stage five. They feed on fluid in the gastrovascular cavities of their host, and grow rapidly. *P. tubulariae* adults are resistant to starvation or feed on detritus when other food is unavailable, but this is unknown in larvae. They appear to resist starvation much as crustacean larvae would. If fed before a period of starvation, the effects of starvation are decreased. These starvation effects are less damaging if the larvae feed first rather than if they are starved immediately after hatching (Anger *et al.* 1981) and the same has been shown for echinoderms (Fenaux *et al.* 1988). Therefore, it seems more critical that food is abundant in early stages of development rather than later in development.

The mating of *Phoxichilidium femoratum* was described by (Loman 1907; Lebour 1947; King 1973). The male climbs on the back of the female then crawls over her head so that their ventral surfaces are opposed. The hooked ovigerous legs of the male fasten to the extruding egg masses and using rotational movements they form the egg mass into a ball as fertilization occurs externally.

Lebour (1947) described mating as occurring in autumn. Each ball of eggs the male carries in his ovigers represents one mating and the entire brood of that female (King 1973). Up to fourteen egg balls have been observed being carried by one male (King 1974). I have also observed as many as fourteen balls of eggs being carried by one male. Development is complete and equal in *Phoxichilidium* spp. (King 1974). The eggs are carried by the male for awhile and then deposited amongst the hydroids to complete their development (Jarvis and King 1978). *P. femoratum* and *P. littorale* have been observed carrying larvae, but this is not usual and the majority tend to release eggs before hatching. There is no evidence in pycnogonids for a pelagic larval stage except in those species where a hydroid medusa is used as a vector (Jarvis and King 1978).

Copulation in most pycnogonids is not a true copulation, but a pairing procedure that enhances the success of external fertilization by ensuring the genital openings are in close proximity at spawning (Jarvis and King 1978). In *Anoplodactylus, Phoxichilidium*, and *Endeis* the male climbs upon the female and over her head to lie beneath her, head to tail. As the eggs are released he rolls them into a ball and glues it to the ovigers (King 1974). The female *Endeis spinosa* releases the contents of a single femur at one time. *Nymphon gracile* releases the contents of two femurs. *Callipallene* produces only one or two eggs in each femur but releases the contents of all femurs at the same time. *Nymphon gracile* males brooding eggs show different staged masses indicating they were acquired over a period of time (King 1974). Jarvis and King (1972) observed *N. gracile* mating in an aquarium. They collected the eggs

from the female as two separate egg masses one placed on each oviger. Other species gather single eggs as in *Callipallene* sp. or an entire brood from one female into a single ball of eggs as in *Phoxichilidium femoratum*. Mating has also been described for *Propallene longiceps* (Nakamura 1981), and *Pycnogonum littorale* (Behrens 1984).

The mating process usually takes a few hours at most, however, *Pycnogonum littorale* maintains mating positions for up to five weeks. The male grabs on to the back of the female and the eggs are collected from the genital openings on the second coxa of the hind legs, ventral side for males and dorsal side for females, in a single mass in which the ovigers are imbedded (Jarvis and King 1972; King 1974). The female releases all the mature eggs at the same time and therefore mates with only one male while in other pycnogonids such as *Nymphon gracile*, the female can mate with three or four males in a single season. After this lengthy mating process the eggs are carried for ten weeks before being deposited (Jarvis and King 1972).

Spermatozoa of *P. littorale* are aberrant. They are non flagellated and unmotile (Arnaud and Bamber 1987). A *P. littorale* female was kept alive and unchanged at the third instar for eleven months in the absence of a male (Arnaud and Bamber 1987). Behrens (1984) reared larvae in the lab on *Clava multicornis*. It took an average of 83 days to go through five molts from protonymphon to juvenile (from (Arnaud and Bamber 1987)). Adults feed on actinians and accumulated detritus (Jarvis and King 1972).

Cleavage of pycnogonid eggs varies. *Phoxichilidium*, *Anoplodactylus*, *Achelia*, and *Pycnogonum* have complete and equal cleavage. *Nymphon* have complete but unequal cleavage and in *Callipallene* the large yolk rich division is initially complete but later only partial (King 1974).

The typical protonymphon has three pairs of appendages with characteristic spines probably used to retain larvae on the adult, attach to a host, or for dispersal (Arnaud and Bamber 1987). Bain (1992) divided pycnogonid larvae into three types. The typical protonymphon hatches and as the body elongates walking legs are added one pair at a time. Examples include: *Tanystylum*, *Pycnogonum*, *Nymphon*, and most Ammotheids. The attaching larva with no larval appendages also adds legs one pair at a time. Examples include: *Propallene*, *Austropallene*, and callipallenids. The third type was called an atypical protonymphon where the larva hatches as a protonymphon but then at first molt all limb buds for walking legs appear at once. Examples include: *Nymphonella*, *Ammothea*, and *Nymphon*. This study shows *Phoxichilidium* also has this atypical protonymphon type.

The protonymphon larvae of *Phoxichilidium femoratum* have hypertrophied claws of the second and third appendages which are modified to form long filaments up to five times length of the body (King 1974). Newly hatched, they can measure sixty to eighty µm across the body, and about the same length (Lebour 1947). They use these appendages to affix themselves to the hydroids, feeding as the adults do, afterwards losing the tendrils in a molt, they pass into the gastral cavity of the hydroid (Thompson 1909). Gegenbauer first

noticed these larvae among hydroids in 1854. They were later found by Allman in 1859, and both these investigators proposed that the eggs were laid in the hydroid polyp. It was Hodge in 1862 who showed that the larva was the stage to enter the gastrovascular cavity (Hilton 1916). As many as five larvae can be found per polyp. The polyp appears unharmed by them (Lebour 1947), except perhaps that the polyp may become slightly elongated (Pyefinch and Downing 1949). I have found up to fourteen larvae packed in a gastrovascular cavity. The "parasite" remains in the polyp until the penultimate larval stage with three pairs of legs and rudiments of a fourth pair (the fifth instar (Arnaud and Bamber 1987)). The larvae are colorless with pink intestinal fluid. They measure sixty to eighty µm across their widest part with conspicuous chelae and proboscis (Lebour 1947). The larvae apparently develop rapidly reaching advanced stages in as few as twenty days (Pyefinch and Downing 1949). They remain in the polyp until the penultimate larval stage which emerges and molts. This young pycnogonid has three pairs of legs and rudiments of the forth pair (Lebour 1947) (figures 4.17-4.20).

Nakamura (1981) cultured *Propallene longiceps* and described development in this non parasitic species. It took approximately five months from egg to adult. Development time to the adult stage was unknown in parasitic forms such as *P. tubulariae* with the exception of the studies by Pyefinch and Downing (1949). Another thread of evidence for this short development time came from the rope collector study. The rope with pycnogonid larvae in hydroid gastrovascular cavities on both ends of the rope showed advanced

larvae and the rope was in the water for twenty eight days. The results and observations presented here indicate development time of little more than twenty days from hatching as protonymphons to breaking out of the hydroid as a juvenile. These results are in agreement with Pyefinch and Downing (1949).

Loss of two developmental stages found in *Propallene longiceps* (Nakamura 1981) and *Ammothea alaskensis* (Okuda 1940) apparently occurs in *Phoxichilidium tubulariae* to speed development from about thirty five days (Nakamura 1981) to less than twenty days. However, Morgan (1891) described the embryology of several species and in doing so found *Pallene* development is an abbreviated version compared to the development of *Phoxichilidium.*

In some species, attachment threads develop after hatching and these larvae may swim for a short time (Russel and Hedgpeth 1990). I found sticky threads present at hatching in *Phoxichilidium tubulariae.* These attachment threads indicate larvae are not great dispersers (Salazar Vallego and Stock 1987; Hedgpeth and Haderlie 1980). The larval appendages secrete the sticky filaments. There is some debate in the literature as to the fate of the larval appendages (Okuda 1940; Nakamura 1981). It is clear that the larval chelifores are the same structures as the adult chelifores. However, the remaining two larval appendages may later become the palps and ovigers or perhaps these structures are created separately and the larval appendages are simply lost. Since *Phoxichilidium* spp. have no palps and the ovigers do not appear until near sexual maturity, it is unlikely the larval appendages become these structures in this species.

Hilton (1916) described the life history of *Anoplodactylus erectus*, and this study showed much in common with this classic study. He found eggs in the summer and early fall in *Tubularia crocea*. Hilton's first stage showed a protonymphon with long tendrils very similar to a *Phoxichilidium* spp. protonymphon. He described the tendrils being lost in a molt and the next two stages show much in common with the larvae found in the gastrovascular cavities in this study. The three pairs of legs then grow out, and after another molt yield his stage five which is very similar to the stage shown hatching out of the hydranth (figures 4.17-4.20). He then found this and later stages clinging to the gonosome or tentacles of the hydroid. This species developed much like *Phoxichilidium tubulariae* described in this study. By the end of November, he also found no larvae (Hilton 1916).

These patterns of larval distribution in time are consistent with Lebour (1947) who found larvae from spring through autumn. *T. larynx* began to decline and larvae could then be found where living *T. larynx* remained. She found as many as five advanced larvae in one polyp and believed the polyps were unharmed, because the presence of larvae did not reduce resistance to copper exposure of *T. larynx* polyps. Although the larvae must break out of the hydranth, destroying it, the colony can quickly regenerate from this hydranth loss. Pyefinch and Downing (1949) found larvae in the gastrovascular cavities in late September and early October. They hypothesized development of the larvae is rapid because colonies collected from a surface that was only immersed for twenty four days showed advanced larvae. They estimated development takes

about twenty days. Another thread of evidence for this short development time came from the rope collector study. The rope with pycnogonid larvae in hydroid gastrovascular cavities showed advanced larvae and the rope was only in the water for a mere twenty eight days.

The molting occurs differently in some groups. *Phoxichilidium tubulariae* sheds more than eight pieces of exoskeleton at each molt, and molts more than seven times during a lifetime. *Pycnogonum littorale* sheds eight pieces of exoskeleton at each molt, while *Nymphon gracile* sheds twelve pieces. Male *Pycnogonum littorale* molt nine times in their lifetime while females molt eleven or twelve times. Growth does occur between moltings by extension of elastic regions at the cuticle joints (Jarvis and King 1972). *Propallene longiceps* undergoes nine molts from hatching to adult (Nakamura 1981).

Some interesting questions remain. Why are the larvae not digested by the hydroid? Nematocysts are used in hydrozoan digestion. Perhaps pycnogonid larvae can resist nematocyst attack. Nematocyst attack was documented in many cases (figures 4.24a-c and 4.25a-c). Pycnogonids do suffer from nematocysts, but apparently not severely enough to be significantly harmed since they actively grab tentacles and other tissue regardless of nematocyst attack.

CONCLUSIONS

Annual population dynamics of *Phoxichilidium* sp. is seasonal. Density of adult animals was greatest in the mid to late summer with reproduction being greatest in July and August. The abundance of pycnogonids peaked as the hydroid population declined. Some populations of this pycnogonid were shown to have two generations in the summer of 1998. Adult migration may play a larger role in distribution of this species than larval dispersal. Since adult pycnogonids are rare in fouling communities during the winter, and adults appear in fouling communities before the *Tubularia* bloom. This type of dispersal has been shown for *Nymphon gracile* (Fage 1932; King and Jarvis 1970; Morgan 1978).

The male pycnogonid loops a portion of the egg mass over his oviger and carries the mass like a purse. The larvae hatch, infect the hydroid, and develop inside the gastrovascular cavity of *Tubularia larynx*. They are free living in the fluid and there is no evidence to suggest they form a cyst or gall. The larvae develop for several molts and then hatch, destroying the hydranth.

Phoxichilidium tubulariae has an atypical protonymphon type development. This fast developmental mode reduces the typical number of molts, and develops rapidly in the gastrovascular cavities of the hydroid host. It decreases development time from the typical 35-40 days to 15-20 days. This developmental strategy is adapted to exploit the seasonal abundance of *Tubularia larynx*.

CHAPTER V

Evolution of larval parasitism in the Pycnogonida

INTRODUCTION

There is an incredible diversity and similarity of marine invertebrate larval forms (McEdward 1995) as well as life-history strategies. Some invertebrates take part in a life-history strategy where they exist as parasitic larvae and are free-living as adults (Davenport 1955). The planulae of the burrowing anemone *Peachia quinquecapitata* on the Pacific coast of North America are parasitic on hydomedusae (Spaulding 1972). The basket star *Gorgonocephalis* develops inside the soft coral *Gersemia* (Patent 1969, 1970a, 1970b). Some other examples of this strategy are: glochidia of fresh water bivalves, nematomorphs, and parasitoid wasps. The larvae of pycnogonid species are ectoparasites, endoparasites, or free-living. Some pycnogonids use cnidarians as larval hosts (Lebour 1947; Child and Harbison 1986).

It has already been established that pycnogonids prey on hydroids and that some species invade hydranths as larvae and encyst during early stages forming a sac or gall in the process. This phenomenon was observed as early as 1844 and was thought analogous to gall formation in some plants (Russel and Hedgpeth

1990). Thompson (1909) states that this life history was discovered in 1854 by Gegenbaur in *Eudendrium*. It was later found in *Coryne eximia* by Allman (1859). Hodge made detailed observations and disagreed with Gegenbaur (1854) in that the larvae entered the hydroid not the egg (Thompson 1909). In 1881, Moseley found capsules with pycnogonid larvae in the stylasterine hydrocoral *Pliobothrus synmetricus* (Thompson 1909).

Brooding is common in chelicerates. Besides male brooding of eggs in the Pycnogonida, scorpions are commonly viviparous and spiders are also known to brood external egg cocoons (Hedgpeth 1978). The brooding of eggs by the male was suggested to have had its origins in a primitively hermaphroditic condition (Jarvis and King 1978). However, the only known hermaphroditic species of pycnogonid is *Ascorhynchus corderoi.* Several gynandromorphic forms have been identified (Child 1978; Child and Nakamura 1982; Nakamura and Child 1983). Gynandromorphs are sexual mosaics where half the body shows male characters and the other half shows female characters.

Life history characters can be used in producing phylogenies (Nakamura 1981). The assumption in using these characters is that life history traits evolve slowly and are good characters for reconstructing evolutionary relationships, but this is not always the case. Wray (1995b) showed developmental changes can occur rapidly, because sea urchins have changed larval feeding mode on several occasions in closely related species. Could a similar situation have occurred in the evolution of pycnogonid life-histories?

"Over the next several years, it will be interesting to see whether uncoupled and punctuated modes of developmental evolution are found in other taxa or in association with other common life history transformations such as the origin of parasitism, coloniality, and brooding." (Wray 1995b)

The purpose of this chapter was to review the current knowledge of pycnogonid larval and adult parasitism. This knowledge was then compared with the life history of *Phoxichilidium tubulariae* (see chapter 4). The life history review was put into an evolutionary framework using morphological and molecular phylogenetic trees (see chapters 1 and 2).

METHODS

Life history information for species from this study (see chapter 4; Lovely 1995) and the literature was collected and an extensive list of characters from the literature were compiled to be used for morphological analysis. The morphological trees were compared to the molecular results to hypothesize an accurate phylogeny. Basic life history information and observations were overlaid onto this phylogeny to examine the possibility that parasitism in the Pycnogonida is polyphyletic.

RESULTS

Most pycnogonid families contain some examples of life histories with a parasitic larva (King 1973). Arnaud and Bamber

(1987) went as far as to say that free living development is uncommon with most species passing through a parasitic stage on or in an invertebrate host. King (1973) synthesized larval associations. A summary of pycnogonid associations is shown on Table 5.1. This table shows internal and external parasitic species in the families Ammotheidae, Phoxichilidiidae, and Callipallenidae. External parasites are shown for Nymphonidae, Endeididae, and Pycnogonidae. Parasitic habits are unknown for the families Colossendeidae, Austrodecidae, and Rhynchothoraxidae. Many of the associations shown are with cnidarians, but echinoderms and molluscs are also common pycnogonid hosts.

The majority of species in the Phoxichilidiidae and the Ammotheidae have parasitic larvae including many endoparasites. Lebour (1947) reviewed the habits of many *Phoxichilidium* and *Anoplodactylus* species that have parasitic larval stages in cnidarians. *Anoplodactylus petiolatus* larvae inhabit polyps of *Campanularia flexuosa* and *Syncoryne* sp. from eight to twelve days before molting and leaving the host. *A. pygmaeus* were reared in the gastrovascular cavities of *Obelia* sp. (King 1973). *Anoplodactylus* sp. can also be found in *Sertularia* polyps in Bermuda (Russel and Hedgpeth 1990). *Phoxichilidium femoratum* larvae have been found in the gastrovascular cavities of *Syncoryne* and *P. tubulariae* in *Tubularia larynx*. *P. virescens* was found in *Coryne* sp. (King 1973).

Endeidae and *Tanystylum* contain species that are external parasites on hydrozoans. Pycnogonidae contains members with external parasites on anemones. *Pallenopsis* (*Bathyallenopsis*) in the family Callipallenidae contain parasites of bathypelagic

scyphomedusae throughout their life-cycle (Child and Harbison 1986). Larvae of an *Ammothea* species have been observed clinging with chelifores to the tentacles and subumbrella of Japanese hydromedusae, and these stages have been described in detail (Okuda 1940). Child and Harbison (1986) described an association between a mesopelagic scyphomedusa *Periphylla periphylla* and adult and juvenile specimens of the pycnogonid *Pallenopsis (Bathypallenopsis) scoparia*. They were found clinging to the subumbrellar surface. It appears all members of this subgenus are parasitic on midwater cnidarians. Almost nothing is known concerning life histories of members of the family Colossendeidae (Arnaud and Bamber 1987). *Ascorhynchus endoparasiticus* has been documented parasitic in the pallial cavity of *Scaphander punctostriatus* from the Azores. It has been suggested that they feed on the rectal contents of the host (Arnaud 1978).

Some species have been shown to parasitise molluscs. *Nymphon parasiticum*, a member of the Family Nymphonidae, has a larval stage that is an external parasite on the foot and cephalic hood of the nudibranch *Tethys leporina* (Arnaud 1978). *Nymphonella tapetis* was described infesting the mantle cavities of two Japanese venerid bivalves (Ohshima 1927). Ohshima (1933) described two species of *Ammothea* as parasites on and in the nudibranch *Armina variolosa*. Stock (1953) found *Ascorhynchus* sp. on the gills of the nudibranch *Aplysia benedicti*. Benson and Chivers (1960) showed an association between *Achelia chelata* and the mussel *Mytilus californianus*. Up to twenty-one parasites of all life history stages of both sexes were found per host. They showed that the pycnogonid

destroyed the soft parts of the mussel including ctenidia and gonads, however, this pycnogonid is also found free living and apparently is not an obligatory parasite.

Ammothea hilgendorfi larvae were found in large galls derived from hydranths of the hydroid *Eucopella everta* (Russel and Hedgpeth 1990). *A. hilgendorfi* was also thought to be a possible ecto-parasite of a holothurian by Ohshima in 1927 (Russel and Hedgpeth 1990). Jarvis and King (1972) described *Pycnogonum littorale* juveniles as ectoparasites on *Tealia felina* in Ireland. They underwent seven larval instars before the metamorphosis to the adult form. Size increased consistently with each molt. They have also been found with the proboscis inserted in *Clava* sp. polyps. Hilton (1934) found *Pycnogonum stearnsi* as ecto-parasites on the anemone *Cribrina xanthogramica* (=*Anthopleura*) and some species in the gastrovascular cavities of *Syncoryne* spp. in Friday Harbor.

Tanystylum duospinum is an ectoparasite. It's larvae can be found attached to hydroids with their chelifores. Threads from the cement glands also aid in attachment to the host. These larvae feed by sucking fluid from the coelenteron and then after a while switching directions and forcing material back into the coelenteron. This species co-occurs with an *Ammothea* species that is an endoparasite. Both species feed on the gut contents of *Eucopella everta* (Russel and Hedgpeth 1990). It is still debated whether multiple species parasitise a single hydranth. Russel and Hedgpeth (1990) described associations of two species of larval pycnogonids, *A. hilgendorfi* and *T. duospinum*, which use different strategies (endo and ecto parasites respectively), with basically the same suctorial

feeding methods. They appear to divide the hydroid colony into non-overlapping resources and thus avoid larval competition.

Direct development from egg to adult is present in a few species of *Pallene* and *Nymphon*, but most species have a protonymphon. *Pycnogonum* protonymphons have long spines and sticky filaments for attachment. *Anoplodactylus* and *Phoxichilidium* do not have cement glands in the chelifores (King 1974). Larvae from species with little yolk leave the ovigers while species with more yolk tend to remain on the ovigers for longer periods of time (King 1973). The development is typically a basic arthropod anamorphic type where larvae hatch with few segments and add segments sequentially after hatching. Sometimes males can be observed carrying larvae but they are typically deposited as eggs or early protonymphons (Jarvis and King 1978).

There are many internal and external parasitic species in the families Ammotheidae, Phoxichilidiidae, and Callipallenidae. Many callipallenids use scyphomedusae as hosts. Ammotheids are found on and in a variety of hosts including cnidarians, echinoderms, and molluscs. Phoxichilids mostly use hydrozoans as hosts. External parasites are shown for Nymphonidae, Endeididae, and Pycnogonidae. Parasitic habits are unknown for the families Colossendeidae, Austrodecidae, and Rhynchothoraxidae.

DISCUSSION

There are three basic pycnogonid larval modes (Bain 1992). The typical protonymphon hatches and as the body elongates

walking legs are added one pair at a time. Examples include *Tanystylum, Pycnogonum, Nymphon*, and most Ammotheids. The attaching larva with no larval appendages also adds legs one pair at a time. Examples include *Propallene, Austropallene,* and callipallenids. The third type was called an atypical protonymphon where the larva hatches as a protonymphon but then at first molt all limb buds for walking legs appear at once. Examples include *Nymphonella, Ammothea, Phoxichilidium,* and *Nymphon*. It seems likely that the atypical mode is derived to speed development in or on a host.

It is postulated that the cause of this internal parasitism is the ingestion by active polyps. Hydroid cell material has been found in these pycnogonid guts (Arnaud and Bamber 1987). Pycnogonids may use an endo-parasitic larval stage as a way to hide their identity, making the hydroid unable to recognize them as predators so they can avoid being attacked as adults. However, nematocysts do continue to fire on larval and adult pycnogonids.

Pycnogonids have used cnidarians as larval hosts since their early evolution, although this association most likely began as an external parasite and the internalization evolved separately. This semi-parasitic life history has evolved multiple times within the Pycnogonida indicating it is a polyphyletic trait. This study also determined brooding of egg masses is a monophyletic trait within the Pycnogonida. The ancestral stock that led to the extant pycnogonids were most likely external parasites on their host. The internalization of the larval stages appears to have happened at least twice, once in the Ammotheidae and another in the Phoxichilidiidae. Therefore, it appears parasitism in general is plesiomorphic, but internal

parasitism is a polyphyletic trait. This plesiomorphic condition of the evolution of larval parasitism is shown on figure 5.1. These summary trees are a synthesis of the trees presented in chapters 1 and 2. An overview of the evolution of larval parasitism in the Pycnogonida is then overlaid on this summary phylogeny.

GENERAL CONCLUSIONS

Eurypterids appear to have been the ancestral chelicerate stock that led to the extant taxa including the Pycnogonida, Xiphosura, and Arachnida (see chapter 1). Although molecular phylogenies did not include the Eurypterida the resulting phylogenies were consistent with this hypothesis. There is an abundance of evidence to indicate pycnogonids are chelicerates, a sister taxon to the living arachnids, and xiphosurans (see chapter 1). The evolutionary relationships between the extant chelicerates and the eurypterids are still unclear and were beyond the scope of this effort.

The morphological analysis presented here (see chapter 1, figure 1.13) supported the Nymphonidae as the most basal pycnogonid family. This is the group thought to be most primitive in much of the literature as well because they have a full complement of chelicerate appendages. The Ammotheidae was found to be paraphyletic. This is not too surprising, considering it is the family with the highest degree of morphological variation. Many ammotheids also resemble the H. A. P. (Hypothetical Ancestral Pycnogonid) (figure i) in many morphological characters. The morphological analysis continues by dividing the remaining pycnogonids into two clades. The first of these two clades contains the Callipallenidae, and Phoxichilidiidae. This clade shares many characters including the reduction of palps while maintaining chelate chelifores. Both of these families show derived developmental

modes as well (see chapter 5). The second clade contains the remaining ammotheids, the Colossendeidae, Austrodecidae, Rhynchothoraxidae, Pycnogonidae, and Endeididae. The molecular phylogenetics of the Pycnogonida (see chapter 2) also supported a paraphyletic Ammotheidae. However, based on 28S sequence results, the Nymphonidae was a derived family and the Ammotheidae was the most basal family. Since the Ammotheidae is paraphyletic and the most morphologically variable family it is likely some portion of this family including the genus *Achelia* is basal to the whole Pycnogonida. The Nymphonidae are morphologically more uniform than the Ammotheidae and are unlikely ancestral.

This study comparing morphological and molecular phylogenetics of the Pycnogonida did not completely answer all questions regarding pycnogonid evolutionary relationships. The morphological and molecular trees did not agree in every detail. While the D3 region of 28S rDNA is a good molecule for evolutionary studies, more research is needed to complete this puzzle. It would be very interesting to see how these results compare with sequence data from other genes. There were also a few gaps in the species available for sequencing. For example no callipallenids, austrodecids, nor rhynchothoraxids were sequenced. Despite the limitations, this analysis of pycnogonid evolutionary relationships using morphological and molecular data was successful in determining aspects of the pycnogonid Bauplan. The H. A. P. (Hypothetical Ancestral Pycnogonid) had a full complement of chelicerate appendages including chelate chelifores, palps, and ovigerous appendages on both sexes. It was also successful in creating trees for

evaluating the evolution of pycnogonid life history traits (see chapter 5). The molecular and morphological trees presented in this study were also basically consistent with Hedgpeth's view of familiar organization.

Phoxichilidium tubulariae Lebour 1947 is not a junior synonym of *Phoxichilidium femoratum* (Rathke 1799). Chapter 3 provides support that *P. tubulariae* is a valid species. Chapter 4 describes the life history of this animal in detail. The annual population dynamics are seasonal. Density and reproduction is highest in the late summer and early fall. Some populations have two generations during the year and adult migration may play an important role in maintaining these dynamics.

The male *Phoxichilidium tubulariae* broods the eggs until they hatch as protonymphons. These larvae then infect the host hydroid, *Tubularia larynx*. They develop quickly, with a reduced number of molts. The decreased development time is adapted to exploit the seasonal abundance of their hydroid hosts.

There are three basic pycnogonid larval modes (Bain 1992). The typical protonymphon hatches and as the body elongates, walking legs are added one pair at a time. Examples include *Tanystylum, Pycnogonum, Nymphon*, and most Ammotheids. The attaching larva with no larval appendages also adds legs one pair at a time. Examples include *Propallene, Austropallene*, and callipallenids. The third type was called an atypical protonymphon where the larva hatches as a protonymphon but then at first molt all limb buds for walking legs appear at once. Examples include *Nymphonella,*

Ammothea, Phoxichilidium, and *Nymphon*. It seems likely that the atypical mode is derived to speed development in or on a host.

Ellsworth Dougherty proposed a "working hypothesis" relating to evolutionary "ideas" (Dougherty 1963), and in doing so foreshadowed many concepts revolutionary to the current trend in studying the evolution of development. This concept is directly relevant to the evolution of parasitic larva in the Pycnogonida. Parasitic lifestyles are a popular "idea" and have evolved on many occasions in metazoans. It appears external parasitic larvae are plesiomorphic in the Pycnogonida, but the internalization of the larval stages has occurred in at least two separate occasions, the first within the Ammotheidae and again in the Phoxichilidiidae. The parasitic life histories present in the aquatic mites also seem to have evolved separately and probably more than once.

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Table 1.1 Summary of pycnogonid classification

Phylum Arthropoda

Subphylum Cheliceriformes

Class Pycnogonida

Order Palaeopantopoda Broili fossils

Order Pantopoda Gerstaecker living

Family	Genera	Species	Chelifore	Palp	Oviger
Ammotheidae Dohrn, 1881 (including Tanystylidae Schimkewitsch, 1913)	27	318	usually achelate	4-10 segments	M, F 9-10 segments
Austrodecidae Stock, 1954 thin annulated proboscis	2	50	none	palps	M, F
Callipallenidae Wilson, 1878 (common in the tropics and rare in polar) (previously Pallenidae but this was a preoccupied name)	25	198	chelate	reduced 3 or 4 segments or absent	M, F 10 segments compound spines
Colossendeidae Hoek, 1881 2 polymerous spp.	6	74	absent or chelate	8-10 segments	M, F 9-10 segments compound spines
Endeididae	1	2	absent	absent	M 7 segments

Family	Genera	Species	Chelifore	Palp	Oviger
Nymphonidae Wilson, 1887 2 polymerous spp.	6	220	chelate	5 segments	M, F 10 segments compound spines
Phoxichilidiidae G. O. Sars, 1891 hydroid feeders	5	113	chelate	absent	M 5-9 segments
Pycnogonidae Wilson, 1878 2 pentamerous spp. external parasites on	2 anemones	53	absent	absent	M 6-9 segments no spines
Rhynchothoracidae Fry, 1978 (found in sand)	1	20	absent	4-6 segments	M, F 4-6 segments

Table 1.2 Key to pycnogonid families

1. Chelifores lacking or with vestigial chelae.....2
1. Chelifores with functional chelae.....7

2. Chelifores lacking.....3
2. Chelae vestigial (except in few genera); palps with 4-10 segments; ovigers 9-10 segments, in both sexes (smaller in female), strigilis small .....Ammotheidae

3. Palps lacking.....4
3. Palps present.....5

4. Without chelifores or palps; ovigers 7 segments, in males only, without strigilis, thin body.....Endeididae
4. Without chelifores or palps; ovigers 6-9 segments, in males only, without strigilis, thick body.....Pycnogonidae

5. Thin annulated proboscis.....Austrodecidae
5. Proboscis lacks annulations.....6

6. Palps and ovigers 9-10 segments; in both sexes.....Colossendeidae
6. Palps and ovigers 4-6 segments; in both sexes...Rhynchothoraxidae

7. Palps lacking or as tiny unsegmented bumps; ovigers 6-segmented, present in males only, without strigilis.....Phoxichilidiidae
7. Palps present, with 1 or 5 segments, or lacking; ovigers usually with 10 segments, in both sexes, usually strong strigilis.....8

8. Palps with 5 segments; ovigers always 10-segmented, with strong strigilis.....Nymphonidae
8. Palps present as single-segmented tubercles, or lacking; ovigers 10-segmented (except *Pallenopsis* females, which sometimes have 9 segments), strigilis weak or strong.....Callipallenidae

Table 1.3 Species used in the cladistic analysis and source information

Family Ammotheidae	
Achelia brevirostris	(Nakamura and Child 1991)
Achelia crurispinifera	Smithsonian
Achelia lagena	(Child 1994)
Achelia orpax	(Nakamura and Child 1983)
Achelia sawayai	(Child 1992b)
Achelia spatula	(Nakamura and Child 1983)
Achelia spinosa	(Hedgpeth 1948)
Ammothea adunca	(Child 1994)
Ammothea appendiculata	(Child 1992b)
Ammothea armentis	(Child 1994)
Ammothea dorsiplicata	(Child 1992a)
Ammothea glacialis	Smithsonian
Ammothea gordonae	(Child 1994)
Ammothea heterosetosa	(Child 1992a)
Ammothea insularis	(Child 1992a)
Ammothea sextaticulata	(Child 1994)
Ammothea spicula	(Nakamura and Child1983)
Ascorhynchus athernum	(Child and Nakamura1982)
Ascorhynchus comatum	(Child 1992a)
Ascorhynchus crenatum	(Child 1992b)
Ascorhynchus cuculum	(Child and Nakamura1982)
Ascorhynchus fusticulum	(Nakamura and Child 1983)
Ascorhynchus glaberrimum	(Nakamura and Child 1983)
Ascorhynchus horologium	(Child 1992b)
Ascorhynchus latipes	(Child 1992b)
Ascorhynchus okai	(Nakamura and Child 1983)
Ascorhynchus paxillum	(Child 1992a)
Ascorhynchus prosum	(Nakamura and Child 1983)
Ascorhynchus serratum	(Child 1992b)
Ascorhynchus simplex	(Nakamura and Child 1991)
Ascorhynchus tuberosum	(Nakamura and Child 1991)
Cilunculus galeritus	(Nakamura and Child 1991)
Cilunculus gracilis	(Nakamura and Child 1991)
Cilunculus haradai	(Nakamura and Child 1983)

Cilunculus sekiguchii (Nakamura and Child 1983)
Cilunculus tubicinis (Child and Nakamura 1982)
Tanystylum birkelandi (Child 1979)
Tanystylum calicirostrum (Child 1979)
Tanystylum cinctum (Child 1992a)
Tanystylum dowi (Child 1979)
Tanystylum mexicanum (Child 1979)
Tanystylum malpelensis (Child 1979)
Tanystylum nabetensis (Nakamura and Child 1983)
Tanystylum oculospinosum (Child 1992b)
Tanystylum orbiculare (Child 1992b)

Family Austrodecidae

Austrodecus breviceps (Child 1994)
Austrodecus calcaricauda (Child 1994)
Austrodecus crenatum (Child 1994)
Austrodecus curtipes (Child 1994)
Austrodecus cestum (Child 1994)
Austrodecus fagei (Child 1994)
Austrodecus glabrum (Child 1994)
Austrodecus glaciale (Child 1994)
Austrodecus macrum (Child 1994)
Austrodecus (Microdecus)*fryi* (Child 1994)
Austrodecus pushkini (Child 1994)
Austrodecus serratum (Child 1994)
Austrodecus varum (Child 1994)
Pantopipetta australis (Child 1994)
Pantopipetta buccina (Child 1994)
Pantopipetta lata (Child 1994)
Pantopipetta longituberculata (Child 1994)

Family Callipallenidae

Callipallene brevirostris (Child 1992b)
Callipallene bullata (Nakamura and Child 1991)
Callipallene panamensis (Child 1979)
Callipallene sagamiensis (Nakamura and Child 1983)
Callipallene solocitatus (Child 1979)
Oropallene dolichodera (Child 1995)
Oropallene metacaula (Child 1995)
Pallenopsis (Pallenopsis) lateralia (Child 1995)
Pallenopsis (Pallenopsis) macronyx (Child 1995)
Pallenopsis (Pallenopsis) notiosa (Child 1992a)

Pallenopsis (Pallenopsis) pilosa	Smithsonian
Pallenopsis (Pallenopsis) schmitti	(Child 1992b)
Pallenopsis (Pallenopsis) truncatula	(Child 1992a)
Pigrogromitus timsanus	(Child 1992b)

Family Colossendeidae

Colossendeis brevirostris	(Child 1995)
Colossendeis concedis	(Child 1995)
Colossendeis elephantis	(Child 1995)
Colossendeis ensifer	(Child 1995)
Colossendeis hoeki	(Child 1995)
Colossendeis media	(Child 1995)
Colossendeis notialis	(Child 1995)
Colossendeis scoresbii	(Child 1995)
Colossendeis scotti	Smithsonian
Dodecolopoda mawsoni	(Child 1995)

Family Endeididae

Endeis nodosa	Smithsonian
Endeis spinosa	(Child 1992b)

Family Nymphonidae

Nymphon aemulum	(Child 1992b)
Nymphon akane	(Nakamura and Child 1983)
Nymphon apheles	(Child 1979)
Nymphon arcuatum	(Child 1995)
Nymphon aritai	(Nakamura and Child 1991)
Nymphon brachyrhynchum	(Child 1995)
Nymphon brevis	(Nakamura and Child 1991)
Nymphon charcoti	Smithsonian
Nymphon chainae	(Child and Nakamura 1982)
Nymphon citerium	(Nakamura and Child 1991)
Nymphon discorsicoxae	(Child and Nakamura 1982)
Nymphon eltaninae	(Child 1995)
Nymphon floridanum	(Child 1992b)
Nymphon forceps	(Nakamura and Child 1991)
Nymphon forticulum	(Child 1995)
Nymphon glabrum	(Child 1995)
Nymphon hadale	(Child and Nakamura 1982)
Nymphon hampsoni	(Child and Nakamura 1982)
Nymphon improcerum	(Nakamura and Child 1991)
Nymphon inferum	(Child 1995)

Nymphon	*infundibulum*	(Nakamura and Child 1991)
Nymphon	*inornatum*	(Child 1995)
Nymphon	*lituus*	(Child 1979)
Nymphon	*longispinum*	(Nakamura and Child 1991)
Nymphon	*macquariensis*	(Child 1995)
Nymphon	*maruyamai*	(Nakamura and Child 1991)
Nymphon	*monothrix*	(Child 1995)
Nymphon	*okudai*	(Nakamura and Child 1991)
Nymphon	*pagophilum*	(Child 1995)
Nymphon	*premordicum*	(Child 1995)
Nymphon	*pumillum*	(Nakamura and Child 1991)
Nymphon	*punctum*	(Child 1995)
Nymphon	*sabellum*	(Child 1995)
Nymphon	*sandersi*	(Child and Nakamura 1982)
Nymphon	*similis*	(Child 1992a)
Nymphon	*simulatum*	(Nakamura and Child 1991)
Nymphon	*spicatum*	(Child and Nakamura 1982)
Nymphon	*tenuimanum*	(Child 1995)
Nymphon	*trituberculum*	(Child 1995)
Heteronymphon	*ponsitor*	(Child and Nakamura 1982)
Pentanymphon	*antarcticum*	(Child 1995)
Sexanymphon	*mirabilis*	(Child 1995)

Family Phoxichilidiidae

Anoplodactylus	*allotrius*	(Child 1979)
Anoplodactylus	*arcuatus*	(Child 1992b)
Anoplodactylus	*batangensis*	(Child 1992b)
Anoplodactylus	*bova*	(Child 1979)
Anoplodactylus	*bruuni*	(Child 1992a)
Anoplodactylus	*californicus*	(Child 1992b)
Anoplodactylus	*carnatus*	(Nakamura and Child 1983)
Anoplodactylus	*dauphinus*	(Child 1992b)
Anoplodactylus	*excelsus*	(Nakamura and Child 1983)
Anoplodactylus	*galetensis*	(Child 1979)
Anoplodactylus	*insignis*	(Child 1992b)
Anoplodactylus	*laciniosus*	(Child 1995)
Anoplodactylus	*lagenus*	(Nakamura and Child 1983)
Anoplodactylus	*lentus*	(Child 1992b)
Anoplodactylus	*lineatus*	(Nakamura and Child 1991)
Anoplodactylus	*maritimus*	(Child 1992b)
Anoplodactylus	*petiolatus*	(Child 1992b)
Anoplodactylus	*pygmaeus*	(Child 1992b)

Anoplodactylus	*reimerae*	(Child 1979)
Anoplodactylus	*speculus*	(Child 1995)
Anoplodactylus	*stellatus*	(Nakamura and Child 1983)
Anoplodactylus	*stri*	(Child 1979)
Anoplodactylus	*tanseii*	(Nakamura and Child 1991)
Anoplodactylus	*velamellus*	(Nakamura and Child 1991)
Anoplodactylus	*vemae*	(Child and Nakamura 1982)
Anoplodactylus	*vulcanus*	(Child 1992a)
Phoxichilidium	*tubulariae*	Personal Observation
Phoxichilidium	*pyrgodum*	(Child 1995)

Family Pycnogonidae

Pentapycnon	*bouvieri*	(Child 1995)
Pentapycnon	*charaoti*	Smithsonian
Pycnogonum	*diceros*	Smithsonian
Pycnogonum	*uedai*	(Nakamura and Child 1983)

Family Rhynchothoraxidae

Rhynchothorax	*architectus*	(Child 1979)
Rhynchothorax	*australis*	(Child 1995)
Rhynchothorax	*barnardi*	(Child 1992a)
Rhynchothorax	*percivali*	(Child 1995)

Order Palaeopantopoda

Palaeoisopus	*problematicus*	(Hedgpeth 1978)

Table 1.4 Character coding used in the cladistic analysis

Character 1-number palp segments:
0, 0-1; 1, 4-8; 2, 9-10

Character 2-palp length vs. proboscis length:
0, palp less than proboscis; 1, palp longer or equal to proboscis

Character 3-palp origin:
0, near oviger; 1, on neck; 2 no palp origin

Character 4-chelifore presence:
0, no chelifore; 1, chelifore present but atrophied; 2, chelifore present

Character 5-number of scape segments:
0, 1, or 2

Character 6-chelae fingers:
0, none; 1, smooth; 2, with teeth

Character 7-chelae fingers:
0, none; 1, meet; 2, overlap

Character 8-size of finger vs. palm:
1, finger equal to palm; 2, finger elongate; 3, palm present but fingers reduced; 0, both absent

Character 9-proboscis shape:
0, pipette shape with annulations; 1, about the thickness of body; 2, stout

Character 10-separation of lateral processes:
0, absent; 1, present

Character 11-pre/post ocular neck:
0, eye posterior to constriction; 1, median eye tubercle; 2, eye anterior to constriction

Character 12-trunk shape:
0, elongate; 1, circular

Character 13-trunk segmentation:
0, absent; 1, present

Character 14-trunk ornamentation:
0, none; 1, median spines

Character 15-opisthosoma shape:
0, rounded; 1, elongate

Character 16-eye tubercle:
0, rounded; 1, tall, elongated, or pointed

Character 17-number oviger segments on male:
0, 0; 1, 6-7; 2, 9-10

Character 18-number oviger segments on female:
0, 0; 1, 6; 2, 9-10

Character 19-compound terminal oviger spines:
0, absent; 1, present

Character 20-oviger terminal claw:
0, absent; 1, present

Character 21-strigilis:
0, absent; 1, present

Character 22-walking leg tarsal shape:
0, stout; 1, elongate

Character 23-accessory claw:
0, absent; 1, present

Character 24-propodal sole spination:
0, homogeneous; 1, heterogeneous

Table 1.5 Morphological matrix used in the cladistics analysis

Nymphon	1	1	1	2	1	2	1	2	1	1	0	0	1	0	1	0	2	2	1	0	1	1	1	0
Heteronymphon	1	1	1	2	1	2	1	2	1	1	0	0	1	0	1	0	2	2	1	0	1	1	0	0
Pentanymphon	1	1	1	2	1	2	1	2	1	1	0	0	1	0	1	0	2	2	1	0	1	1	0	0
Sexanymphon	1	1	1	2	1	2	1	2	1	1	0	0	1	0	1	0	2	2	1	0	1	1	0	0
Colossendeis	2	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	2	2	1	0	1	1	0	0
Dodecolopoda	2	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	2	2	1	0	1	1	0	0
Rhynchothorax	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	0	2	2	0	1	0	0	1	0
Austrodecus	1	1	1	0	0	0	0	0	0	1	1	0	1	1	1	1	1	1	0	0	0	0	1	0
Pantopipetta	1	1	1	0	0	0	0	0	0	1	1	0	1	0	1	1	2	2	0	1	1	0	0	0
Pycnogonum	0	0	2	0	0	0	0	0	1	1	1	1	1	1	1	0	2	0	0	1	0	0	0	0
Pentapycnon	0	0	2	0	0	0	0	0	1	1	1	1	1	1	1	0	2	0	0	1	0	0	0	0
Endeis	0	0	2	0	0	0	0	0	1	1	1	0	1	0	0	0	2	0	0	0	0	0	0	0
Anoplodactylus	0	0	2	2	1	1	2	2	1	1	2	0	1	0	0	0	1	0	0	0	0	0	1	1
Phoxichilidium	0	0	2	2	1	1	2	1	1	1	2	0	1	0	0	0	1	0	0	0	0	0	1	1
Calliopallene	0	0	2	2	1	2	1	1	1	1	0	0	1	0	0	1	2	2	1	0	1	0	1	1
Oropallene	1	0	1	2	1	2	1	1	1	1	0	0	1	0	0	0	2	2	1	1	1	0	1	1
Pallenopsis	0	0	1	2	2	1	1	1	1	1	0	0	1	0	1	0	2	2	1	0	1	0	1	1
Pigrogromitus	0	0	2	2	1	1	1	1	1	1	1	0	1	0	1	0	2	2	1	0	1	0	1	1
Achelia	1	1	1	1	1	0	0	0	1	0	1	1	0	0	1	0	2	2	1	0	1	0	1	1
Ammothea	1	1	1	2	1	1	1	3	1	1	1	0	1	1	1	0	2	2	0	0	1	0	1	1
Ascorhynchus	2	1	1	2	1	1	1	3	2	1	1	0	1	1	1	1	2	2	1	1	1	0	0	0
Cilunculus	1	1	1	1	2	0	0	0	2	1	1	0	1	0	1	1	2	2	1	0	1	0	1	1
Tanystylum	1	1	1	1	1	0	0	0	2	0	1	1	0	0	1	1	2	2	1	0	0	0	1	1
Palaeoisopus	?	1	0	2	1	1	1	1	1	1	1	0	1	0	1	0	2	2	0	0	0	1	0	0

Table 2.1 Organisms sequenced for the molecular analysis

Taxon	Collection Location
Crustacea	
Lithodes maia	New Hampshire, U. S. A.
Pagurus longicarpus	New Hampshire, U. S. A.
Cancer borealis	New Hampshire, U. S. A.
Arachnida	
Phalangium opilio	New Hampshire, U. S. A.
Latrodectus mactans	Dr. Tillinghast laboratory, U. N. H.
Dermaceutor variabilis	New Hampshire, U. S. A.
Omartacarus sp.	New Hampshire, U. S. A.
Xiphosura	
Limulus polyphemus	New Hampshire, U. S. A.
Pycnogonida	
Pycnogonum littorale	Maine, U. S. A.
Endeis spinosa	Arrabida, Portugal
Colossendeis megalonyx	Arrival Heights, Antarctica
Colossendeis robustus	Cape Armitage, Antarctica
Nymphon australe	Granite Harbor, Antarctica
Nymphon grossipes	New Hampshire, U. S. A.
Ammothea gracialis	Cape Evans, Antarctica
Ammothea spinosa	Granite Harbor, Antarctica
Cilunculus sp.	Arrival Heights, Antarctica
Anoplodactylus lentus	Christmas Bay, Texas, U. S. A.
Phoxichilidium tubulariae	New Hampshire, U. S. A.
Achelia chelata	Pt. Argula, California, U. S. A.
Achelia echinata	Arrabida, Portugal

Table 5.1 Summary of pycnogonid associations

Taxon	Host	Source
Family: Ammotheidae		
Nymphonella tapetis	in *Paphia philippinarum*	(King 1973)
Lectythorhynchus hilgendorfi	on *Holothuria lubrica*	(King 1973)
L. marginatus	in *Aglaophenia latirostris*	(King 1973)
Ammothea sp.	in galls in *Coryne* sp. and on the nudibranch *Armina varidosa*	(King 1973)
Achelia alaskensis	in hydromedusae	(King 1973)
Ascorhynchus endoparasiticus	*Scaphander punctostriatus*	(Arnaud 1978)
Family: Nymphonidae		
Nymphon parasiticum	on the opisthobranch *Tethys leporina*	(King 1973)
Family: Callipallenidae		
Pallenopsis (Bathyallenopsis)	bathypelagic scyphomedusae	(Child and Harbison 1986)
Pallenopsis (Bathypallenopsis) *scoparia*	mesopelagic schyphomedusa *Periphylla periphylla*	(Child and Harbison 1986)

Family: Phoxichilidiidae

Anopoldactylus erectus	in *Tubularia* sp.	(King 1973)
A. exiguus	in galls on *Coryne* and *Podocoryne*	(King 1973)
A. pygmaeus	in *Obelia* sp.	(King 1973)
A. petiolatus	in *Campanularia flexuosa* and *Syncoryne* sp.	(Lebour 1947)
Anoplodactylus sp.	in *Sertularia* sp.	(Russel and Hedgpeth 1990)
Phoxichilidium femoratum	in *Syncoryne* sp.	(Lebour 1947)
P. tubulariae	in *Tubularia* sp.	(Lebour 1947)
P. virescens	in *Coryne* sp.	(King 1973)

Family: Endeididae

Endeis spinosus	on *Obelia* sp. medusae and polyps	(King 1973)

Family: Pycnogonidae All external parasites

Families: Colossendeidae, Austrodecidae, and Rhynchothoraxidae Parasitic habits are unknown.

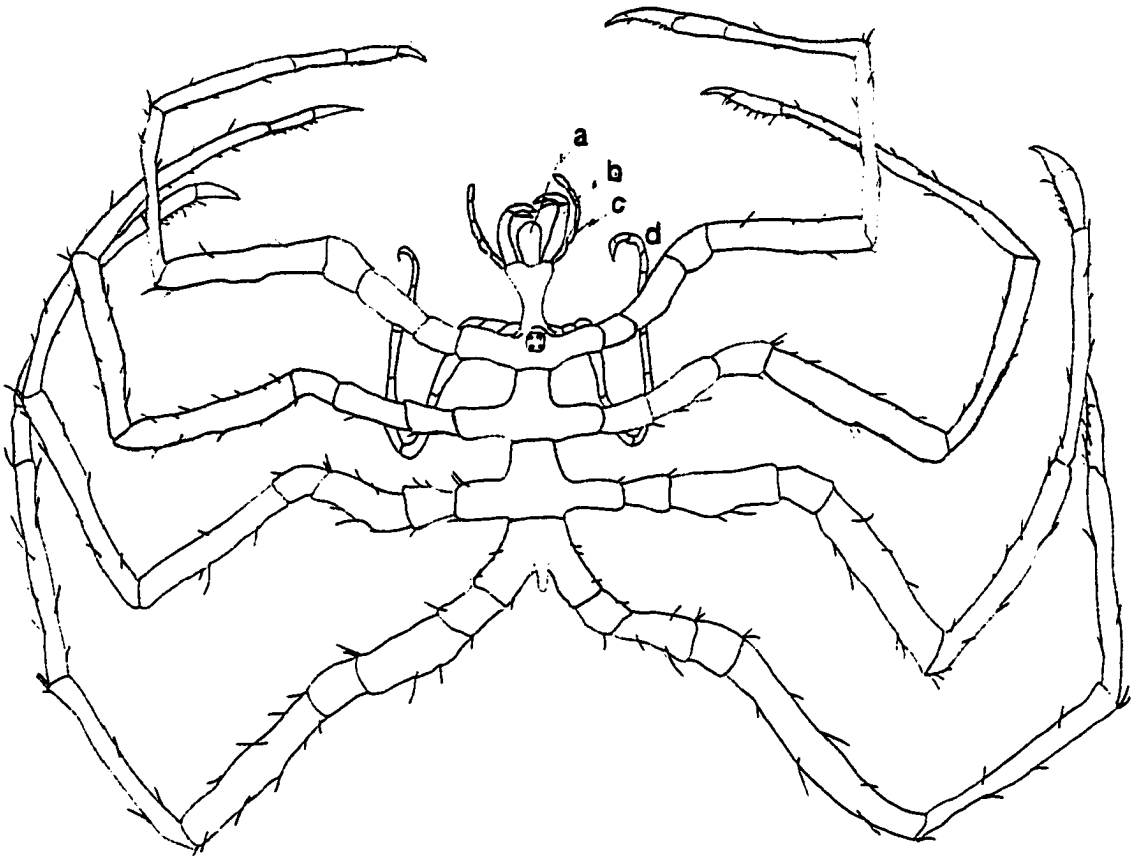


Figure i Diagram of a generalized pycnogonid:
a) proboscis b) chelifore c) palp d) oviger

Figure 1.1a-1.1b Scanning Electron Micrographs of pores in pycnogonid cuticle

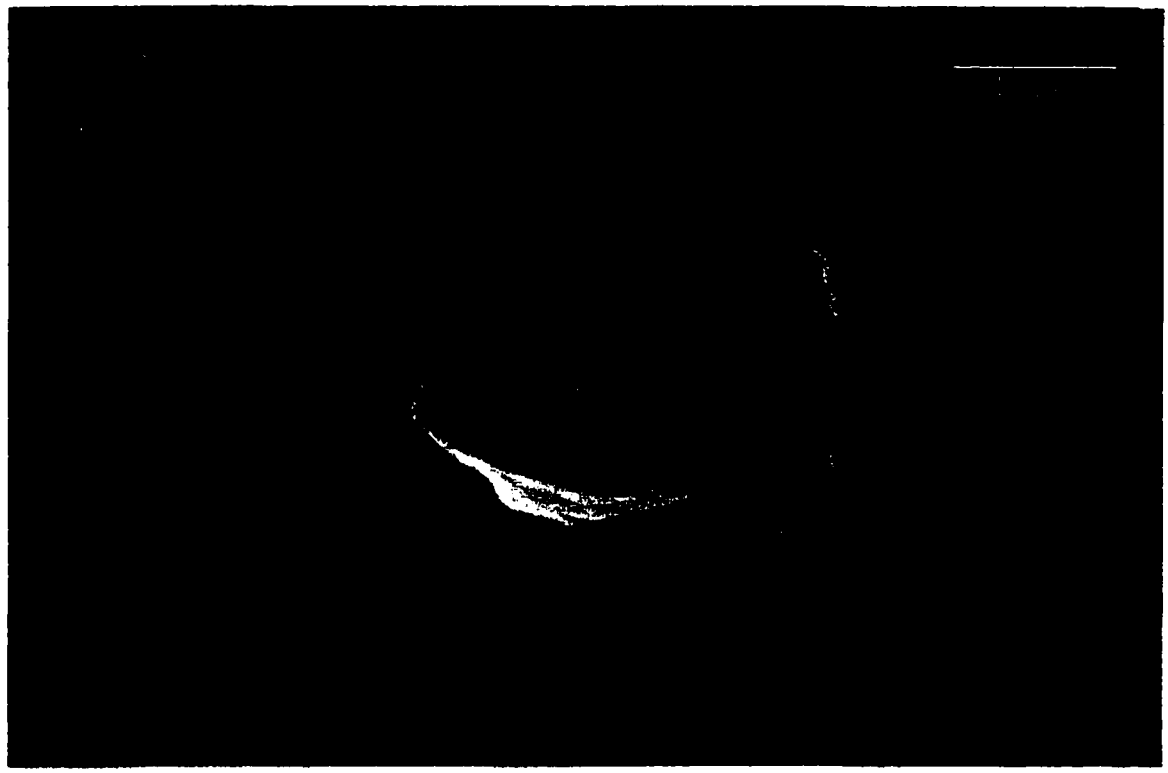


Figure 1.1a

Figure 1.1b

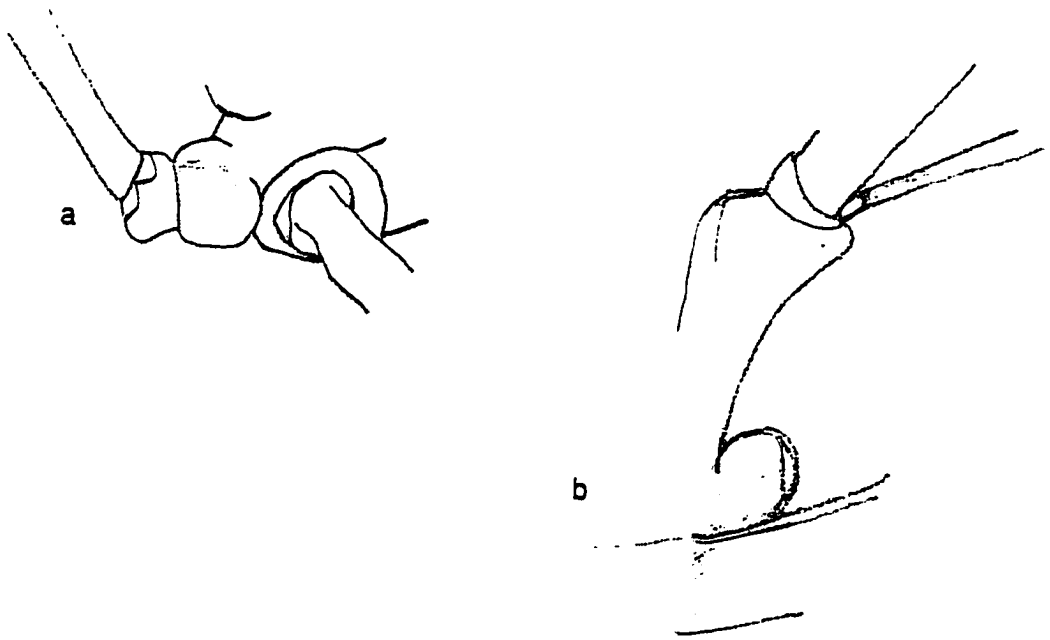


Figure 1.2 a) palp origin near oviger b) palp origin on neck

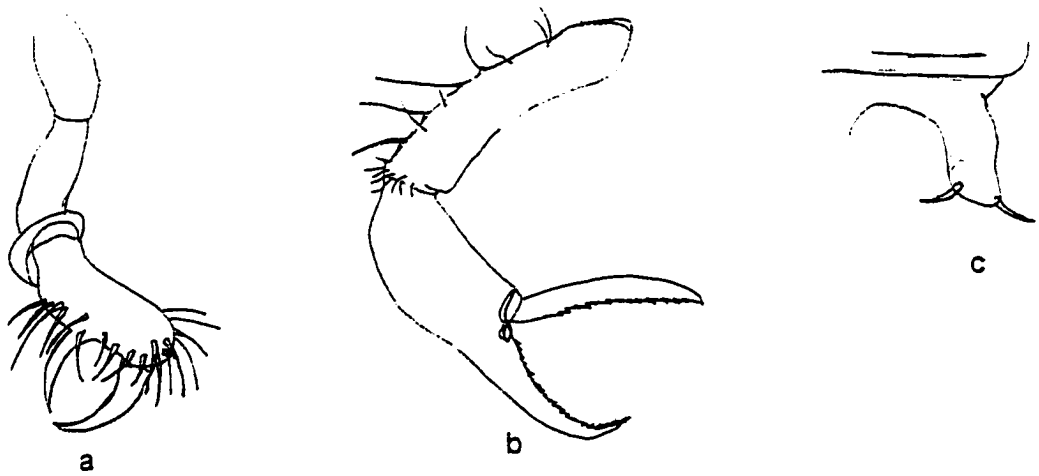


Figure 1.3 chelifore: a) lateral b) anterior c) atrophied

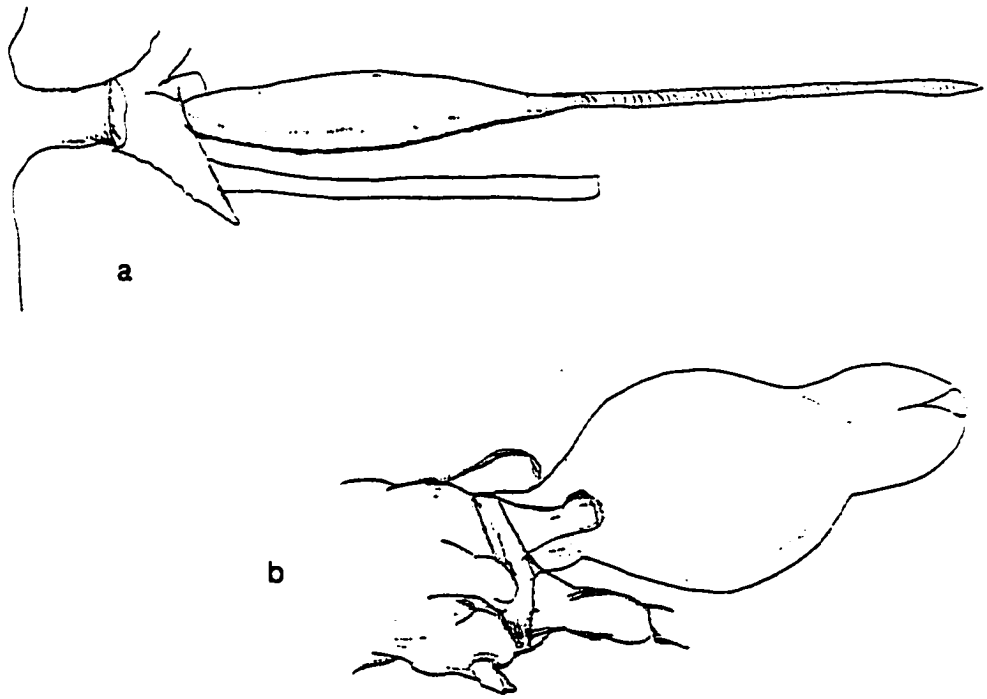


Figure 1.4 proboscis shape: a) pipette shape with annulations b) stout

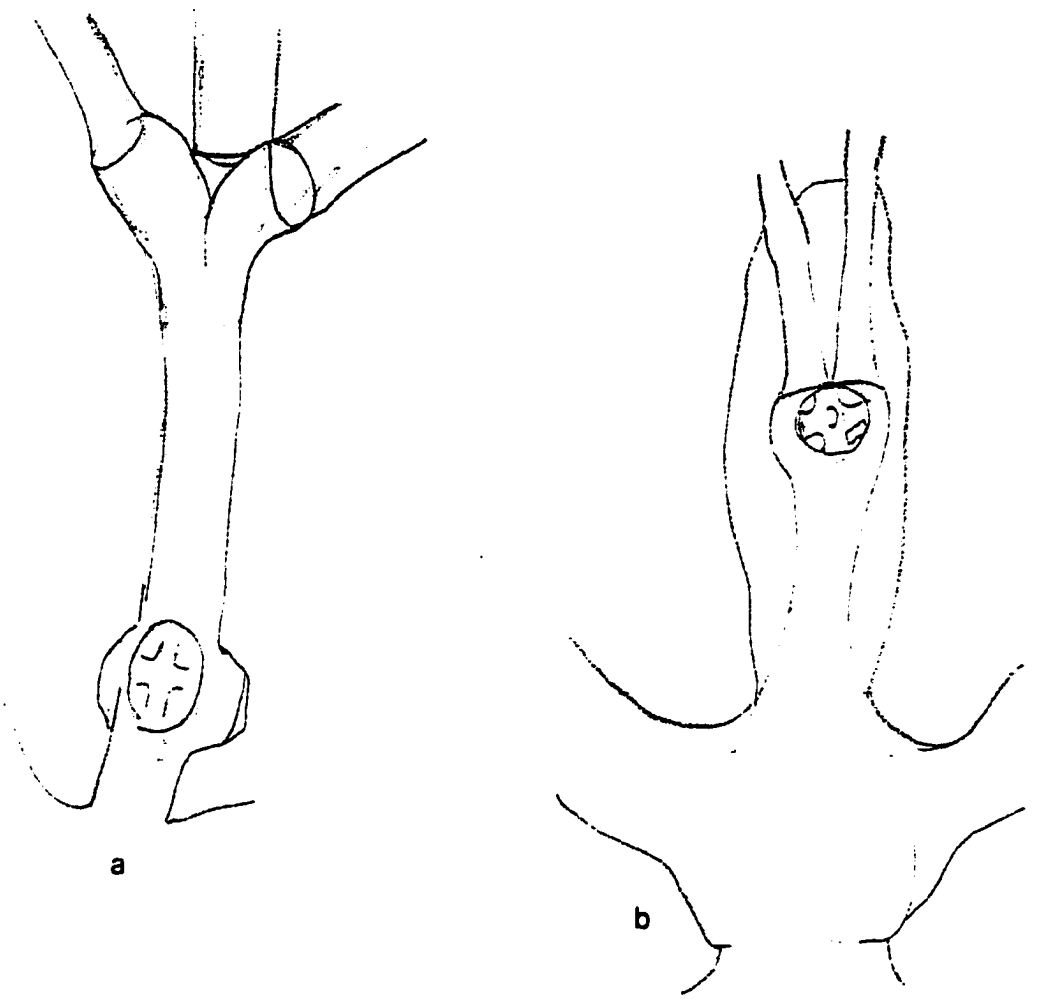


Figure 1.5 a) eye posterior to constriction b) eye anterior to constriction

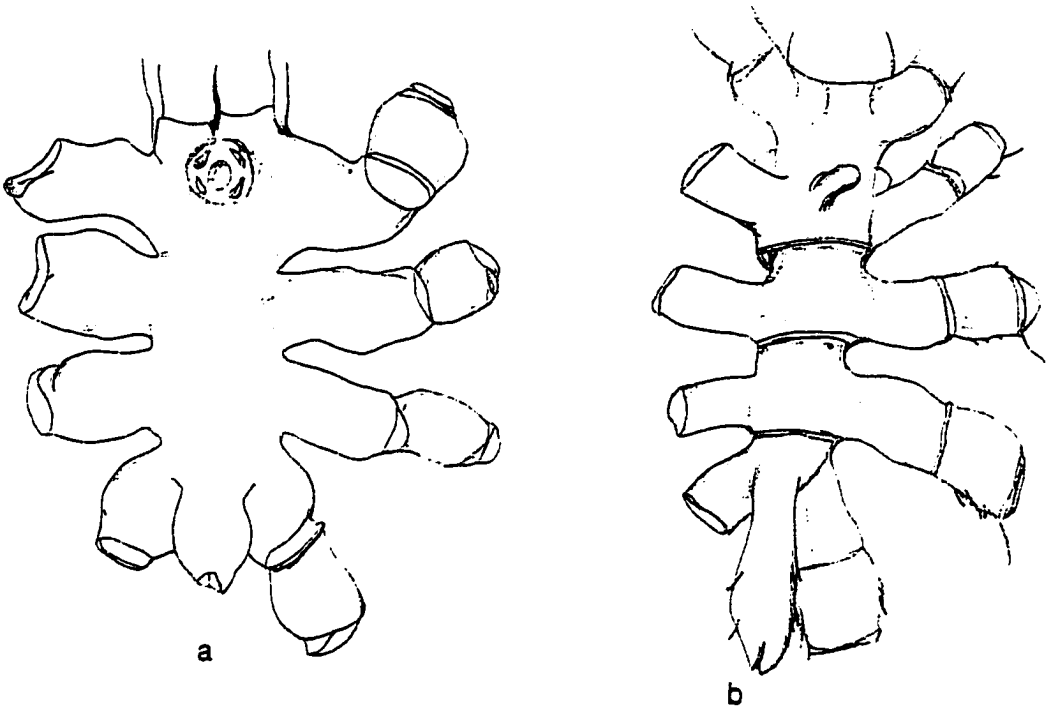


Figure 1.6 a) no trunk segmentation. no ornamentation
b) trunk segmentation

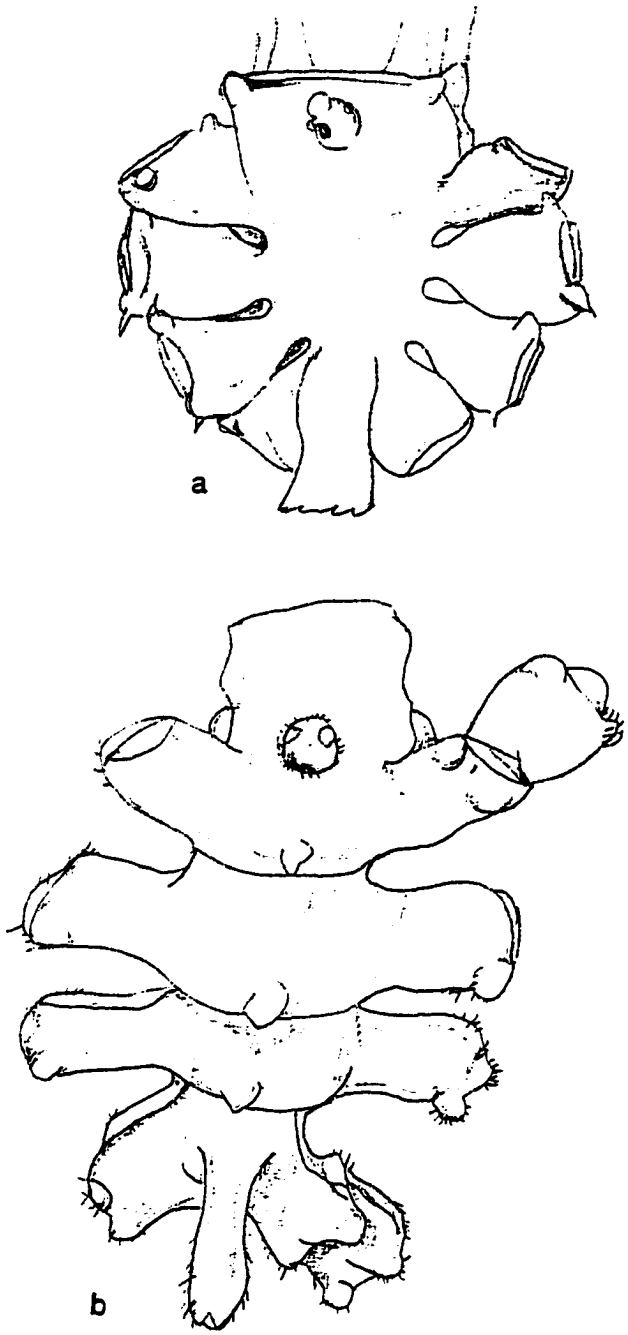


Figure 1.7 a) circular trunk b) elongate trunk with median spines

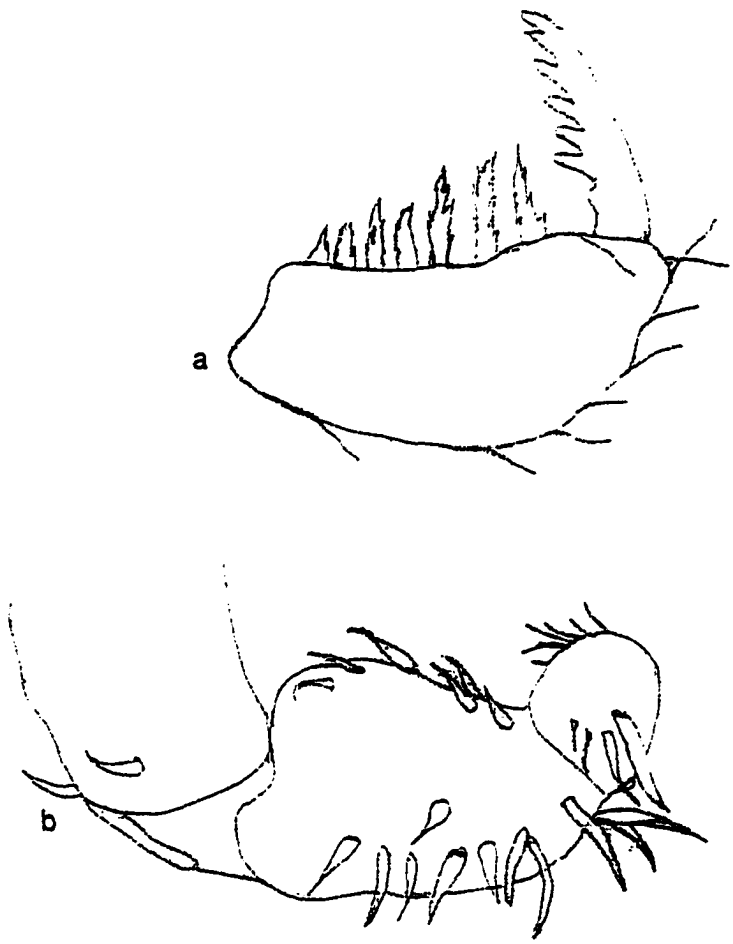


Figure 1.8 a) compound oviger spines with terminal claw
b) simple oviger spines without terminal claw

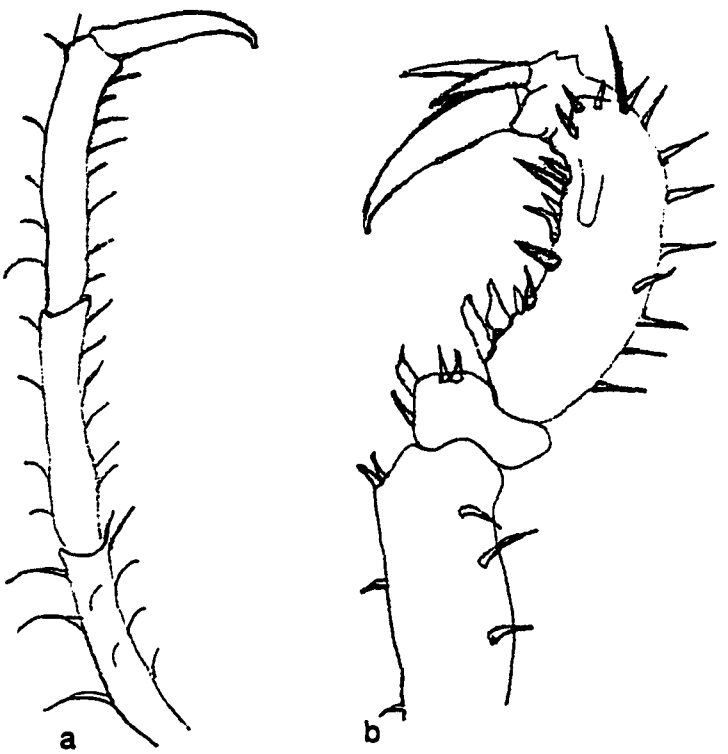


Figure 1.9 a) tarsus elongate, without accessory claws, homogeneous propodal sole spination
b) tarsus stout, with accessory claws, heterogeneous propodal sole spination

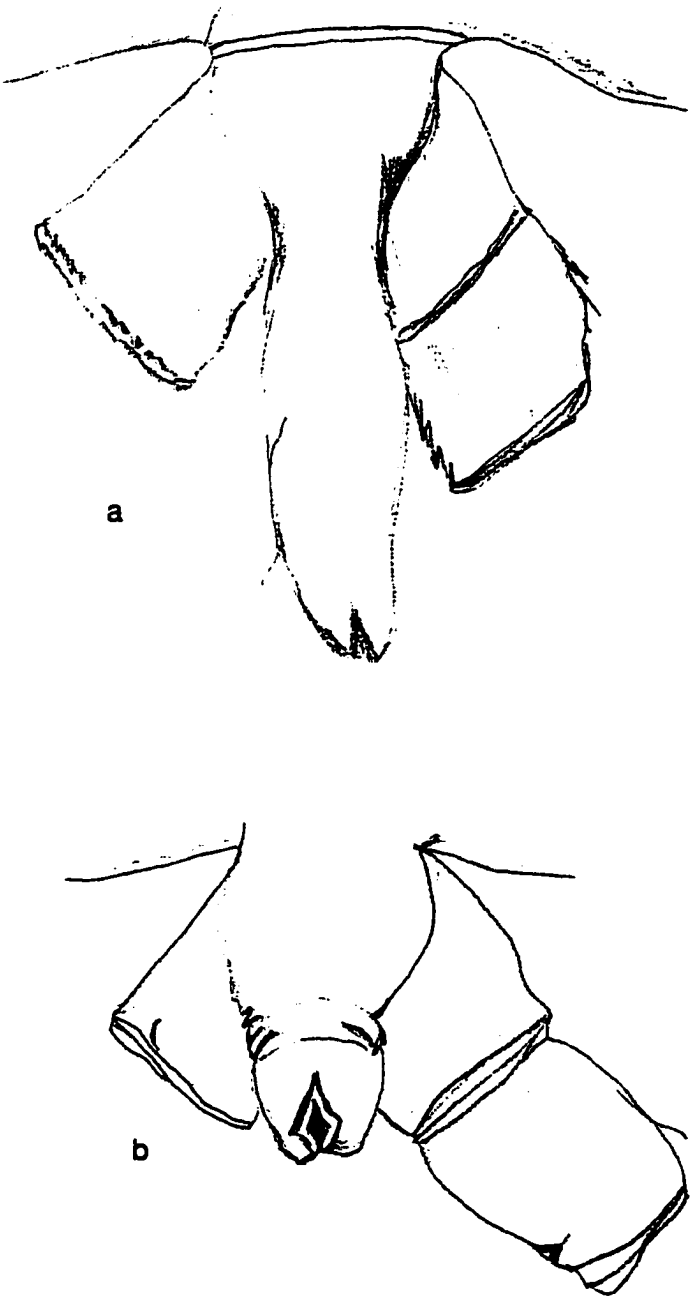


Figure 1.10 opithosoma shape a) elongate b) round

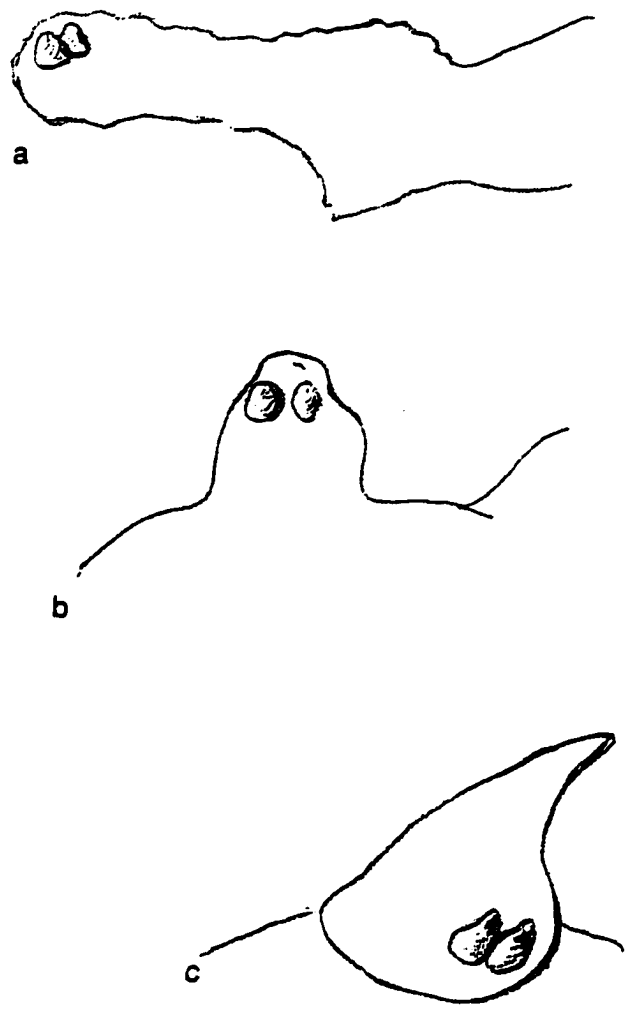


Figure 1.11 eye tubercle a) elongate b) round c) pointed

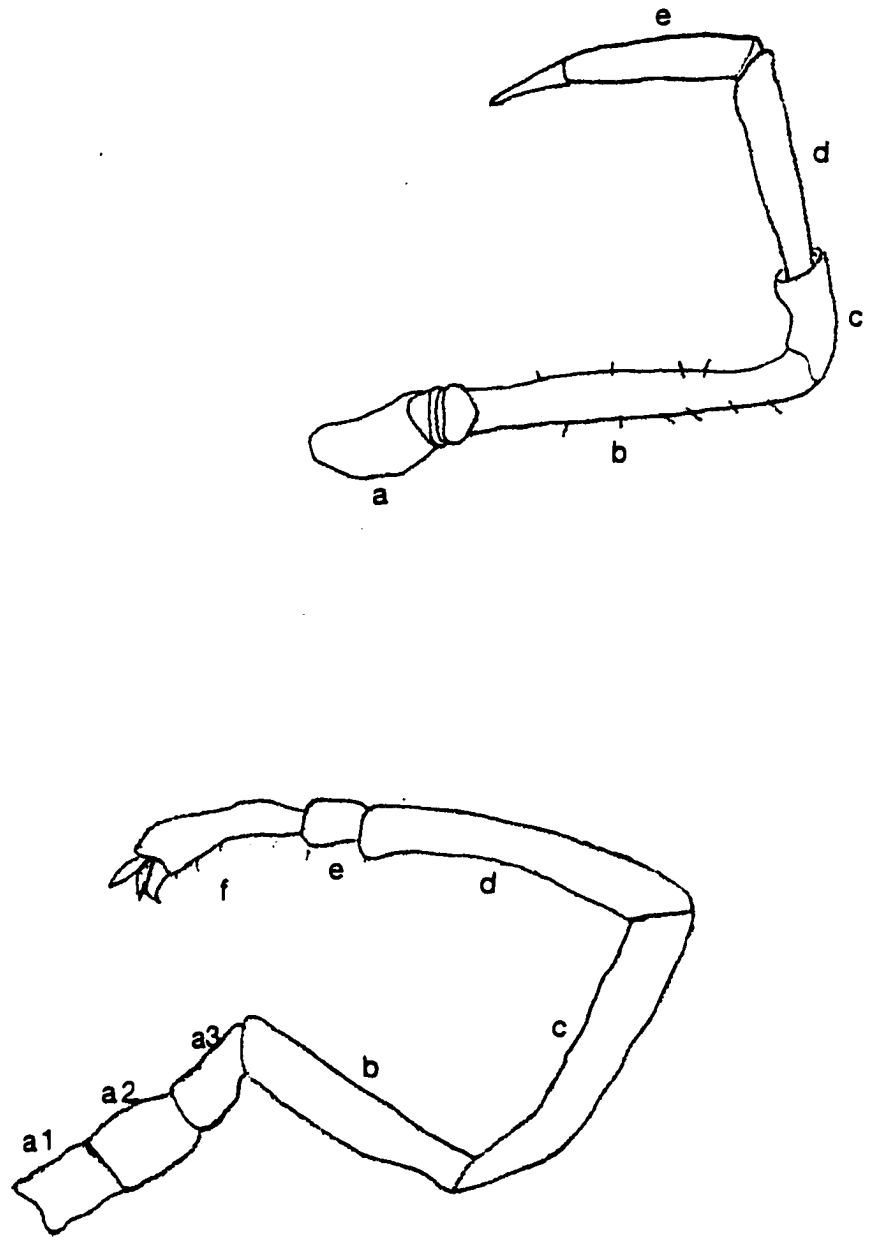


Figure 1.12 pycnogonid leg parts; a) coxa b) femur c) tibia d) tarsus e) propodus vs. arachnid leg parts; a) coxa b) femur c) patella d) tibia e) metatarsus f) tarsus

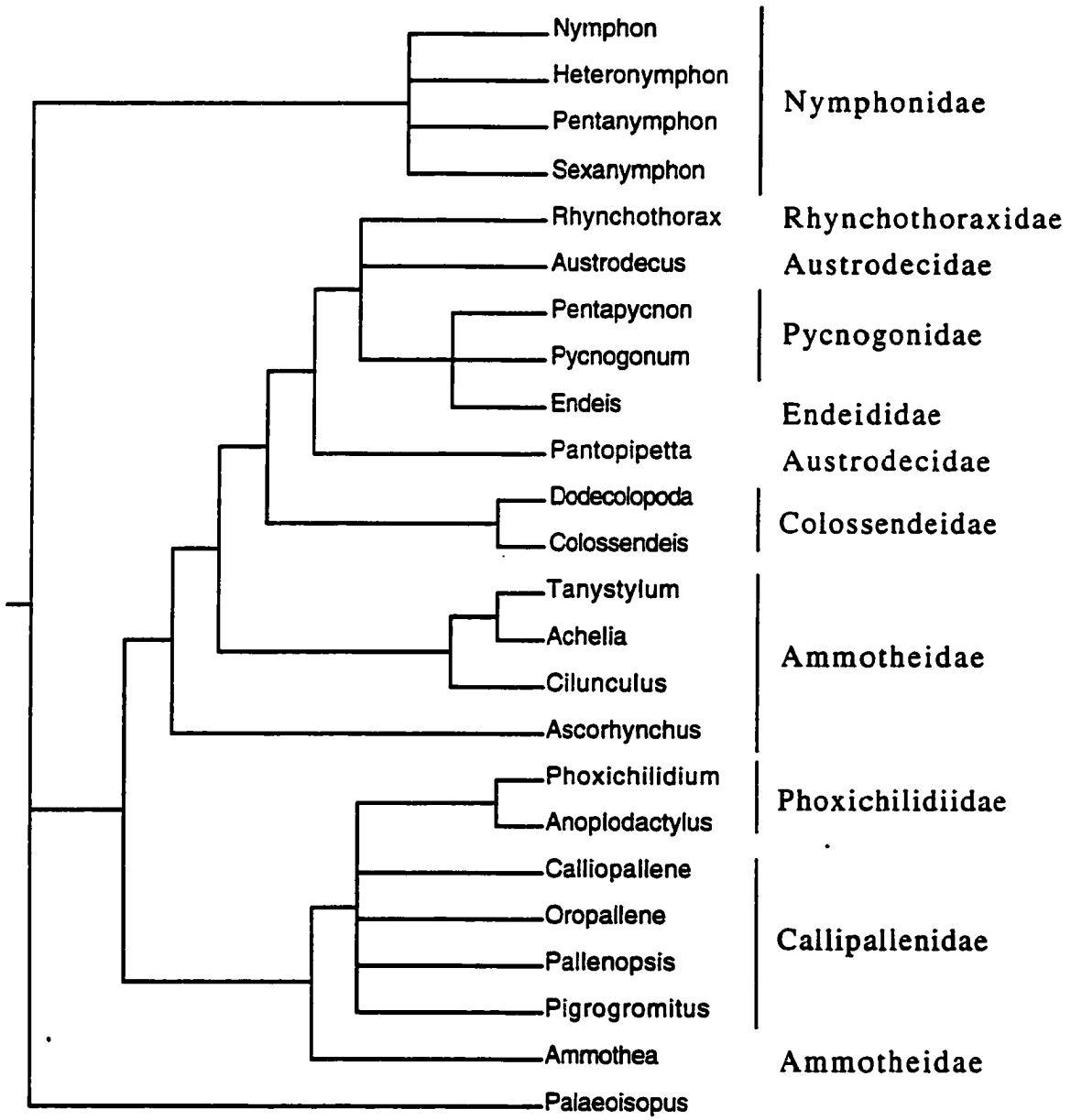


Figure 1.13 Strict concensus of the 15 most parsimonious morphological trees

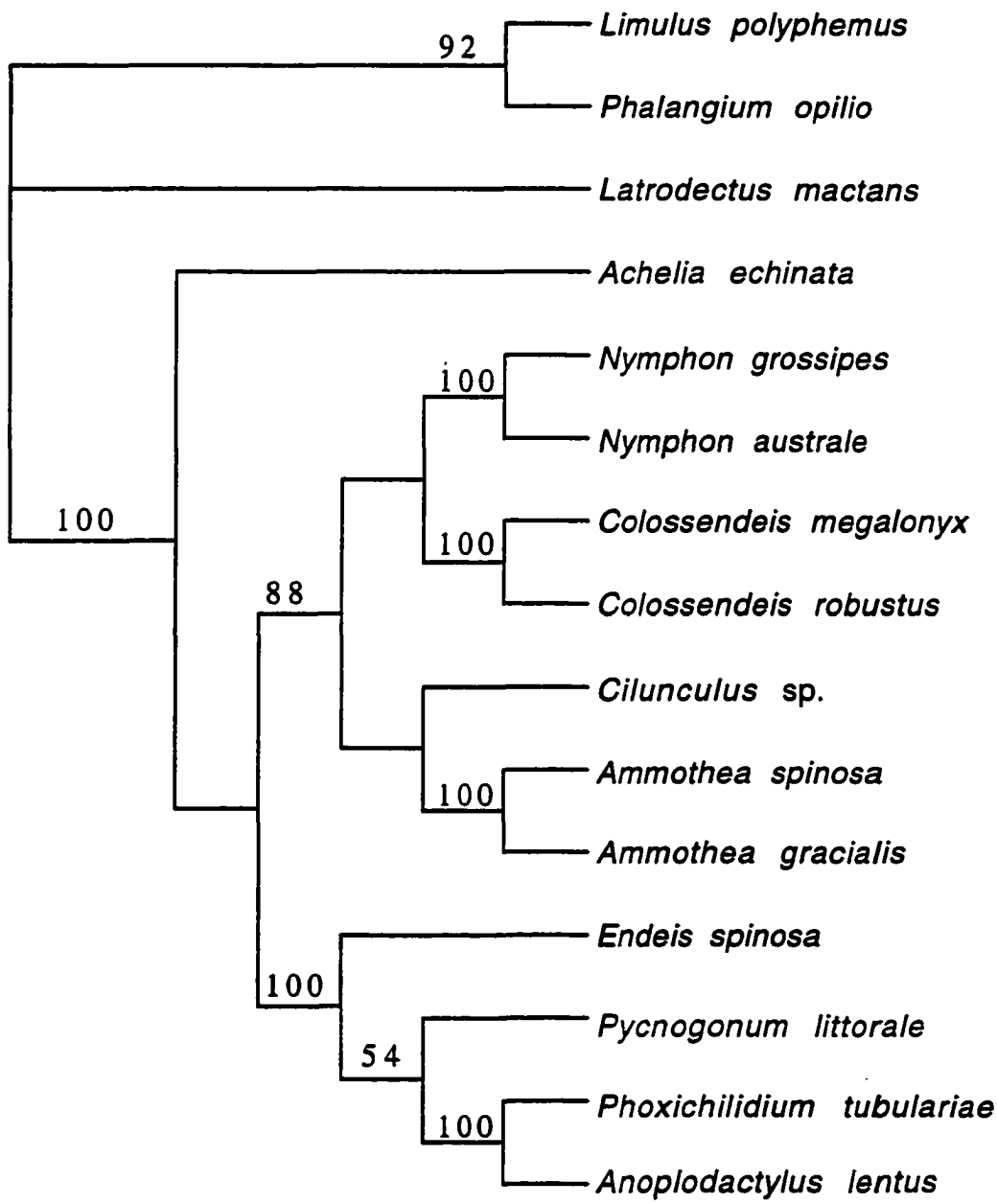


Figure 2.1 Single shortest distance based phylogeny of the Pycnogonida. Numbers represent bootstrap values (1000 replications).

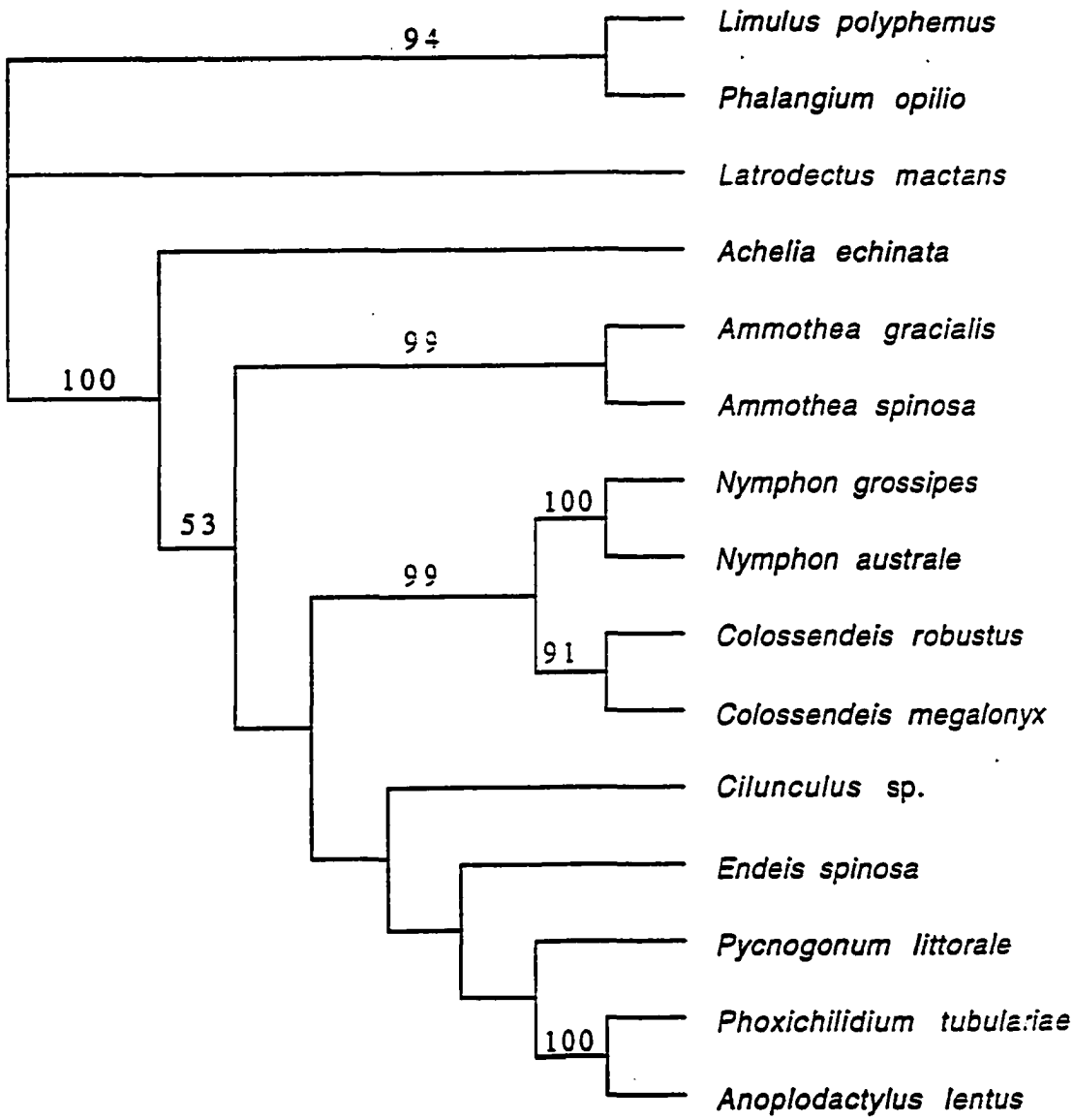


Figure 2.2 Single most parsimonious phylogeny of the Pycnogonida. Numbers represent bootstrap values (1000 replications).

Figure 3.1 Scanning electron micrograph of propodus and claws 146x

Figure 3.1

Figure 3.2 Scanning electron micrograph of chelifores and proboscis

Figure 3.2

Figure 4.1 *Tubularia larynx* hydranth squashed using light microscopy a) 100x b) 400x

Figure 4.1

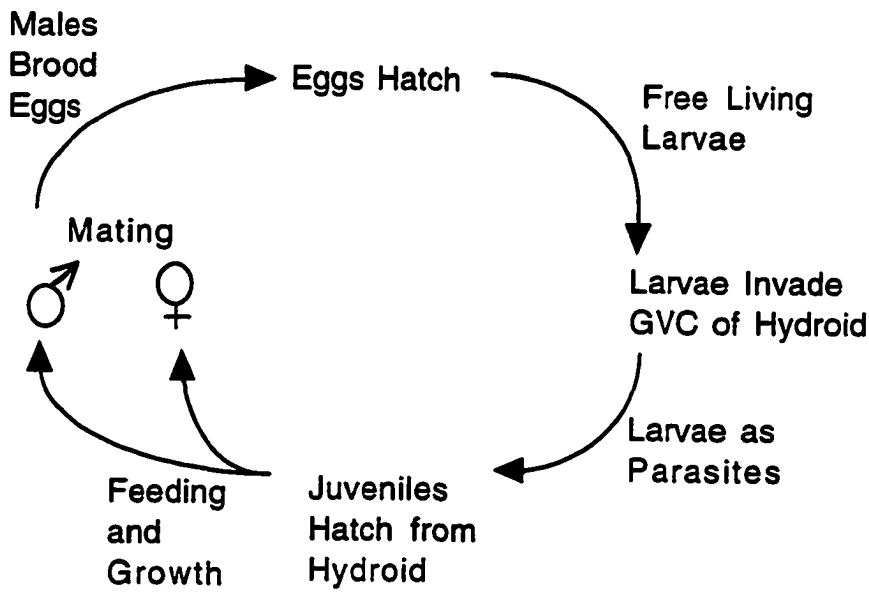


Figure 4.2: Life History of *Phoxichilidium tubulariae*

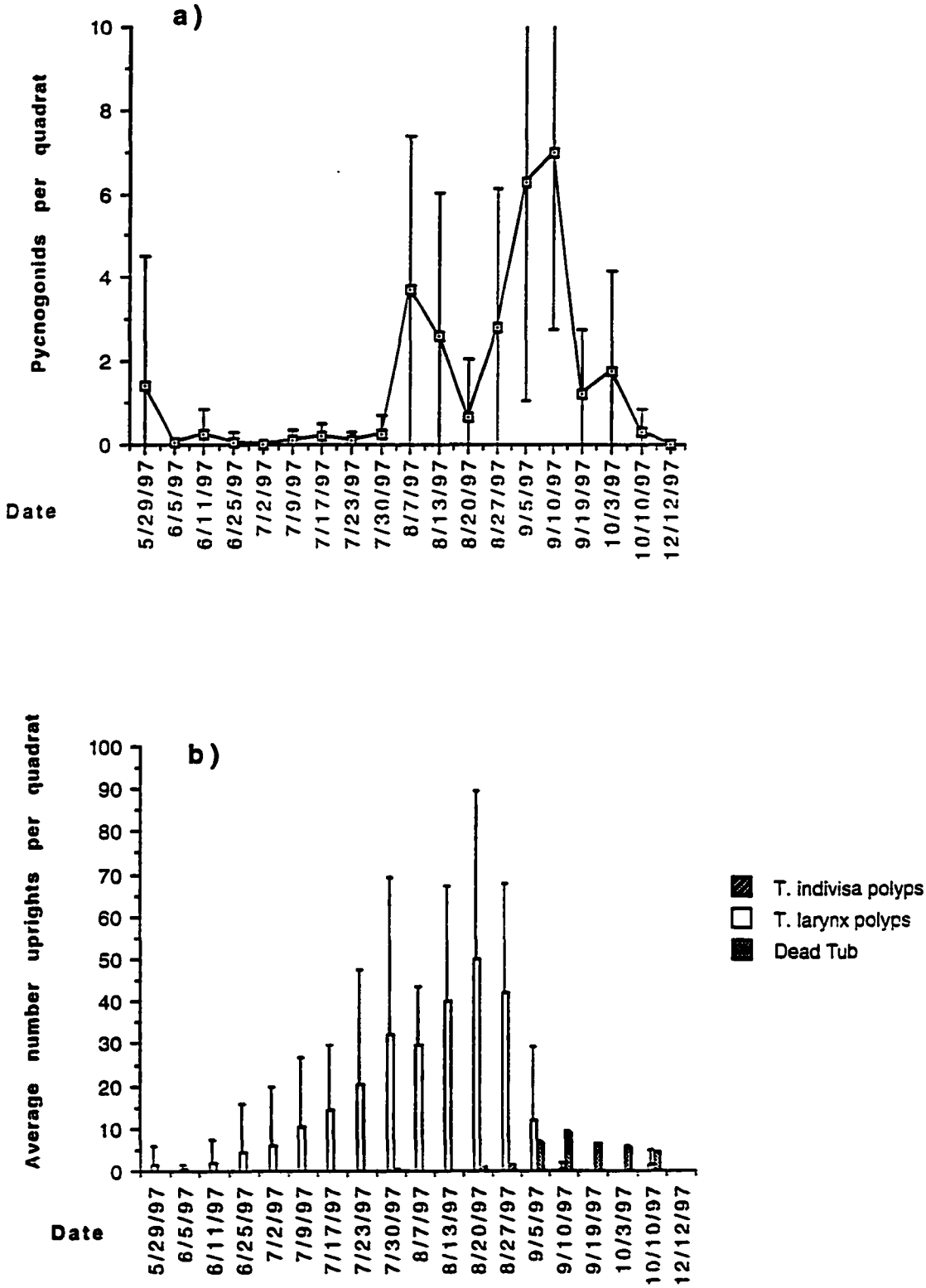


Figure 4.3 Predator/Prey Abundance
Coast Guard Floats 1997

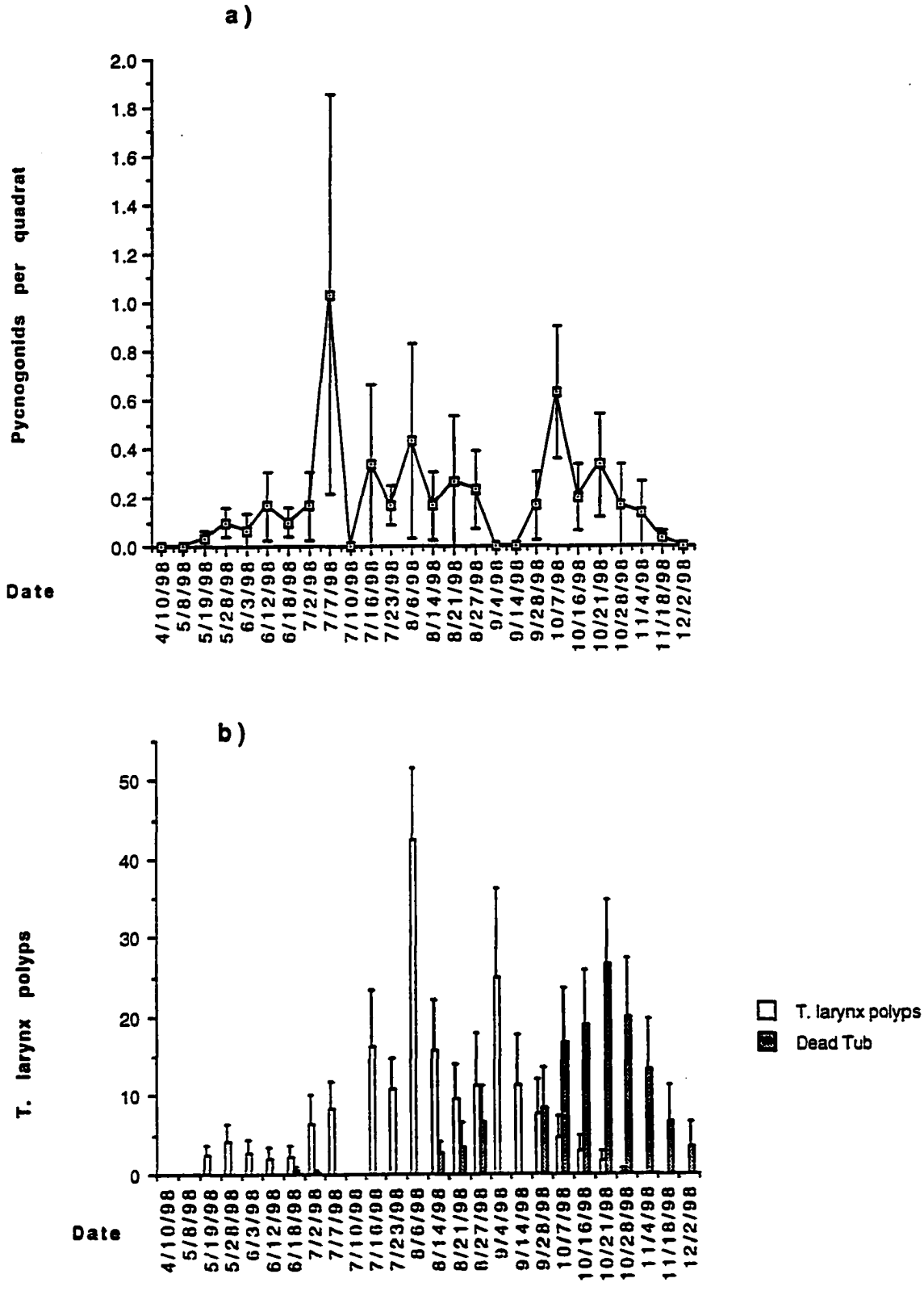


Figure 4.4 Predator/Prey Abundance Coast Guard Floats 1998

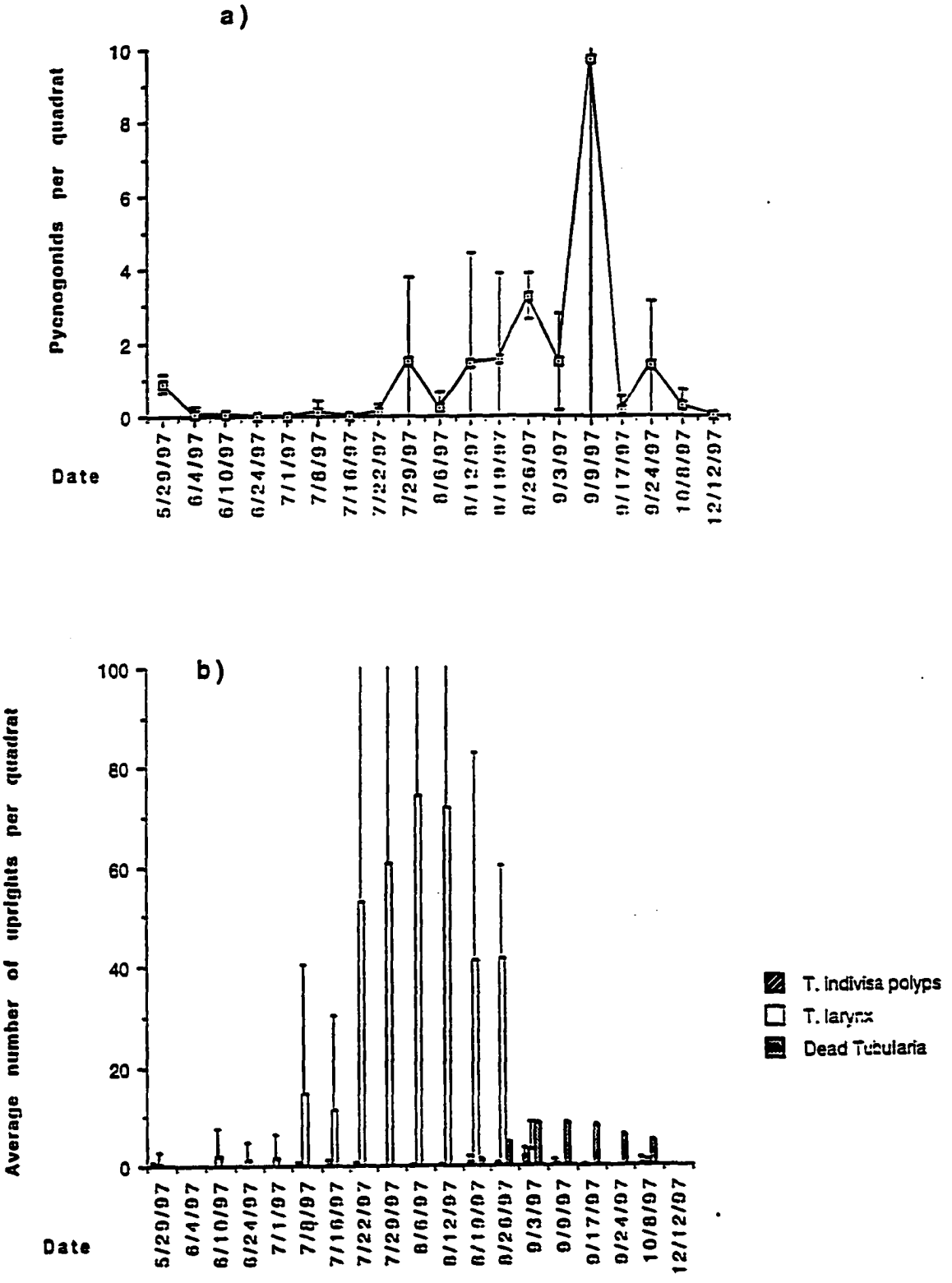


Figure 4.5 Predator/Prey Abundance
Fishing Pier Floats 1997

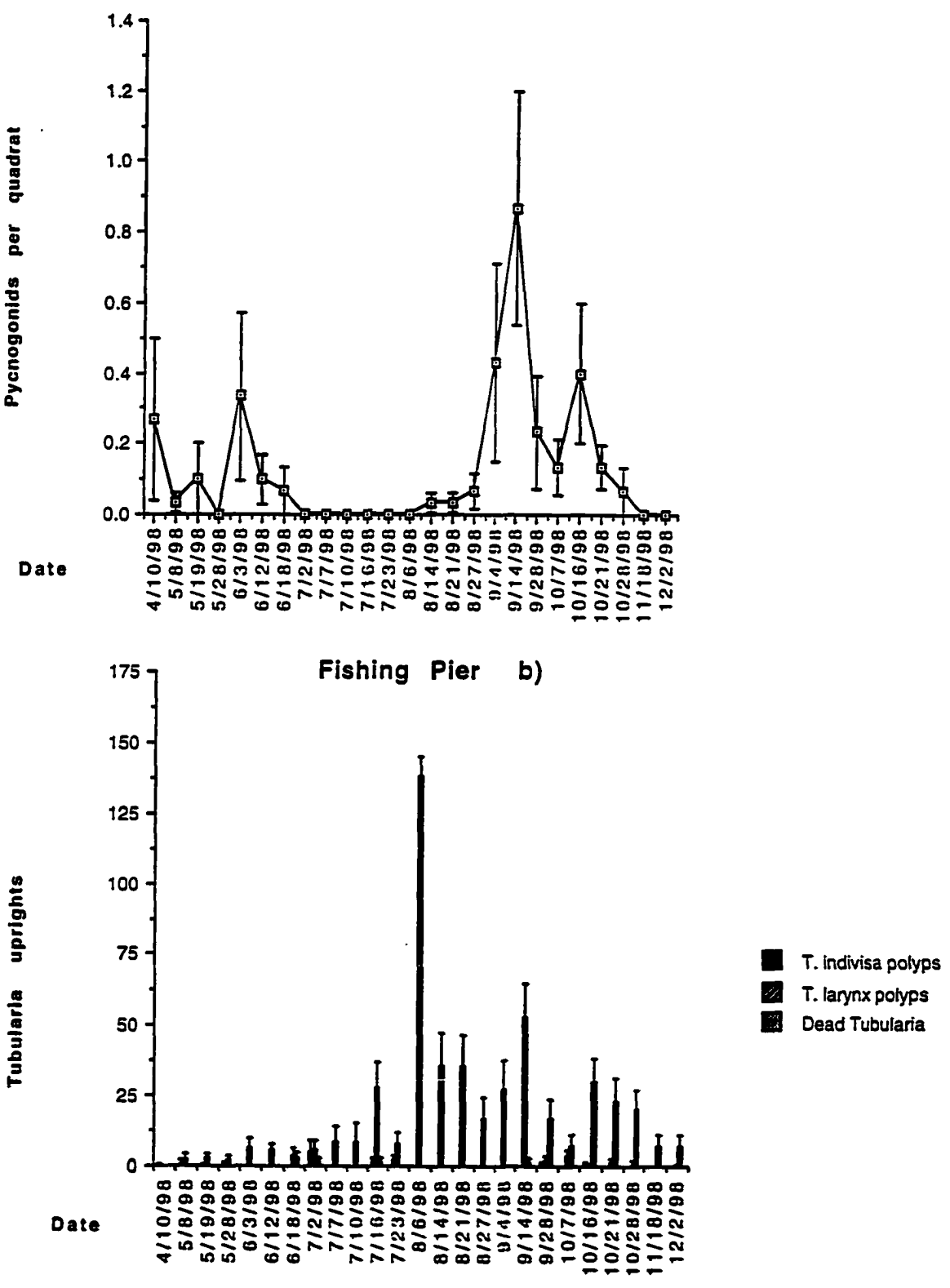


Figure 4.6 Predator/Prey Abundance

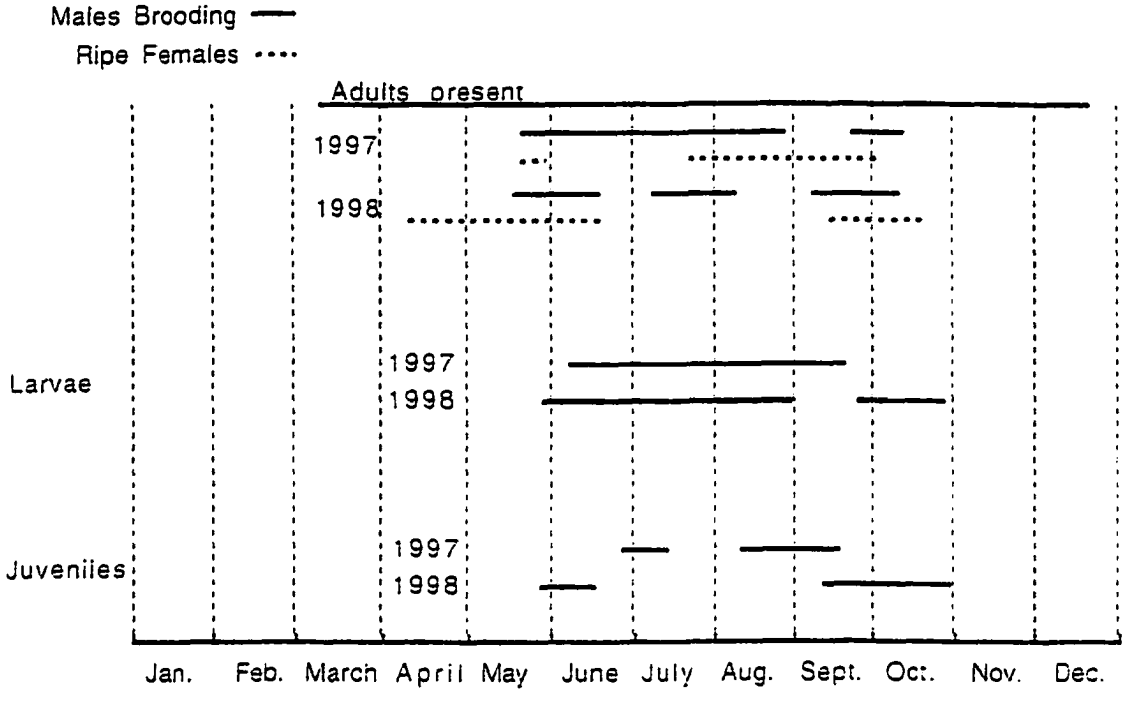
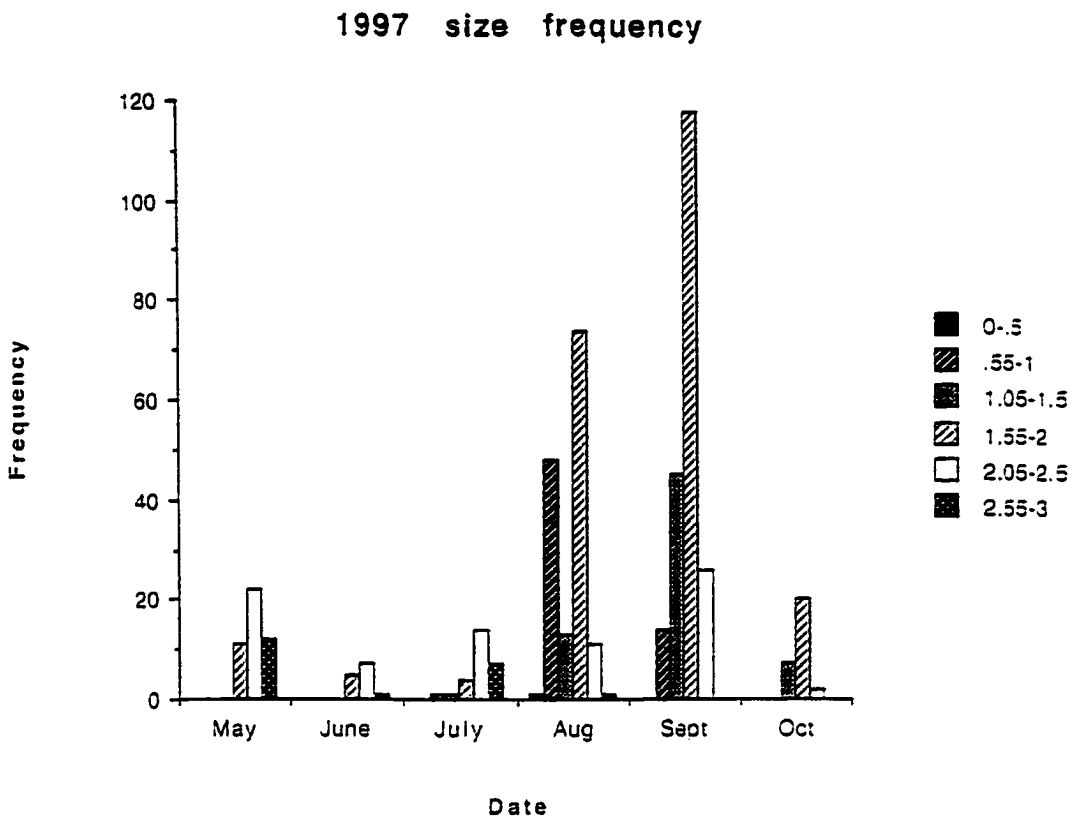


Figure 4.7 Reproductive Status Diagram



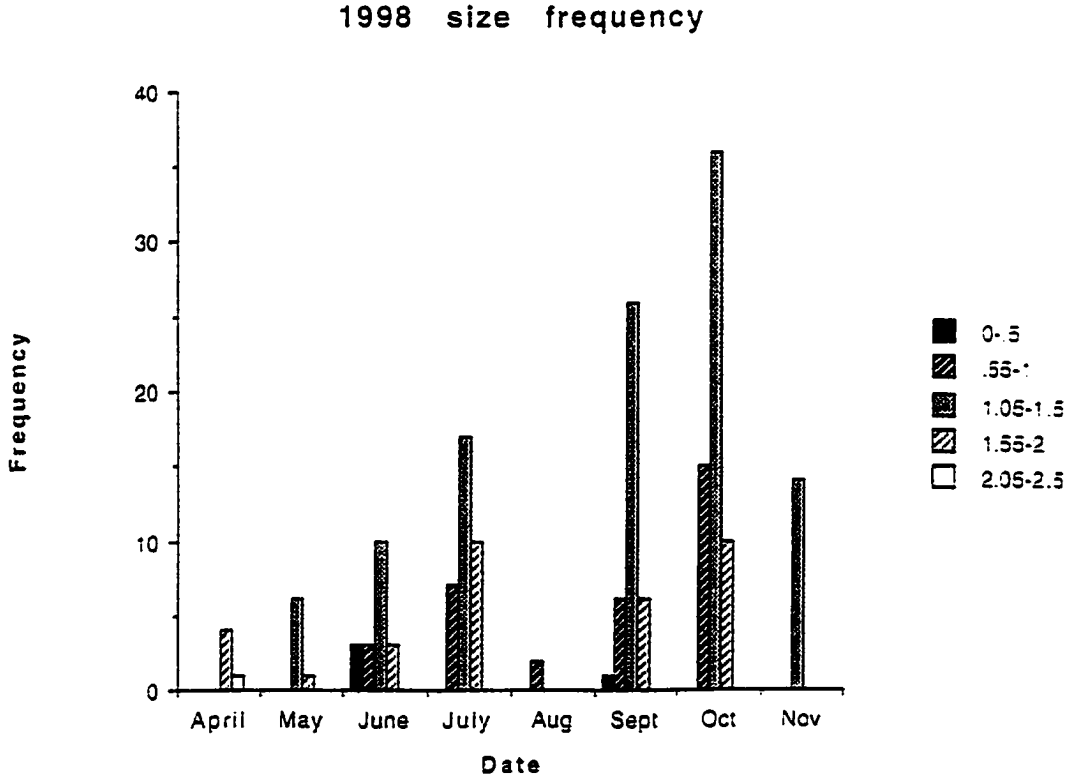
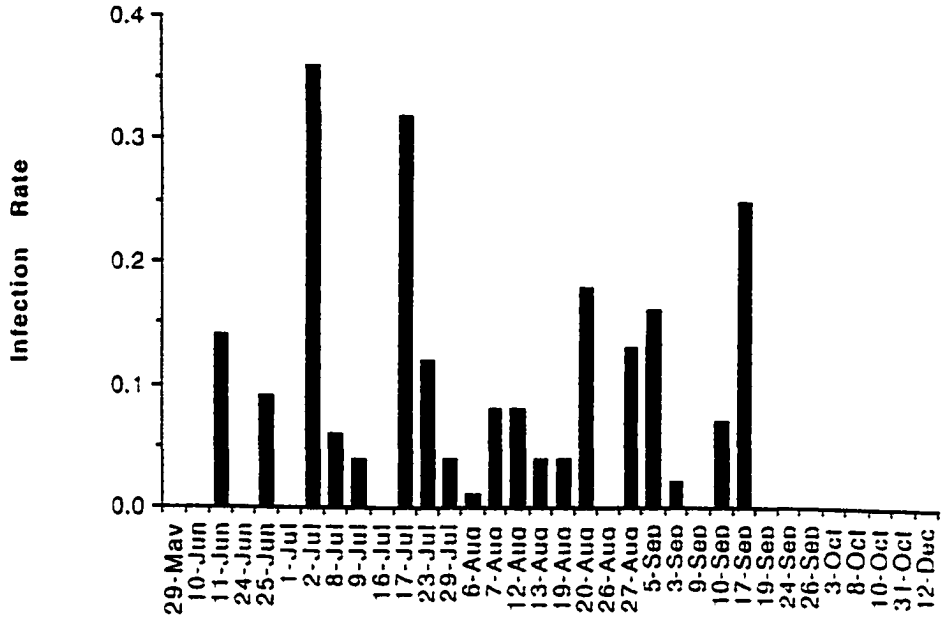


Figure 4.8 Size Frequency Distributions



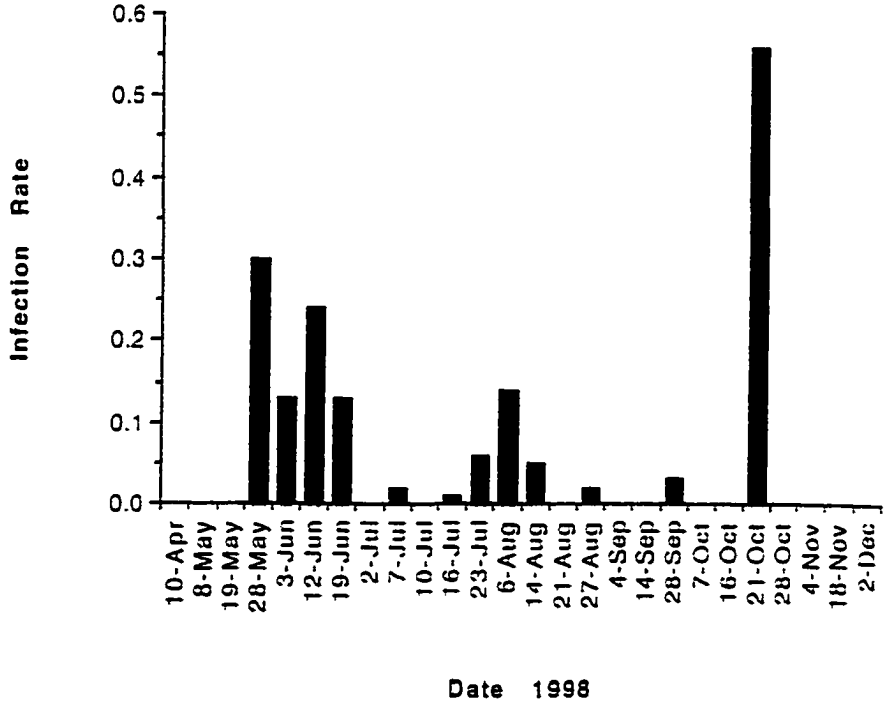


Figure 4.9 Hydroid Infection Rates

Figure 4.10a-4.10b Scanning electron micrographs of male brooding egg masses

Figure 4.10a

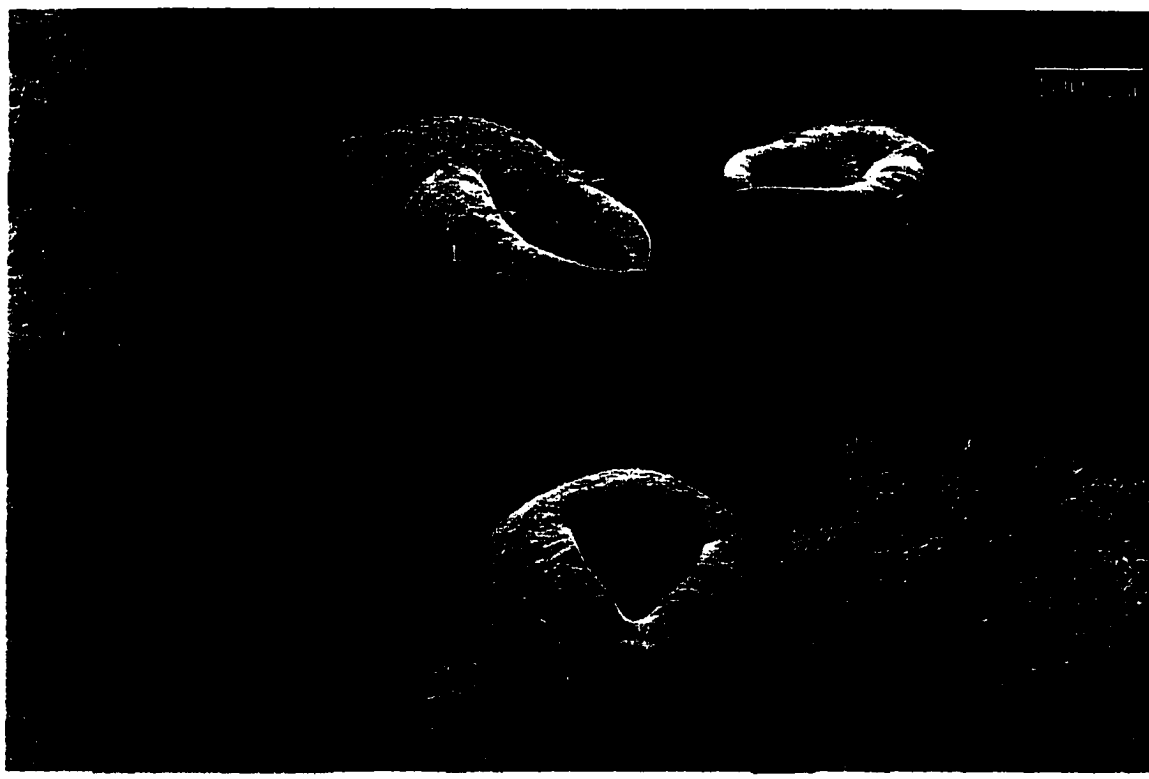


Figure 4.10b

Figure 4.11a-4.11b Scanning electron micrographs of male brooding egg masses a) 197x b) 86x

Figure 4.11a

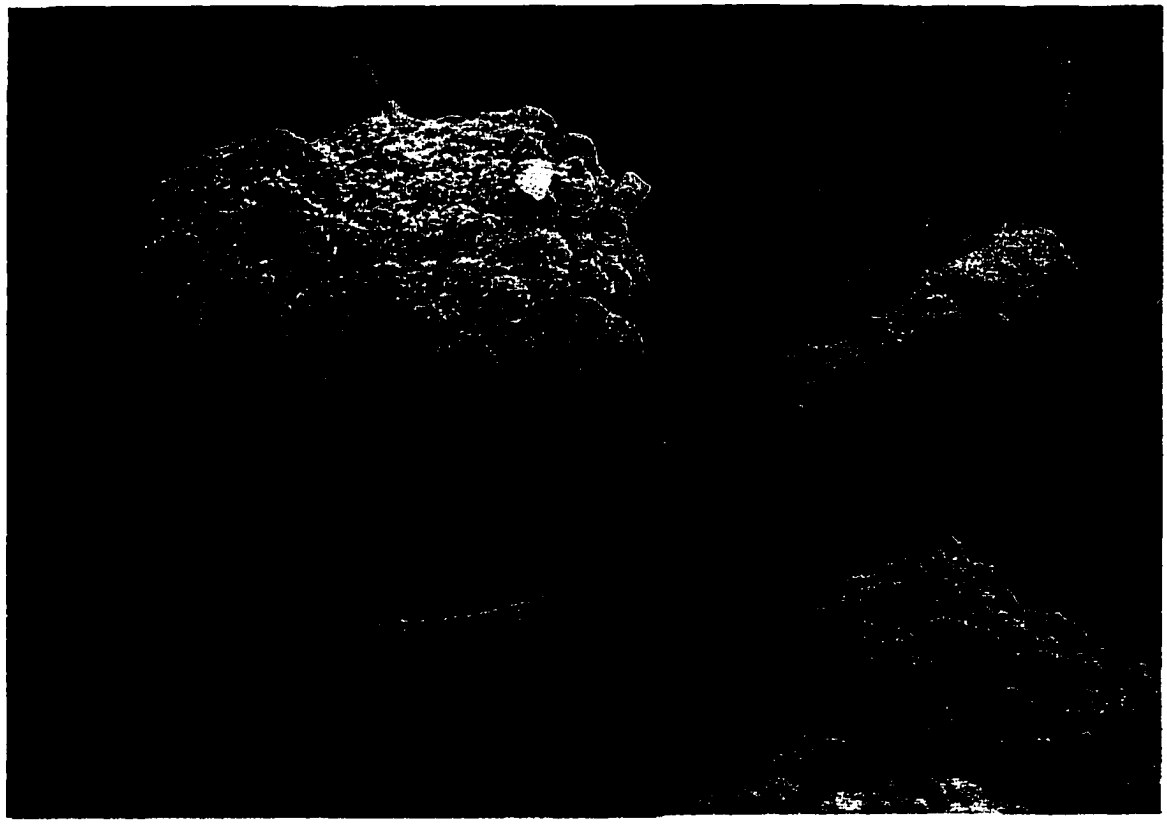


Figure 4.11b

Figure 4.12 Scanning electron micrograph of protonymphon

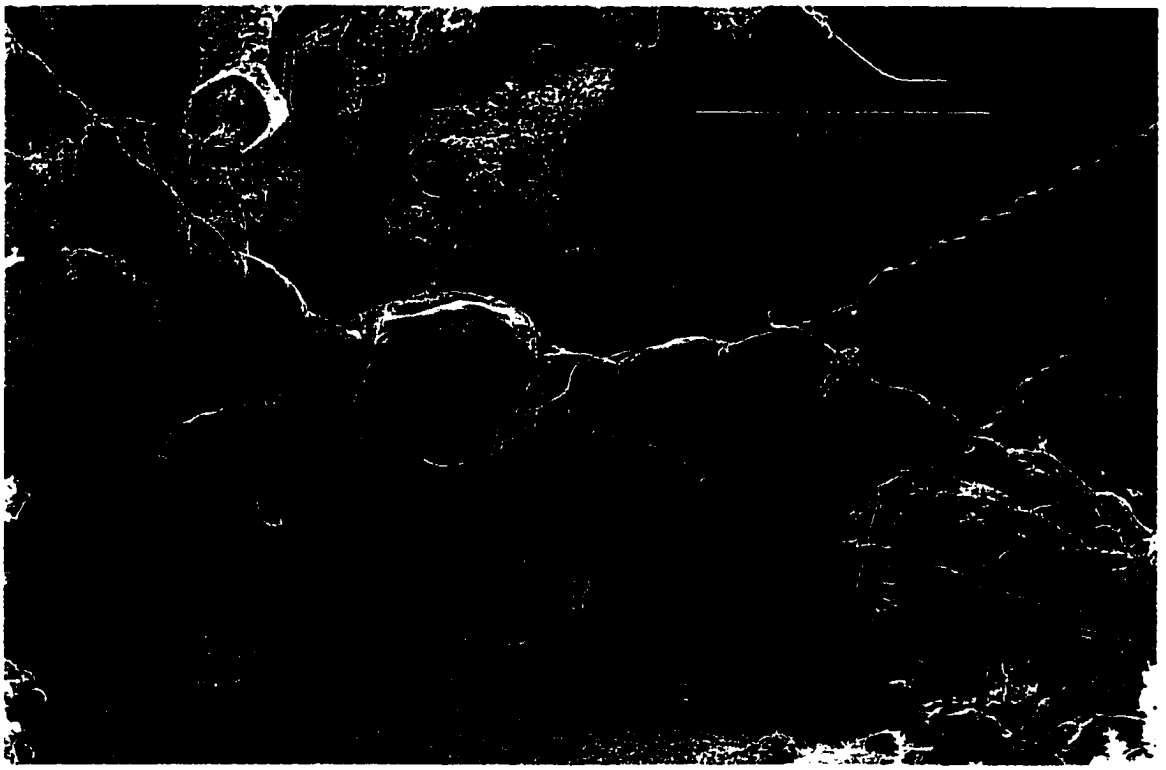


Figure 4.12

Figure 4.13 Scanning electron micrograph of protonymphon filament 2970x

Figure 4.13

Figure 4.14a-4.14b	Scanning electron micrographs of stage three larva
Figure 4.14c	Scanning electron micrograph of the posterior limb-buds of a stage three larva
Figure 4.14d	Scanning electron micrograph of a molted cuticle of a stage three larva

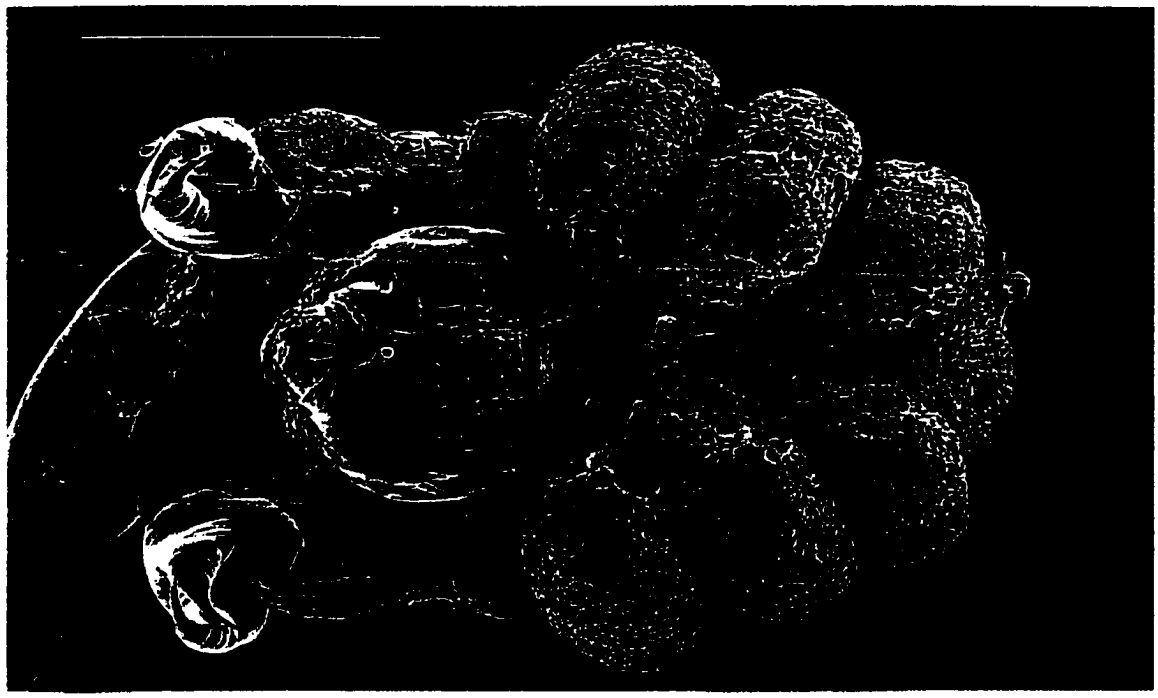


Figure 4.14a

Figure 4.14b

Figure 4.14c

Figure 4.14d

Figure 4.15a-4.15b Scanning electron micrographs of stage four larva disected out of the hydranth

Figure 4.15a

Figure 4.15b

Figure 4.16a-4.16c Scanning electron micrographs of stage four larva outside the hydranth

Figure 4.16a

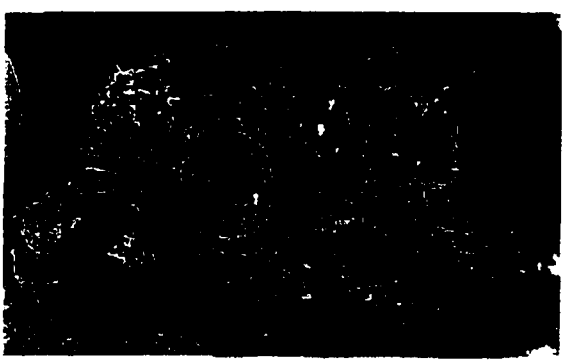


Figure 4.16b

Figure 4.16c

Figure 4.17 Scanning electron micrograph of a pre-hatching juvenile (stage five) dissected out of a hydranth 28x

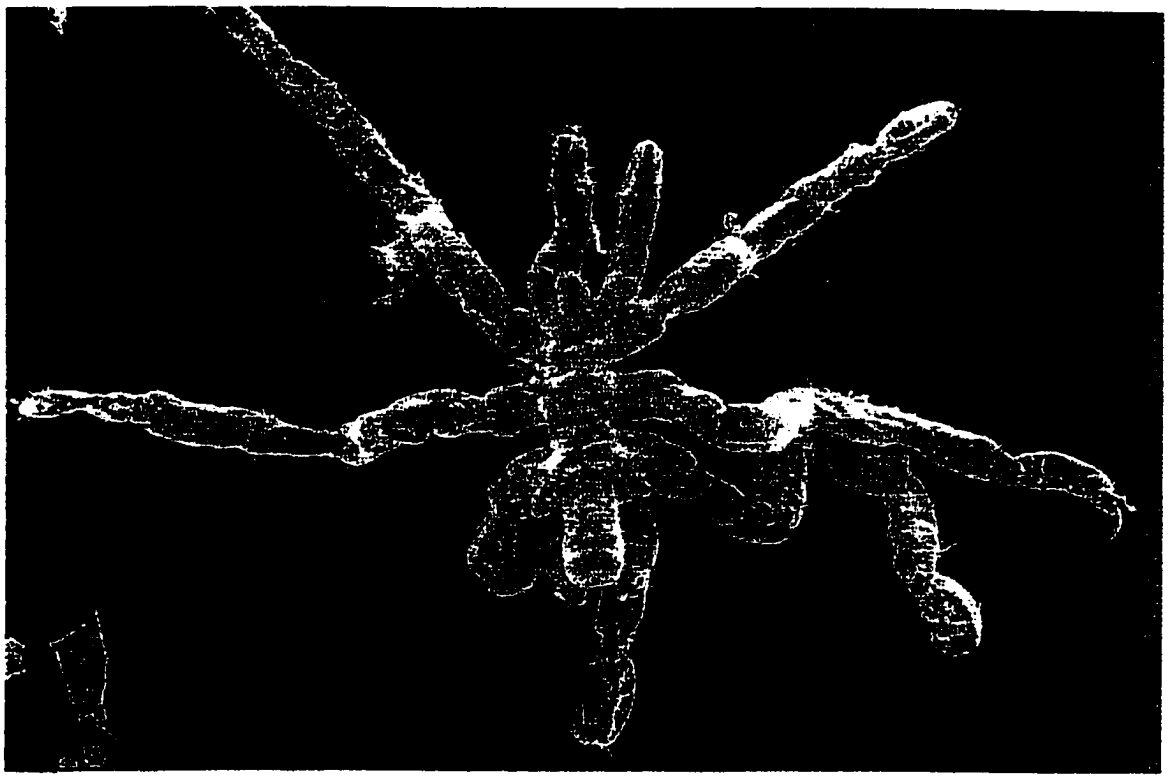


Figure 4.17

Figure 4.18 Scanning electron micrograph of hatching out of a hydranth. Notice the most posterior pair of walking legs protruding from the top of the hydranth and an anterior walking leg sticking out the bottom of this hydranth. This animal was caught while emerging from the hydranth.

Figure 4.18

Figure 4.19 Scanning electron micrograph of a post-hatching juvenile

Figure 4.19

Figure 4.20 Scanning electron micrograph of a post-hatching juvenile

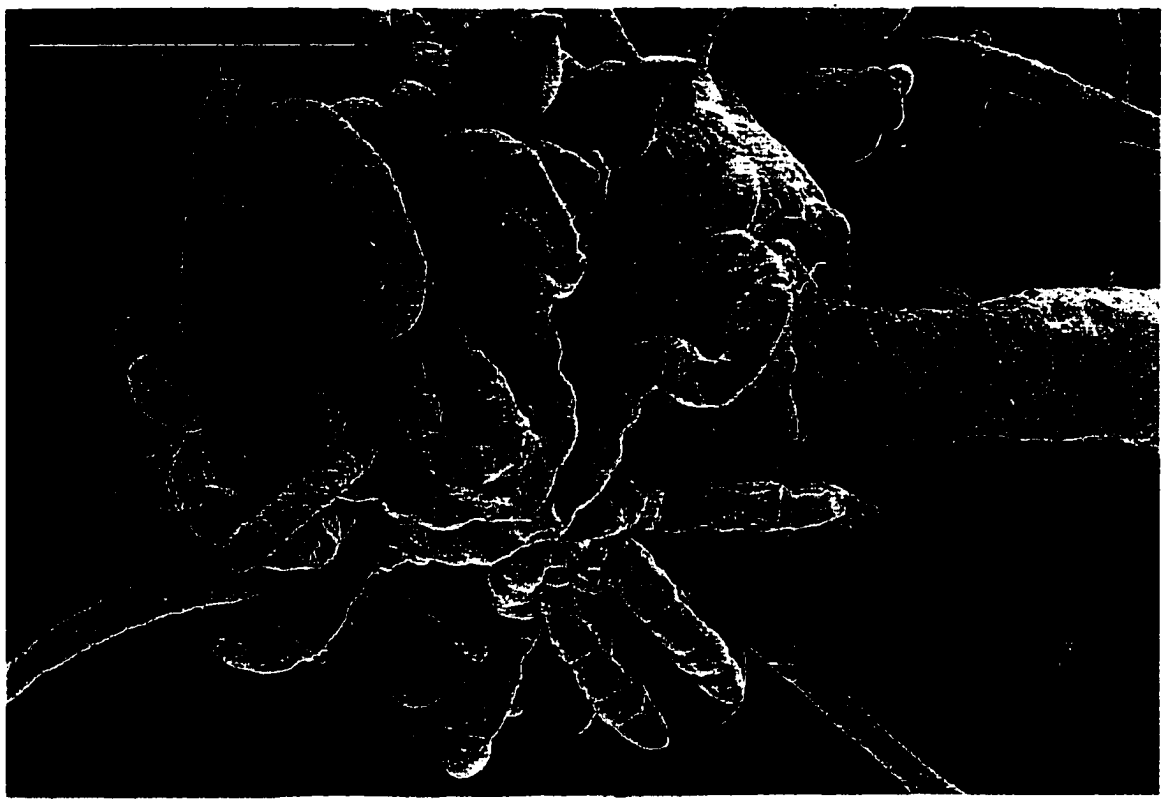


Figure 4.20

Figure 4.21a Scanning electron micrograph of a post-hatching juvenile showing the open anus

Figure 4.21b Scanning electron micrograph of a mouth

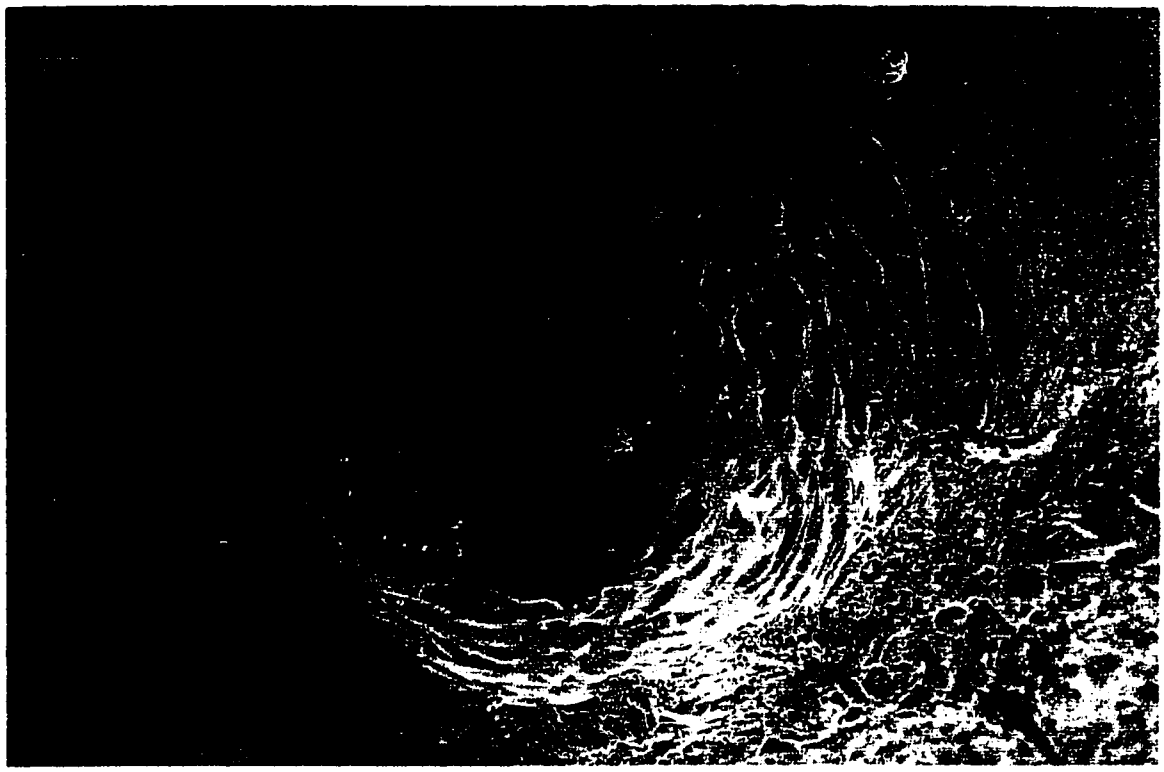


Figure 4.21a

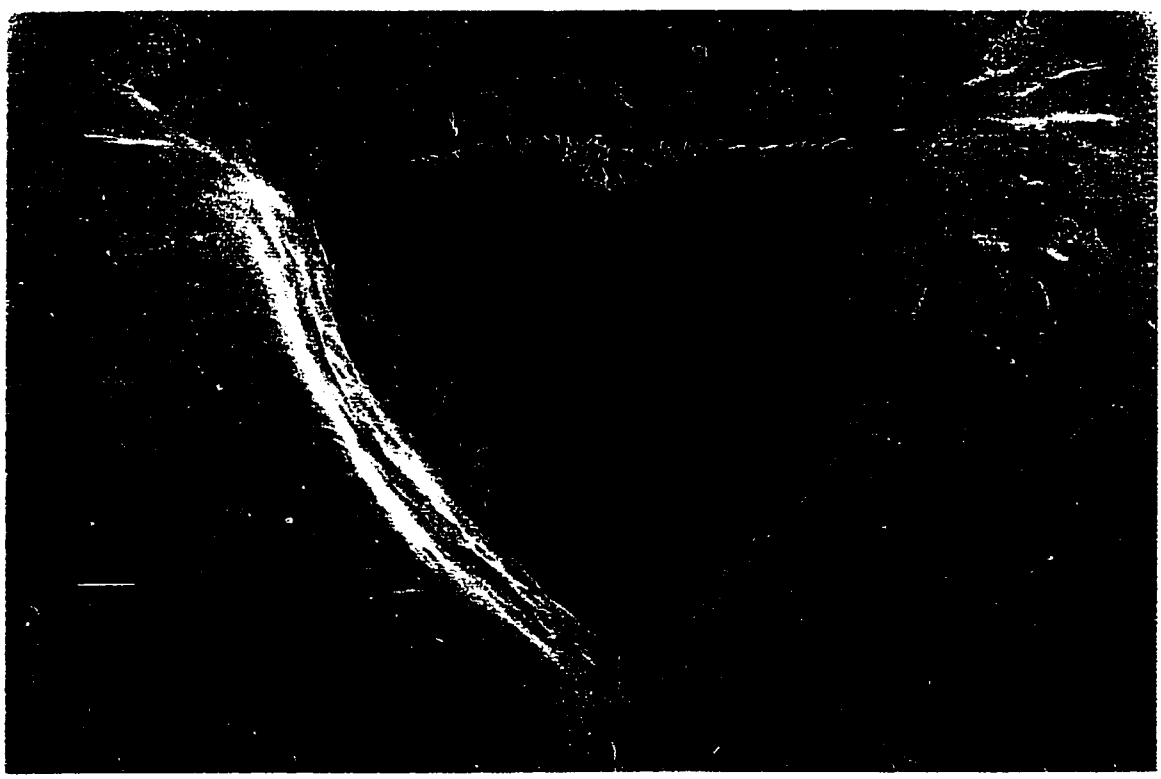


Figure 4.21b

Figure 4.22a-4.22d Scanning electron micrograph of male gonopores

Figure 4.22a

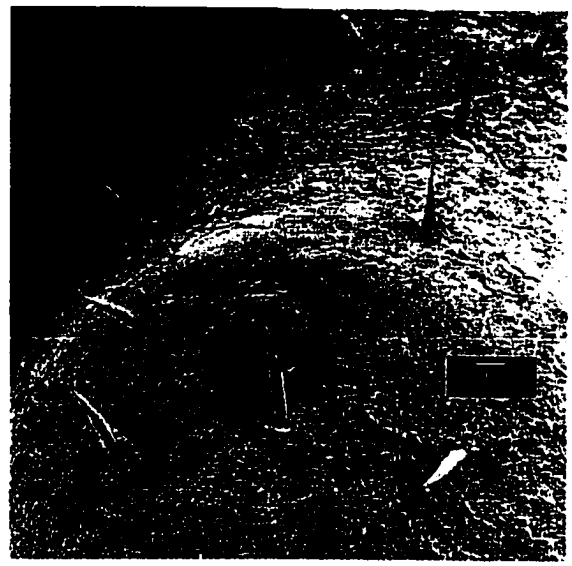


Figure 4.22b

Figure 4.22c

Figure 4.22d

Figure 4.23a-4.23c Scanning electron micrographs of female gonopores

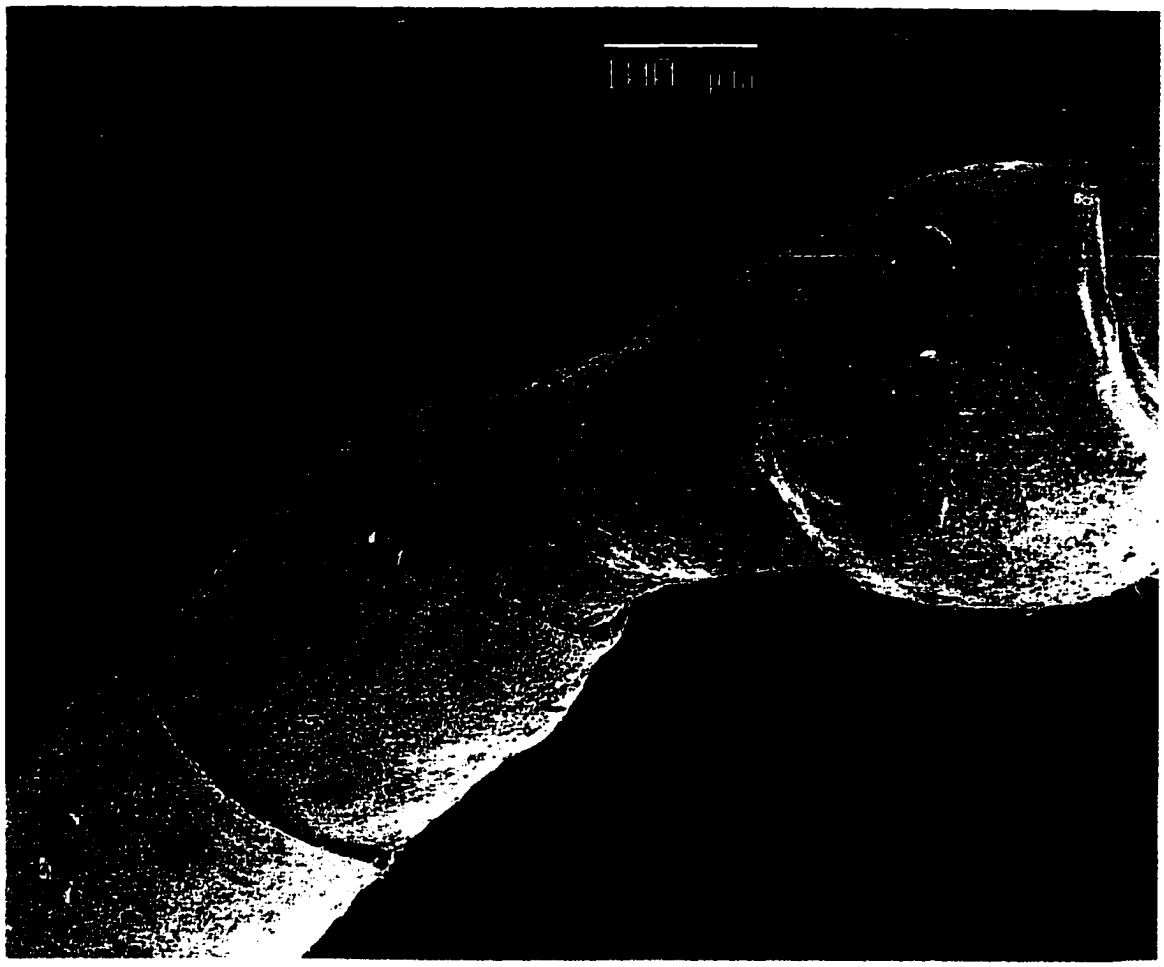


Figure 4.23a

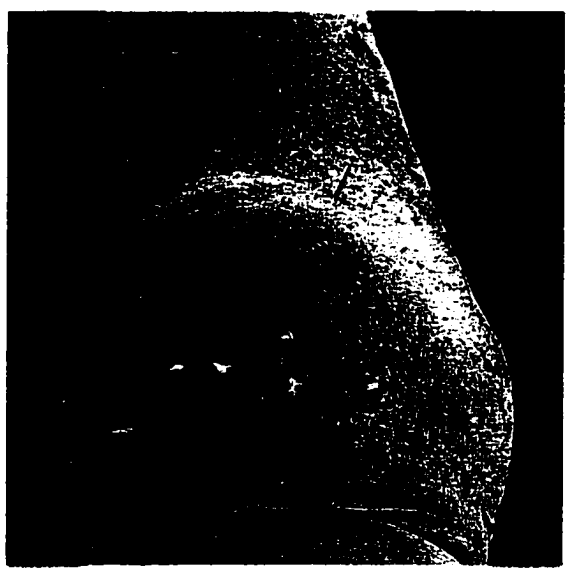


Figure 4.23b

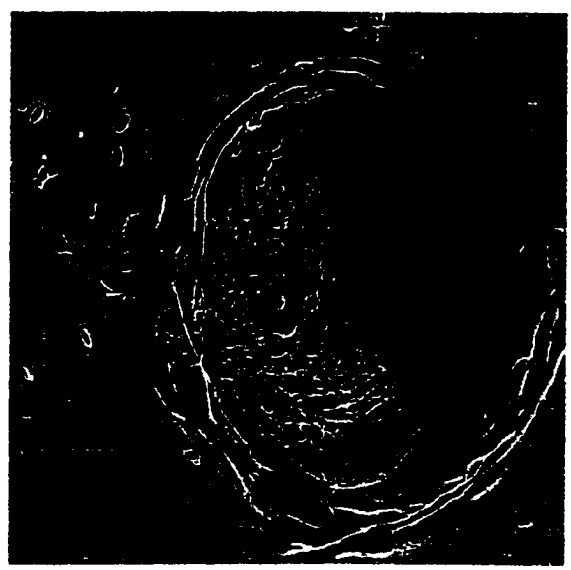


Figure 4.23c

Figure 4.24a-4.24c Scanning electron micrographs of hydroid nematocysts attacking a pycnogonid

Figure 4.24a

Figure 4.24b

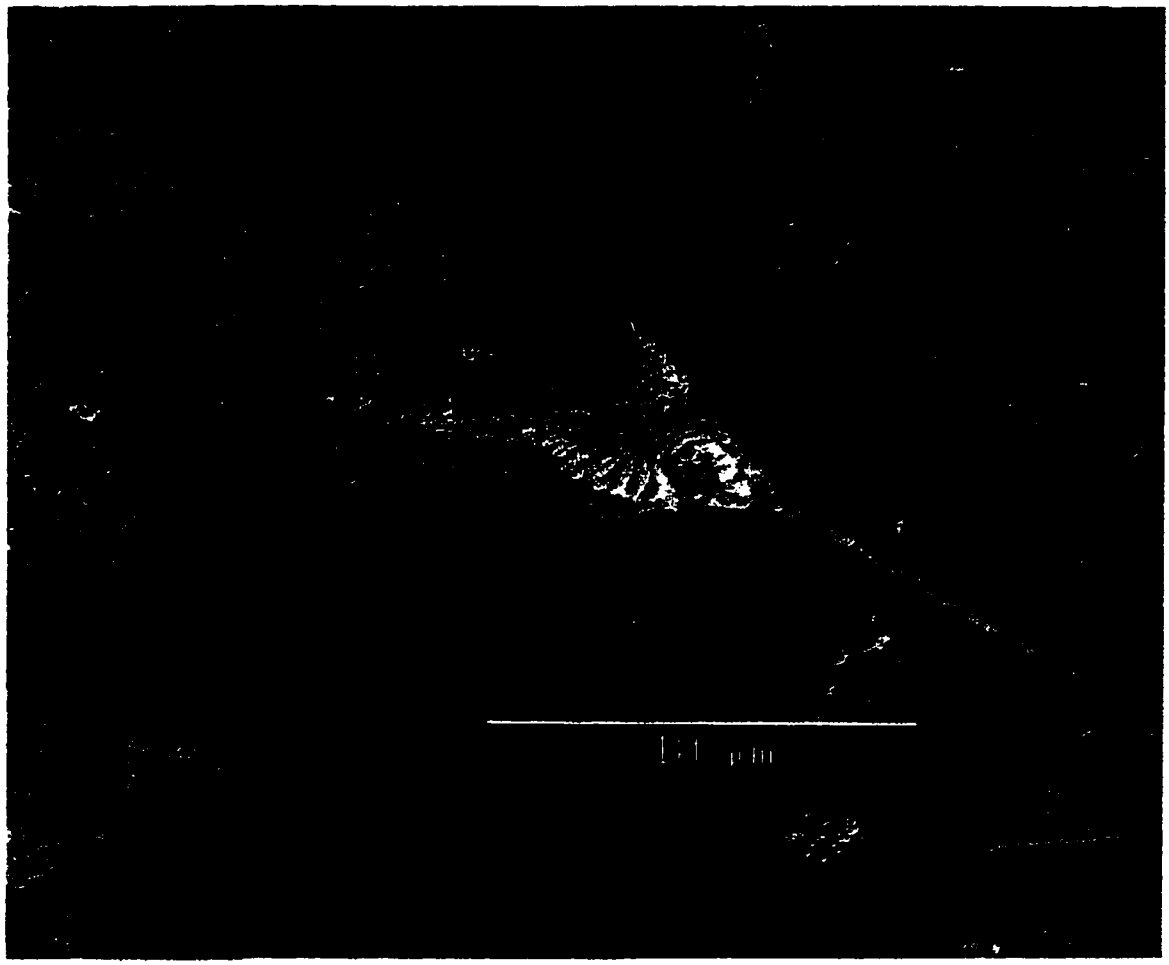


Figure 4.24c

Figure 4.25a-4.25b Scanning electron micrographs of hydroid nematocysts attacking a pycnogonid

Figure 4.25c Scanning electron micrograph of a pycnogonid grabbing a tentacle with chelifores

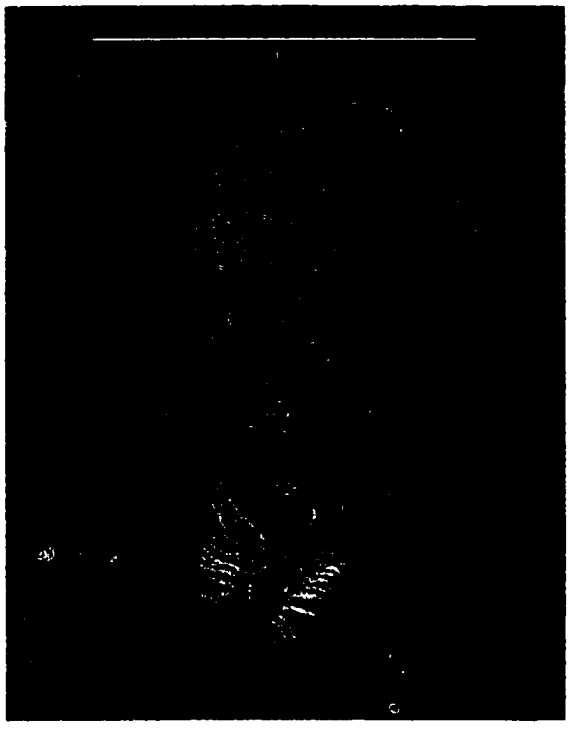


Figure 4.25a

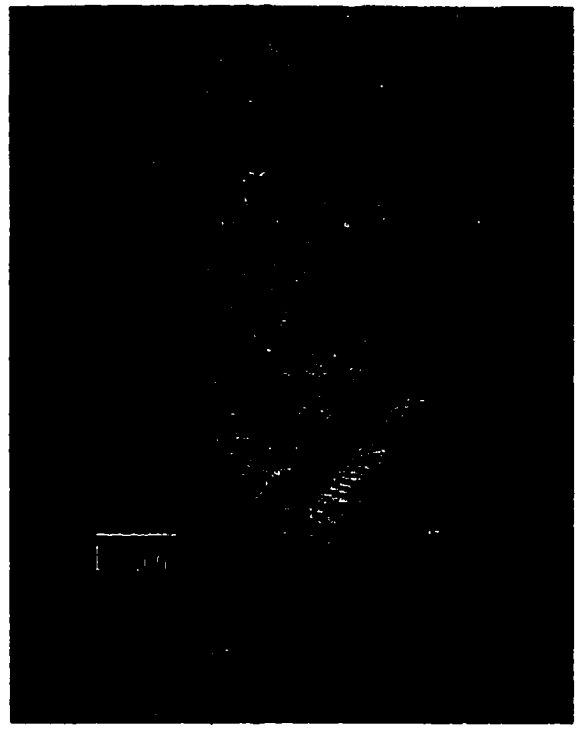


Figure 4.25b

Figure 4.25c

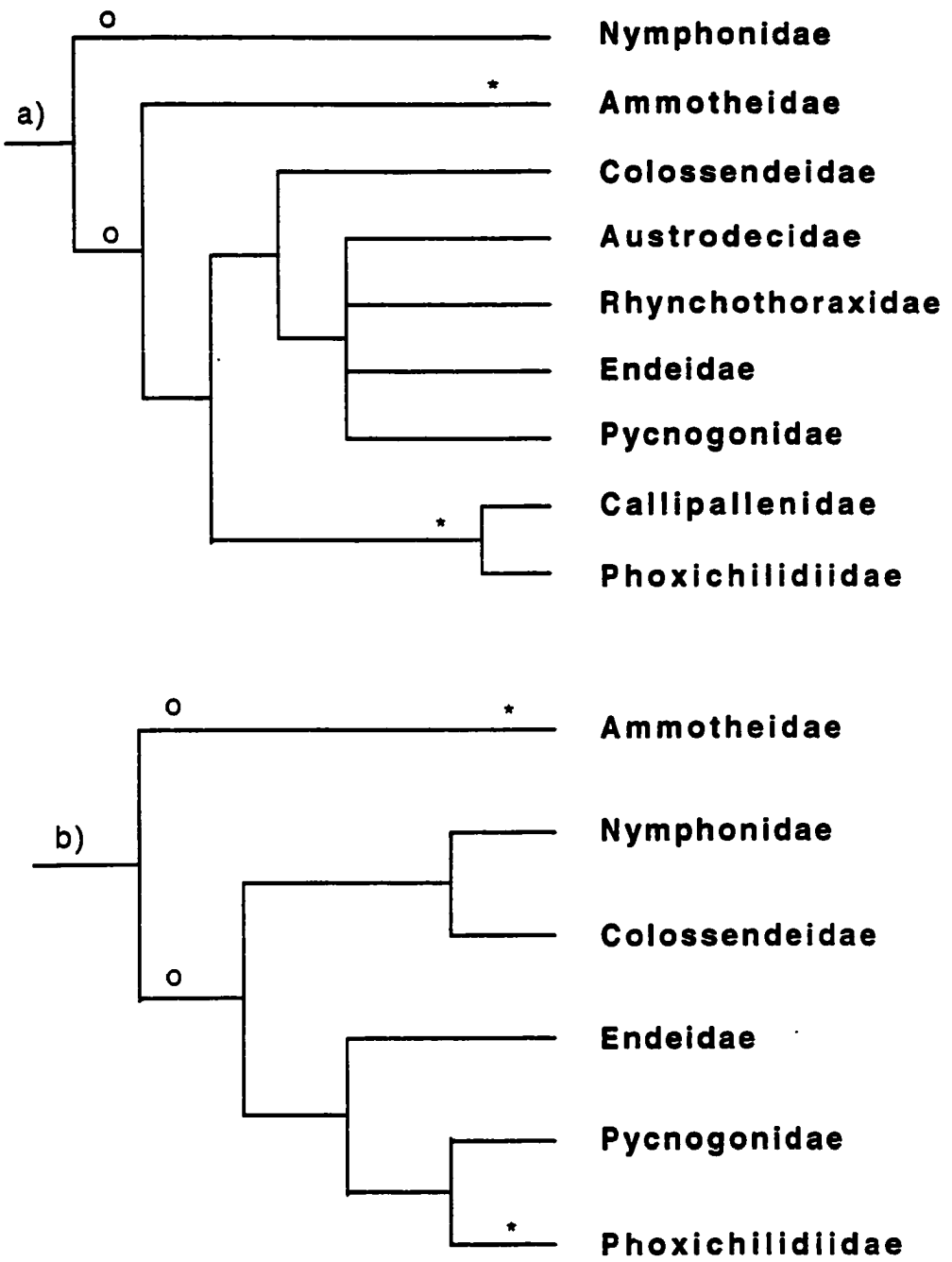


Figure 5.1 Summary trees and overview of the evolution of larval parasitsm in the Pycnogonida based on a) morphology and b) 28S rDNA

o= external parasitic life histories present

*= internal parasitic life histories present