SIPHONOSTOMATOIDS INFECTING SELECTED MOBULIDS (Rajiformes: Mobulidae) OFF THE KWAZULU-NATAL COAST

by

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DELARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Zoology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Lebepe, M.C. (Miss) **Date**

1. Abstract

Considering South Africa's richness in aquatic species, very little knowledge exists regarding copepods that are symbiotic on hosts ranging from invertebrates to marine mammals. In order to have any indication of the existing biodiversity of this group of organisms in South African waters, a thorough investigation of all possible hosts needs to be conducted, which in turn will most likely increase the number of recorded symbiotic copepods considerably. The current descriptive study was done in an effort to contribute to a larger study of metazoan parasites of elasmobranch hosts along the KwaZulu-Natal coast of South Africa. A total of 40 (31 *Mobula kuhlii*; two *Mobula eregoodootenkee* and seven *Manta alfredi*) mobulids were examined for infection by symbiotic copepods at the KwaZulu-Natal Sharks Board (KZNSB). More than 90% of all examined hosts were infected with different types of symbiotic siphonostomatoids. Collected copepod specimens were fixed and preserved in 70% ethanol and studied with both the stereo- and light microscopes using the wooden slide technique. Some selected specimens were further studied using Scanning Electron Microscopy (SEM) to elaborate on ill-defined features. A total of 13 different species of the order Siphonostomatoida distributed over five families were identified. The five families include Eudactylinidae (*Eudactylina oliveri*, *Eudactylina diabolophila* and *Nemesis* sp.); Caligidae (*Caligus crysophrys*i, *Pupulina* sp. 1, *Pupulina* sp. 2; *Pupulina* sp. 3, Unidentified sp. 1, Unidentified sp. 2 and Unidentified sp. 3); Kroyeriidae (*Kroeyerina mobulae*); Dichelesthiidae (*Anthosoma crassum*) and Cecropidae (*Entepherus laminipes*). Two of the 13 species (*E. laminipes* and *A. crassum*) are monotypic and were therefore easily identified. *Eudactylina oliveri* exhibited a prevalence of 75% and 100%; mean intensity of 42 and 130 parasites per host and a mean abundance of 32 and 130 individuals per host while *Pupulina* sp. 1 exhibited a prevalence of 61.29% and 100%; mean intensity of 41 and 5 individuals per host and a mean abundance of 2 and 5 individuals per host on *M. kuhlii* and *M. eregoodootenkee* respectively. Component populations of *E. oliveri* and *Pupulina* sp. 1 exhibited an aggregated distribution pattern on their examined hosts.

The phylogenetic relationship between nine caligid species (three known *Pupulina* species, three collected *Pupulina* species and three Unidentified sp. species as in-group) with *Caligus glandifer* as out-group was determined and analysed using a morphological dataset (40 characters) from previous and current descriptions. The exhaustive search with PAUP* retained a single most parsimonious tree with a tree length (TL) = 85; consistency index (CI) = 0.7; retention index (RI) = 0.7; homoplasy index (HI) = 0.3 and a rescaled consistency index (RCI) = 0.5. Bootstrap support for the estimated clades was mostly low with values less than 95%. The phylogenetic hypothesis of the 10 caligid species presented in the current study was derived from the phylogenetic analysis of the information for adult females and is therefore not intended to be a definitive theory but should be treated as a testable hypothesis that can be further analysed using more data. The current study provides the first record of *C*. *chrysophrysi*, *Pupulina* sp. 1, *Pupulina* sp. 2, *K*. *mobulae* and *E*. *laminipes* on *M*. *kuhlii*; *E*. *oliveri*, *Pupulina* sp. 1, *Pupulina* sp. 2 and *Pupulina* sp. 3 on *M*. *eregoodootenkee*; and *E*. *diabolophila*, *Nemesis* sp., *C*. *chrysophrysi*, *E laminipes*, *A*. *crassum* and the three Unidentified species on *M*. *alfredi* frequenting the east coast of South Africa and thus contributes to the knowledge of our marine biodiversity. Mobulid hosts were not carefully studied for copepod infection previously and the copepods that were reported from the mobulids were probably found by chance. Therefore future investigation into the symbiotic siphonostomatoids of more mobulid hosts and other host species may result in more reports of symbiotic Copepoda from South African waters.

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Chapter 1: General Introduction

1.1 Marine invertebrates: Copepods

In order to successfully conserve the South African marine environment and its diversity of life, it is essential to understand the biology, systematics and behaviour of the marine biota. South Africa is very rich in aquatic species, but still very little knowledge about the status of the marine biodiversity in South African waters exist (Gibbons 1999; 2000; 2010). Even less is known about the biology and distribution of parasitic invertebrates in South African waters (Dippenaar 2005), out of which the largest and most diverse are represented by copepods (Bush *et al*. 2001; Benz & Bullard 2004). Copepods are small crustaceans incorporated within the phylum Arthropoda and they form part of the zooplankton in both the marine and freshwater ecosystems (Gibbons 1999; Bush *et al*. 2001; Ho 2001; Martin & Davis 2001). They are considered to be the most common and most abundant metazoans in both the marine and freshwater environments (Ho 2001; Martin & Davis 2001). These small organisms are characterized by the absence of compound eyes, worm-like segmented bodies with five pairs of cephalic appendages, seven pairs of thoracic appendages and a limbless abdomen (Dudley & Illg 1991; Bush *et al*. 2001) and their body sizes can range from about 0.2 mm to about 10 mm depending on the type of species encountered (Walter & Boxshall 2008). Furthermore, as an adaptation mechanism, some of the parasitic copepods have developed increased body sizes (Bush *et al*. 2001). Thus, in copepods, the structure, morphology and number appendages have been widely used as diagnostic features in their identification and classification (Dudley & Illg 1991; Martin & Davis 2001; Caira & Healy 2004).

In 1981, more than 8000 copepod species were identified from across the world's oceans (Griffiths 1999). The number increased to more than 11500 in 2001 (Ho 2001). Currently there are 15341 known copepod species described across the world (Walter & Boxshall 2008). The numbers tend to increase and decrease over time as a result of newly described species and the synonomization of misidentified species (Boxshall 2010). Though most copepods are predominantly free-living, some do have symbiotic relationships with a diversity of hosts ranging from sponges to marine mammals (Ho 2001). However, like most marine invertebrates, the distribution and diversity of symbiotic copepods are not well known, particularly for those that occur in South African waters, primarily as a result of the limited number of active experts in the field (Gibbons 1999; 2000; 2010). In 2001, only 10% of researchers from the World Association of Copepodologists were actively working on symbiotic copepods (Ho 2001), while currently in South Africa there is only one Copepodologist actively working on symbiotic copepods of chondrichthyes (S.M. Dippenaar pers.comm.).

1.2 Symbiotic Copepods

Most symbiotic copepods are represented by ectoparasitic species, thus they are either found attached to the external body surfaces of their hosts or may prefer to colonize more sheltered microhabitats such as the gills, nostrils and the mouth cavity of their hosts (Huys & Boxshall 1991; Ho 2001). However, some mesoparasites have also been reported from various host species and examples include *Carnifossorius* Deets & Ho, 1988, a mesoparasite of *Rhina ancylostoma* and females found within the family Sphyriidae Wilson, 1919 and Lernaeopodidae Milne Edwards, 1840 (Kabata 1979; Deets & Ho 1988; Benz *et al*. 2007). Since symbiotic copepods have a need to attach to their variety of hosts, they often lack some of the characteristics that define the Copepoda in general (Bush *et al*. 2001). As such, their bodies have undergone morphological changes which include reduced locomotory appendages, fused body segments with no evidence of segmentation remaining and they have modified appendages or have developed new ones as a result of their symbiotic lifestyles (Dudley & Illg 1991; Bush *et al*. 2001). Symbiotic copepods can be regarded as host specialists or host generalists; and in terms of Darwinian fitness, host generalists can survive equally well on a variety of host species while host specialists only occur on one or a few host species (Bush *et al*. 2001; Boxshall & Halsey 2004). However, like most parasites, most symbiotic copepods exhibit specificity for certain types of hosts, thus they are restricted to members of a certain genus or family of hosts (Kabata 1979; Benz 1994; Deets 1994; Bush *et al*. 2001). Furthermore, most symbiotic species exhibit an aggregated distribution pattern on their hosts and examples include the eudactylinids and kroyerids (Benz & Dupre 1987; Dippenaar *et al*. 2008; Dippenaar *et al*. 2009).

Most of the symbiotic copepods are found within the Siphonostomatoida (with about 2150 species) and Poecilostomatoida (with about 2300 species), (Walter & Boxshall 2008; Boxshall 2010). The Siphonostomatoida and the Poecilostomatoida are the only copepod orders that have representatives infecting some marine fishes, while the most successful symbiotic copepods infecting fishes and elasmobranchs are represented by members of the Siphonostomatoida (Benz 1994; Benz & Bullard 2004). The largest number of symbiotic copepods reported (with more than 2150 described species) therefore belongs to the order Siphonostomatoida (Walter & Boxshall 2008). Representatives of the Siphonostomatoida are characterized by a siphon, which is a tube-like mouth resulting from the fusion of the labium and labrum, armed with saw-like mandibles that are used when feeding (Benz & Dippenaar 1998; Bush *et al*. 2001; Boxshall & Halsey 2004). In 2004, there were 38 families of Siphonostomatoida identified worldwide (Boxshall & Halsey 2004). The current number of families identified within the Siphonostomatoida has increased to 41 families (Walter & Boxshall 2008; Boxshall 2010).

Of the 41 families, 24 occur in symbiosis with invertebrates, while 17 are in symbiosis with vertebrates (Tang & Newbound 2004; Dippenaar 2005; Dippenaar & Jordaan 2007). Twelve of the 17 families form symbiotic relationships with sharks, rays and chimaeras, which are all members of Chondrichthyes (Dippenaar 2005). Out of the more than 2150 siphonostomatoid copepods reported worldwide, less than 10% have been reported from South African waters (Dippenaar & Jordaan 2007). Thus, up to 2007, siphonostomatoids in symbiosis with elasmobranchs (41 examined host species) from South African waters included only 35 species which belong to 19 genera distributed over 7 families (Dippenaar 2005; Dippenaar & Jordaan 2007). According to Compagno (1999), South Africa is very rich in aquatic species and has about 210 chondrichthyan species. These 210 species are probably infected with a large diversity of symbiotic siphonostomatoids. Therefore, an investigation of all possible hosts is likely to increase the number of reported siphonostomatoids in South African waters significantly (Dippenaar 2005). Thus marine symbiotic copepods are in urgent need of attention to be able to get any indication of the existing biodiversity of this group of animals in southern African waters (Dippenaar 2005; Dippenaar & Jordaan 2007).

1.3 Why study symbiotic siphonostomatoids

Copepods are considered keystones or anchors of the aquatic food webs (Martin & Davis 2001; Caira & Healy 2004). They are sensitive bio-indicators of global and local climate change, key providers in the aquatic ecosystem while some are symbionts of economically important aquatic fish (Martin & Davis 2001; Caira & Healy 2004). Some symbiotic copepods are major pests in both the marine and freshwater aquaculture industries around the world (Ho 2001). Therefore, care should be taken to ensure that attention is paid to the symbiotic copepods that occur physically in close association with all the major groups of aquatic organisms (Ho 2001). Research studies focusing on the biology and diversity of symbiotic copepods will contribute a lot towards an understanding of these small creatures (Dippenaar 2005; Dippenaar & Jordaan 2007). Worldwide few people work actively on symbiotic copepods (Ho 2001) and even fewer are active in South Africa (Gibbons 2010). Due to the large gap in the knowledge concerning the diversity and distribution of symbiotic copepods worldwide, the study of these individuals should be the focus of research studies in modern biology (Ho 2001) and since most aquatic organisms have the ability to host symbiotic copepods (Bush *et al*. 2001), it then becomes clear that there are many more species to be discovered, identified and classified (Benz & Bullard 2004).

1.4 Biosystematics in Copepodology

According to Benz (2005), the best beginning to a study of any group of organisms is to have knowledge of their morphology and systematics. Systematics classifies organisms according to their close morphological and molecular similarities (Cotyeri 1995; Latch & Ivy 2009) while phylogenetic systematics or cladistics center on the construction of monophyletic groupings of selected taxa (Cotyeri 1995; Hills *et al*. 1996; Page & Holmes 1998). Phylogenetic systematics has mostly been applied to free-living taxa and it is only recently that this method was applied in the science of parasitology (Deets 1994). The first authors to investigate the relationships of parasitic copepods by applying phylogenetic systematics using morphological characteristics were Cressey, Collete and Russo in 1853 (Deets 1994). Subsequent applications of cladistics in determining the relationships of symbiotic copepods include works by Deets & Ho (1988), Ho (1990), Deets (1994), Benz (1994) and

Boxshall & Huys (1998). Knowledge of the taxonomic and phylogenetic relationships of organisms obtained from morphological systematics permits the reconstruction of organismal phylogenies, which are tree-like structures that show the evolutionary relationships of organisms (Page & Holmes 1998; Latch & Ivy 2009). Phylogenies are very useful in organizing the knowledge of biodiversity and classification structuring, which provide insight on the evolutionary history of organisms. The most common technique used for reconstructing phylogenies is based on the criterion of maximum parsimony (Mabee 2000).

Parsimony assumes that the hypothesis that requires fewer explanations is a more defensible hypothesis, thus the simplest explanation is the best explanation (Nixon 1999; Mabee 2000). Phylogenetic analysis using parsimony (PAUP) employs maximum parsimony to determine the most parsimonious tree, which is the tree that has the fewest number of evolutionary changes for all characters derived from a common ancestor (Mabee 2000). Furthermore, morphological characters provide a source of data for taxonomic research on different organismal taxa and help to determine the direction of evolution while it also provides distinctions between species (Al-Saqhir 2010). Morphological details of small organisms are often ignored by observers, but are the key to understanding their physiology, ecology, behaviour, distribution and systematics (Bush *et al*. 2001; Benz 2005). Applying the science of discovering, identifying and classifying organisms is essential to the understanding of the distribution and diversity of symbiotic copepods on a large diversity of hosts (Gibbons 1999, 2000; Benz & Bullard 2004). Unfortunately, in general the morphology of symbiotic copepods from elasmobranch hosts, a small percentage of which are the devil rays (Mobulidae Rafinesque, 1810), is poorly known (Benz & Bullard 2004).

1.5 Hosts: Mobulidae

The mobulid rays are represented by pelagic species widely distributed in warm temperate and tropical areas across the world’s oceans (Compagno & Last 1999; Last & Stevens 2004; Casas *et al*. 2006; White *et al*. 2006; Marshall *et al*. 2009). They are characterized by large pectoral fins that form a wing-like disc, a broad trunk, a slender whip-like tail and an elongated snout with a rostral fin or “cephalic horn” on either side (Compagno & Last 1999). The ray family, Mobulidae is currently divided into two genera, namely *Mobula* Rafinesque, 1810 and *Manta* Bancroft, 1828 (Compagno & Last 1999; White *et al*. 2006; Osmar *et al*. 2008; Marshall *et al*. 2009; Marshall *et al*. 2011). This family comprises of 11 known species distributed over the two genera *Manta* (with two species) and *Mobula* (with nine species), (Compagno & Last 1999; White *et al*. 2006; Marshall *et al*. 2009; IUCN 2012; Marshall *et al*. 2011). Previous records (Compagno *et al*. 1989; Compagno & Last 1999; White *et al*. 2006; Harding & Bierwagen 2009; IUCN 2012) indicated the genus *Manta* to be monotypic, thus it was considered to be represented by a single species, namely *Manta birostris* (Donndorff, 1798) which occurs in the Indian Ocean. However, there is at least a second species of *Manta*, *Manta alfredi* (Krefft, 1868), also occurring in the Indian Ocean (Marshall *et al*. 2009; Romanov 2010; Marshall *et al*. 2011). There is also a possibility of a third species of *Manta* occurring in the Atlantic Ocean but research to validate this species is still ongoing (A.D. Marshall pers. comm.).

There are a few distinct characteristics that separate the two mobulid genera; with *Manta* species being larger in size, with long head fins and a terminal mouth (Harding & Bierwagen 2009; Marshall *et al*. 2009; Romanov 2010; Marshall *et al*. 2011), while *Mobula* members are smaller, have short head fins and a ventral mouth (Sciara 1988; Compagno & Last 1999; Casas *et al*. 2006; Romanov 2010). The mobulids are mostly solitary, but can also occur in small groups (Sciara 1988; Casas *et al*. 2006; White *et al*. 2006; Marshall *et al*. 2009; Romanov 2010). All the species of the Mobulidae are currently listed as either endangered, near threatened or vulnerable by the IUCN Red List for Threatened Animals (IUCN 2012). These mobulid rays feed by filtering out planktonic animals and small fishes that pass through their large mouths and get caught on the filter plates of their internal gill openings (Sciara 1988; Compagno & Last 1999; Harding & Bierwagen 2009). In terms of evolution, *Mobula* species represent the oldest lineage while *Manta* species represents the most recent within the family Mobulidae (Nishida 1990; Lovejoy 1996; Kashiwagi *et al*. 2012). According to Romanov (2010), the biology of mobulids remains poorly known, primarily as a result of lack of comprehensive identification of these species in the field. Furthermore, the mobulid rays are very uncommon, rarely seen or caught and thus not well studied (Compagno *et al*. 1989; Compagno & Last 1999; Michael 1993; White *et al*. 2006; Romanov 2010). Seven of the 11 species of mobulid rays, namely *Manta birostris*; *Manta alfredi*; *Mobula kuhlii* (Valenciennes, in Müller & Henle, 1841); *Mobula japanica* (Müller & Henle, 1841); *Mobula eregoodootenkee* (Bleeker, 1859); *Mobula tarapacana* (Philippi, 1892); *Mobula thurstoni* (Lloyd, 1908), reportedly frequent South African waters (Compagno 1999; Marshall *et al*. 2009; IUCN 2012).

1.6 Siphonostomatoida reported from Mobulidae

Symbiotic siphonostomatoids reported from mobulids worldwide include members of the families Caligidae Burmeister, 1835; Kroyeriidae Kabata, 1979 and Eudactylinidae Wilson, 1922 (Kabata 1979; Boxshall & Halsey 2004). Members of Caligidae reported from mobulids include *Pupulina flores* van Beneden, 1892 from *Manta birostris* caught in Azores and Lemon Bay, Florida (Atlantic Ocean) (Wilson 1935) and *Mobula hypostoma* (Bancroft, 1831) caught in the Galapagos Islands (Pacific Ocean) (Wilson 1935); *Pupulina minor* Wilson, 1952 and *Pupulina brevicauda* Wilson, 1952 both reported from *Mobula thurstoni* and *Mobula mobular* (Bonnaterre, 1788) caught off California (Pacific Ocean) (Wilson 1952) and Trivandrum, India (Indian Ocean) (Pillai & Padmanabhan 1963; Pillai 1964). Eudactylinids reported from mobulids include *Eudactylina oliveri* Laubier, 1968 from *Mobula thurstoni*, *Mobula eregoodootenkee* and a *Mobula* sp. captured off Anacap Island (Pacific Ocean) (Laubier 1968), Mexico (Atlantic Ocean) (Deets 1994) and Nosy Bè, Madagascar (Indian Ocean) (Deets 1994); *Eudactylina vasquitillae* Deets, 1994 from *Mobula tarapacana* off Punta Arena de la Ventana (Atlantic Ocean) (Deets 1994) and *Eudactylina diabolophila* Deets, 1994 from *Manta birostris* captured off the Pacific Ocean's Sand and Line islands (Deets 1994). Reports of Kroyeriidae species from mobulid hosts include *Kroeyerina elongata* Wilson, 1932 from *Mobula thurstoni* caught near east Canada (Atlantic Ocean) (Kabata 1979) and *Kroeyerina mobulae* Deets, 1987 from *Mobula japanica* and *Mobula lucasana* both caught off St Peters Port (Atlantic Ocean) (Deets 1987).

Previous records of symbiotic siphonostomatoids infecting mobulids from South African waters include *Eudactylina oliveri* on *Mobula kuhlii*; *Anthosoma crassum* (Abildgaard, 1794) and *Entepherus laminipes* Bere, 1936 both found on *Manta birostris*; and *Caligus elongatus* Von Nordmann, 1832 found attached to the gill lamellae of *Mobula kuhlii* caught off the KwaZulu-Natal coast (Indian Ocean)

(Dippenaar 2005; Dippenaar & Jordaan 2007). By studying the distribution and diversity of symbiotic copepods on mobulids along the South African coast, a small but significant contribution will be made towards knowledge concerning the symbiotic copepods of Chondrichthyes as a group and the biodiversity of these small organisms in South African waters and may also give some insights into the host biology.

1.7 Purpose of the study

1.7.1 Aim: To investigate the symbiotic copepods on selected members of the ray family Mobulidae.

1.7.2 Objectives:

a. To examine caught mobulids for the presence of symbiotic copepods.

b. To identify collected copepods by:
    - i. comparing them with previously described species,
    - ii. dissecting and drawing selected specimens where necessary,
    - iii. describing the dissected specimens or elaborate on ill described features,
    - iv. and doing a cladistics analysis of specific species.

c. To investigate their ecological aspects by determining the:
    - i. prevalence,
    - ii. mean intensity,
    - iii. mean abundance,
    - iv. and distribution/dispersion of the collected species.

1.8 Significance and motivation of the study

The current study will contribute a small but significant proportion towards the broader study in the investigation of the diversity of marine invertebrates along the South African coast. Despite South Africa's richness in aquatic species, only limited knowledge exists on the status of marine parasitic invertebrates that occur in symbiosis with the wide variety of possible hosts. A broader knowledge of the biology, systematics and behaviour of parasitic invertebrates, as well as the effects they have on their hosts will in turn help with the successful conservation of the South African aquatic biota. Information gathered about the symbiotic siphonostomatoids may also contribute towards our knowledge of the host biology.

CHAPTER 2: METHODOLOGY

2.1 Sampling

The facilities of the KwaZulu-Natal Sharks Board (KZNSB) provide a very rare and unexploited opportunity for research on marine symbiotic copepods in South Africa. This institution is responsible for netting popular swimming beaches in South Africa in an effort to prevent shark attacks. A total of 40 mobulid hosts (31 *Mobula kuhlii*; two *Mobula eregoodootenkee* and seven *Manta alfredi*) were caught as accidental by-catch of the KZNSB bather-protection nets from various localities.

Mobula eregoodootenkee: two hosts off Richards Bay (28.48S 32.06E) caught during September 2004.

Mobula kuhlii: six hosts off Umdloti (29.40S 31.08E) caught during April 2004, December 2010, January 2011 and February 2012; one host off Umhlanga Rocks (29.43S 31.05E) caught during December 2011; one host from Ansteys Beach (29.55S 31.01E) caught during January 2010; one host off St. Michaels (30.50S 30.24E) caught during May 2006; seven hosts off Durban (29.51S 31.00E) caught during April 2004, December 2005; December 2009, January 2010, November 2010, December 2010 and December 2011; one host off Brighton Beach (29.56S 31.01E) caught during January 2011; one host off Warner Beach (30.05S 30.52E) caught during January 2011; one host off Winkelspruit (30.06S 30.51E) caught during December 2010; three hosts off Umgababa (30.09S 30.50E) caught during December 2005, January 2010 and November 2011; four hosts off Karridene (30.07S 30.37E) caught during January 2011 and November 2011; four hosts off Park Rynie (30.19S 30.44E) caught during January 2000 and January 2011; one host off Hibberdene (30.34S 30.34E) caught during April 2009 and one host off Umzumbe (30.32S 30.51E) caught during January 2011.

Manta alfredi: one host off Karridene (30.07S 30.37E) caught during January 2011; two hosts off Park Rynie (30.19S 30.44E) caught during October 2004 and January 2011; one host off Margate (30.52S 30.21E) caught during February 2009; one host off Ramsgate (30.53S 30.20E) caught during August 2010 and one host off San Lameer (30.57S 29.20E) caught during August 2010. Caught host specimens were examined for the presence of symbiotic copepods at the KZNSB headquarters in

Umhlanga Rocks. The entire external body surface was examined for infection by symbiotic siphonostomatoids. Thereafter, the gills and nostrils of the caught mobulid hosts were dissected out and thoroughly examined for infection by symbiotic siphonostomatoids. Collected siphonostomatoid specimens were then fixed and preserved in 70% ethanol. All collected specimens, including specimens collected before the onset of the current study by S.M. Dippenaar, were examined in the laboratory at the University of Limpopo.

2.2 Data Collection

2.2.1 Morphological data

The wooden slide technique (Humes & Gooding 1964) was used to study the morphological features of collected copepod specimens with both the stereo- and light microscopes. Copepods were cleared in lactic acid in which a pinch of lignin pink was dissolved, dissected, drawn and measured with the use of an ocular micrometer. Drawings were made with the aid of drawing tubes. Some specimens were studied using the Scanning Electron Microscope (SEM) at the University of Limpopo, Medunsa Campus. The specimens were cleaned with small brushes and consecutively dehydrated in 70%, 80%, 95% and 100% ethanol. Dehydration was followed by immersion of the specimens in a small volume of hexamethyldisilanzane for one and a half days. The specimens were placed under a vacuum to remove excess hexamethyldisilanzane, and then mounted on metal stubs, sputter coated in Gold-palladium and viewed with the Scanning Electron Microscope.

2.3 Data Analysis

2.3.1 Ecological statistics

For each of the collected species, the prevalence (number of infected mobulids by a specific copepod species/total number of mobulids examined X 100); the mean intensity (total number of specific collected parasites/the number of infected mobulids) and the mean abundance (total number of specific parasite species/total number of mobulids examined) were determined according to Bush *et al*. (1997). Additionally the distribution/dispersal of some species' component population was determined by calculating the sample mean ($\bar{x}$) and the variance (s^2), according to Bush *et al*. (2001).

Anatomical terminology conforms to that of Boxshall (1990) and Kabata (1979), while host nomenclature is according to Compagno (1999) and Michael (1993).

CHAPTER 3: Family Caligidae Burmeister, 1835

3.1 Introduction

Established in 1835 by H. Burmeister, Caligidae is considered the largest family of the Siphonostomatoida (Kabata 1979; Boxshall & Montu 1997; Boxshall & Halsey 2004; Ho & Lin 2010). It comprises of more than 450 species distributed over 34 accepted genera with another one, *Calistes* Dana, 1852, listed as a *genus inquirendum* (Boxshall & Halsey 2004; Boxshall 2010). The total number of genera includes those previously placed in the family Euryphoridae synonymized by Boxshall and Halsey in 2004 (Walter & Boxshall 2008). Members of Caligidae predominantly parasitize marine fish, a small percentage of which are elasmobranchs (Kabata 1979; Ho *et al*. 2000; Boxshall & Halsey 2004; Venmathi & Ohtsuka 2008; Ho & Lin 2010). The various members of this family inhabit the external body surfaces, the mouth and oral cavity, the gill lamellae and filaments of their hosts (Kabata 1979; Benz 1994; Boxshall & Montu 1997; Boxshall & Halsey 2004). Representatives of this family are characterized by a broad, dorsoventrally flattened cephalothorax where the first three segments and the first three pairs of legs are incorporated into the cephalothorax and the fourth thoracic segment is free (Kabata 1979; Boxshall & Montu 1997; Boxshall & Halsey 2004; Ho & Lin 2004; Ho & Lin 2010). Caligids are however closely associated to five other Siphonostomatoida families, together known as the caligiforms, all having the broad, dorsoventrally flattened cephalothorax (Kabata 1979; Benz 1994; Ho & Lin 2004). These families include Cecropidae Dana, 1849, Dissonidae Yamaguti, 1963, Euryphoridae (now synonymized with Caligidae), Pandaridae Milne Edwards, 1840 and Trebiidae C.B Wilson, 1905 (Kabata 1979; Dojiri 1983; Boxshall & Halsey 2004; Ho & Lin 2010). Members of Caligidae can be distinguished from other caligiforms by the single free fourth thoracic segment and the lack of dorsal plates on this segment (Kabata 1979; Boxshall & Halsey 2004; Ho & Lin 2004; Ho & Lin 2010).

The caligid copepods are well known as economically important parasites in the aquaculture industry (Ho & Lin 2002; Ho & Lin 2004; Venmathi & Ohtsuka 2008) and several species have emerged as major pathogens and serious pests in farmed and wild fin-fish in the commercial aquaculture facilities (Todd 2007; Boxshall & El-Rashidy 2009). Caligid copepods are predominantly external symbionts of these commercially important fishes (Boxshall & Montu 1997; Ho *et al*. 2000; Luque & César 2000). Of the 35 genera in this family, the genus with the highest number of species is *Caligus* Müller, 1785 with well over 262 known species (Kabata 1979; Benz 1994; Boxshall & Montu 1997; Luque & César 2000; Boxshall & El-Rashidy 2009; Ho & Lin 2010) followed by *Lepeophtheirus* with more than 107 species (Ho & Lin 2010; Morgan *et al*. 2010; Boxshall 2010).

3.2 Caligidae collected from examined mobulids

3.2.1 *Caligus* Müller, 1785

Introduction

In addition to the 262 described species, there are seven other species described within this genus listed as *nomen nudum* (Boxshall 2010). *Caligus* apparently, was the second fish parasitic copepod ever to be mentioned in scientific literature and is therefore seen as the stereotypical copepod parasite of fish that everyone envisions (Williams & Williams 1996; Vinoth *et al*. 2010). Members of this genus can infect a broad range of hosts but are predominantly parasitic on marine teleosts (Kabata 1979; Benz 1994; Tang & Newbound 2004; Ho & Lin 2010). Most species are strictly host specific and they often accumulate in small areas that may provide easy feeding and protection (Kabata 1979; Dojiri 1983; Benz 1994; Williams & Williams 1996; Ho & Lin 2004). Of the 269 known species of *Caligus*, only 17 have been reported from elasmobranch hosts with four occurring solely on elasmobranchs (Tang & Newbound 2004; Morgan *et al*. 2010). Twenty nine out of the 269 species have been reported from the coast of South Africa (Grobler *et al*. 2004; Dippenaar 2005). Only a single species (*Caligus elongatus* von Nordmann, 1832), was reported from elasmobranch hosts *Carcharias taurus* (Rafinesque, 1810), *Carcharhinus obscurus (*Lesueur, 1818) and *M*. *kuhlii* off the east coast of South Africa (Dippenaar 2005; Dippenaar & Jordaan 2007). Records of *Caligus* species reported from mobulids across the world's oceans include *C*. *alalongae* Kroyer, 1963 from *Mobula rochebrunei* (Vailant, 1879) and *C*. *rufimaculatus* Wilson, 1905 from *Mobula* sp. (Tang & Newbound 2004). The only record of a *Caligus* species reported from a mobulid frequenting South African waters is thus *C*. *elongatus* (Dippenaar 2005; Dippenaar & Jordaan 2007).

Morphologically, *Caligus* specimens are very similar to those of *Lepeophtheirus,* with a single distinct characteristic (the presence or absence of lunules) separating the two genera from each other (Kabata 1979; Dojiri 1983). *Caligus* species occur in warm temperate waters while *Lepeophtheirus* species occur in colder waters (Dojiri 1983). *Caligus* specimens can be distinguished from other caligids by the presence of lunules on the front of the cephalothorax, the H-shaped grooves on top of the cephalothorax, a cephalothorax that is usually circular and egg strings that are longer than the rest of the body (Kabata 1979; Dojiri 1983; Williams & Williams 1996).

3.2.1.1 *Caligus chrysophrysi* Pillai, 1985

Material collected: From *M. kuhlii* 44 ♀♀ and five ♂♂ --- 30 ♀♀ and five ♂♂ from four hosts off Umdloti (29.40S 31.08E) caught during January 2010, January 2011 and February 2012 respectively; one ♀ from one host off Umhlanga Rocks (29.43S 31.05E) caught during December 2011; one ♀ from one host off Ansteys Beach (29.55S 31.01E) caught during January 2010; nine ♀♀ from three hosts off Durban (29.51S 31.00E) caught during January 2010 and December 2011 and three ♀♀ from two hosts off Karridene (30.07S 30.31E) during January and November 2011 respectively. From *M. alfredi* 10 ♀♀ and one ♂ --- two ♀♀ from a host off Karridene (30.07S 30.31E) caught during January 2011 and eight ♀♀ and one ♂ from a host off San Lameer (30.57S 29.20E) caught during August 2010.

All the specimens were found on the external body (skin) surfaces and gill arches of the examined hosts.

Morphological analysis:

According to Pillai (1985) *C. chrysophrysi* is commonly found and easily distinguishable. It is distinguished from its congeners by having nearly parallel limbs of the sternal furca, the shape and size of the claws on all three segments of the exopod of leg 2, the armature of exopod segment 3 of leg 1, and the slender elongate setae of the caudal rami. The first record of *C. chrysophrysi* is from *Rhabdosargus sarba* (Forsskål, 1775) caught off Kerala, India (Pillai 1985).

Distribution statistics of component population:

Caligus chrysophrysi exhibited a prevalence of 35.34% and 28.57%, a mean intensity of 5 and 6 individuals per host and a mean abundance of 2 on *M. kuhlii* (Table 1; Figs. 1, 2, 3) and *M. alfredi* (Table 3; Figs. 1, 2, 3) respectively.

3.2.1.2 Remarks

Although considered a commonly occurring species by Pillai (1985), *Caligus chrysophrysi* has only been reported from *R. sarba*. The current study provides the first record of this species from an elasmobranch host worldwide. Thus, *C. chrysophrysi* is reported for the first time from *M. kuhlii* and *M. alfredi* frequenting the east coast of South Africa. Dippenaar and Jordaan (2007) reported *C. elongatus* from *C. taurus*, *C. obscurus* and *M. kuhlii* caught in South African waters. The specimens examined from *M. kuhlii* (and possibly the other two hosts) were however misidentified. Thus the specimens of *Caligus* encountered on *M. kuhlii* examined in the present study are *C. chrysophrysi* and not *C. elongatus* as reported by Dippenaar and Jordaan (2007). The identification of the examined specimens by Dippenaar and Jordaan (2007) was based on the description of *C. elongatus* by Parker (1969), (S.M. Dippenaar, pers. comm.). According to Parker (1969), *C. elongatus* can easily be distinguished by the strong diverting tines (with rounded tips) of the sternal furca and the appearance of the four terminal spines of the third exopodal segment of leg 1, where the outermost spines (spine 1 and the twice as long spine 4) are simple and the two middle spines are bifurcated at mid-length. *Caligus chrysophrysi* however, also possesses the same armature of the third exopodal segment of leg 1, as do most species within this genus (Pillai 1985; Ho & Lin 2004; D. Tang, unpubl. data). Furthermore the tines of the sternal furca in *C. chrysophrysi* are nearly parallel and not diverging (Pillai 1985; D. Tang, unpubl. data). *Caligus chrysophrysi* was thus identified and distinguished from *C. elongatus* based on descriptions by Pillai (1985) and the unpublished figures by Tang (D. Tang, unpubl. data). *Caligus chrysophrysi* can easily be distinguished by the stout posterior lobe of the maxillule; the shape and size of the spines of the three exopod segments of leg 2; the long hairs that fringe the lateral border of all three leg 2 endopod segments; the shape, size and armature of the four terminal spines of leg 1 exopod segment 3 and the extremely long, slender, pinnate setae of the caudal rami

(Pillai 1985; D. Tang, unpubl. data). Thus, *C. elongatus* does not infect *M. kuhlii* and the current study therefore represents the first record of *C. chrysophrysi* from *M. kuhlii*, *M. alfredi* and from the east coast of South Africa.

Caligus chrysophrysi exhibited a higher prevalence on *M. kuhlii* (35.34%) (Table 1; Fig. 1) than on *M. alfredi* (28.57%) (Table 3; Fig. 1) and has a mean intensity of 5 individuals per host on *M. kuhlii* (Table 1; Fig. 2) and 6 individuals per host on *M. alfredi* (Table 3; Fig. 2).

3.2.2 ***Pupulina*** **van Beneden, 1892**

Introduction

Discovered and first described by P.J. van Beneden in 1892 from specimens acquired in Azores, the genus *Pupulina* has always been inadequately known (Wilson 1935; Wilson 1952; Dojiri 1983). In 1899, Bassett-Smith denied the validity of *Pupulina* and consequently placed it in the well-known genus *Lepeophtheirus* (Wilson 1935). The discovery of species similar to van Beneden's specimens on mobulids off the Dry Tortugas and the Galapagos Islands in 1929, subsequently led to the validation of van Beneden's claims that *Pupulina* is an independent genus (Wilson 1935; Wilson 1952). Van Beneden described *Pupulina flores* as the type species and it was recorded for a second time by Bere in 1936 (Wilson 1952). According to Wilson (1935) the original description by van Beneden was incomplete which then led to the re-description of *P. flores* after 40 years. Thereafter two new species, *Pupulina minor* and *Pupulina brevicauda* were described by Wilson (1952). These were collected from the giant devil ray *Mobula lucasana* caught off the coast of California (Wilson 1952). Therefore, the genus presently contains three accepted species (Boxshall 2010). All the reports of *Pupulina* species are from members of the devil ray family Mobulidae from various localities (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964; Dojiri 1983; Pillai 1985). This therefore suggests that members of this genus are specific to *Mobula* species (Wilson 1952; Dojiri 1983).

Members of this genus can be recognized and clearly distinguished from other caligid species by the general habitus (Wilson 1935; Wilson 1952; Dojiri 1983; Pillai 1985). Some of the features distinguishing *Pupulina* specimens are the presence of a dentiform process attached to the ventral surface immediately posterior to the maxillipeds; the presence of posterolateral processes on the genital complex of the female; the well-developed endopod of leg 1; the distinctly 3-segmented rami of leg 3; the armature on the exopod of leg 3 and the armature of leg 4 (Wilson 1952; Dojiri 1983; Pillai 1985). The only character that *Pupulina* shares with other members of Caligidae is the absence of lunules and while most members possess a sternal furca, *Pupulina* does not (Wilson 1935; Wilson 1952; Dojiri 1983; Pillai 1985). Overall, the genus *Pupulina* is very unique and all its members are easily distinguishable from each other (Wilson 1952; Dojiri 1983; Pillai 1985).

3.2.2.1 *Pupulina* sp. 1

Material collected: From *M. kuhlii* 78 ♀♀ --- 11 ♀♀ from four hosts off Umdloti (29.40S 31.08E) caught during April 2004 and January 2011; seven ♀♀ from one host off Umhlanga Rocks (29.43S 31.05E) caught during December 2011; 28 ♀♀ from five hosts off Durban (29.51S 31.00E) caught during April 2004, November 2010, December 2010 and December 2011; three ♀♀ from one host off Winkelspruit (30.06S 30.51E) caught during December 2010; 15 ♀♀ from three hosts off Karridene (30.07S 30.37E) caught during January and November 2011; five ♀♀ from two hosts off Park Rynie (30.19S 30.44E) caught during April 2000 and January 2011; six ♀♀ from one host off Hibberdene (30.34S 30.34E) caught during April 2009 and three ♀♀ from one host off Umzumbe (30.32S 30.51E) caught during January 2011. From *M. eregoodootenkee* nine ♀♀ --- nine ♀♀ from 2 hosts off Richards Bay (28.48S 32.06E) caught during September 2004.

All collected specimens were found on the external skin surfaces, gill arches and gill rakers of their hosts.

Adult female (Figs. 4 - 9):

Overall body length including setae of caudal rami about 6.63 mm. Cephalothorax (Fig. 4a, b) composed of head fused with first three thoracic segments, posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 4a, b) nearly circular, longer than wide, posterior margin ovally extended, reaching fourth free thoracic segment. Fourth thoracic segment wider than long, without ornamentations. Genital complex (Fig. 4a, b) almost square, half as broad as cephalothorax, anterolateral corners extending slightly anteriorly, without posterolateral expansions. Abdomen (Fig. 4a, b) overall length 1.18 mm; indistinctly 2-segmented, sparsely spinulated; segment 1 long, slender; segment 2 less than half the length of segment 1. Caudal rami (Figs. 5a; 9a) almost ½ as long as abdomen (0.65 mm, excluding setae), sparsely spinulated, with one pinnate seta laterally about mid-length and five long, apical (three pinnate, two naked) setae.

Antennule (Fig. 4c) 2-segmented; segment 1 broad with 25 plumose setae on anterodistal surface; segment 2 slender, elongate, armed with 13 naked setae (one seta at mid-length, two sub terminal setae, eight terminal setae and two aesthetascs). Antenna (Figs. 4d; 8c, d) 3-segmented (4-segmented in previous descriptions); coxa (segment 1) armed with blunt, posteromedial process; basis (segment 2) broad, with small, flat, protruding pad on inner margin; subchela (segment 3) long, strongly curved claw (Fig. 8c) with sharp tip armed with one naked seta on protrusion proximally and one slender, naked seta at mid-length; post-antennal process (Fig. 4e), slightly curved tubercle armed with two papillae each bearing three long setules plus an inner papilla with three long setules. Mouth tube (Fig. 8a, b) typical of caligids, longer than wide with intrabuccal stylet and strigil (Fig. 5d); mandible (Fig. 5b, c) with 11 teeth. Maxillule (Figs. 5e; 8a, e) biramous; palp a small papilla, bearing two short and one long naked setae; endite a large, simple, sub-triangular spiniform process; post-maxilluliary process (Figs. 5e; 8a, e) a small dentiform process attached to the ventral surface of cephalothorax medial to tip of endite. Maxilla (Figs. 5f; 8f) branchiform, 2-segmented; syncoxa (lacertus) long, armed with short, blunt process proximally; basis (brachium) slender, armed with a serrated membranous flabellum and a flat setuled ridge distally with a serrated longer calamus and shorter canna. Maxilliped (Figs. 5g; 8f) 2-segmented; protopod (corpus) long, broad, unarmed; subchela short, armed with naked seta proximally and a short, sharply curved terminal claw; post-maxillipedal process (usually attached to ventral surface near base of maxilliped in other *Pupulina* species) not observed.

Armature of rami of legs 1-4 (Figs. 6a, b; 7a, b; 9b, c, d, e, f) as follows (Roman numerals indicating spines and Arabic numerals setae):

	Exopod	Endopod
Leg 1	0-0; IV-3	0-0; 0-3
Leg 2	I-1; I-1; II-6	0-1; 0-2; 0-6
Leg 3	I-1; I-1; III-4	0-0; 0-2; 0-4
Leg 4	I-0; I-0; IV-0	absent

First three pairs of legs biramous with 3-segmented rami (except leg 1 with 2-segmented rami); sympods of legs 1 and 2, 2-segmented, each with one inner pinnate seta and one outer pinnate seta, not observed on leg 3 sympod.

Leg 1 (Figs. 6a; 9b, c) exopod segment 1 with rows of setules on inner margin; segment 2 with four (two serrated; one setuled; one naked with a bifurcate tip) (Fig. 9c) spines terminally and three long pinnate setae; endopod segment 1 broad, tapering apically, unarmed; segment 2 small, curved inwards, armed with three long pinnate setae terminally and bearing setules on lateral margin. Leg 2 (Figs. 7a; 9d) sympod with striated membrane; exopod segments 1 and 2 each armed with one large, serrated outer spine (extending across surface of next segment) and one long inner pinnate seta; segment 3 small, with two distal spines and six pinnate setae; endopod segments 1 and 2 each armed with a semi-circular lamina on lateral margin lined with rows of fine setules; segment 1 small, with one inner pinnate seta; segment 2 broad, with two large, inner pinnate setae distally and fine setules along inner margin; segment 3 small, armed with six (three long, three short) pinnate setae. Leg 3 (Figs. 7b; 9e) exopod segments 1 and 2 each bearing one outer spine and one long, inner pinnate seta; segment 3 small, armed with three, naked apical spines, four pinnate setae distally and lined with fine setules on outer lateral margin; endopod segment 1 armed with extremely large semi-circular lamina (covers entire surface of first two exopodal segments and half of endopod segment 2), lined with rows of fine setules; segment 2 extended laterally into smaller semi-circular velum lined with fine setules on border and two large inner pinnate setae distolaterally; segment 3 small, armed with four pinnate setae. Leg 4 (Figs. 6b; 9f) uniramous, 3-segmented; sympod large, armed with scattered spinules dorsally and one distolateral pinnate seta; segments 1 and 2 slender, each armed with one setuled spine bearing a pectinate membrane at base (segment 1 with short spinules on dorsal margin); segment 3 armed with three setuled terminal spines and one, small naked terminal seta with pectinate membrane at base. Leg 5 (Fig. 7c) small, papilla-like process armed with three pinnate setae. Leg 6 absent/not observed.

Remarks:

Pupulina sp. 1 marks the first *Pupulina* species reported from *M*. *kuhlii* and *M*. *ergoodootenkee* worldwide as well as from South African waters. *Pupulina* sp. 1 shares most of its characteristics with the three described species, but can be distinguished from them by the outward appearance of the general habitus and the shape and size of the genital segment. *Pupulina* sp. 1 can be distinguished from *P*. *flores*, *P*. *minor* and *P*. *brevicauda* by possessing a genital segment with anterolateral corners slightly extending anteriorly and lacking posterolateral expansions; while all three described species lack anterolateral expansions. Furthermore, *P*. *flores* and *P*. *mino*r both have posterolateral corners slightly extending posteriorly while *P*. *brevicauda* possesses posterolateral corners that are extending well beyond the tips of the caudal rami. *Pupulina* sp. 1 also has a 2-segmented abdomen, a character which it shares with both *P*. *minor* and *P*. *brevicauda*, while *P*. *flores* has a 3-segmented abdomen. *Pupulina* sp. 1 also possesses an abdomen as long as the genital segment while the abdomen is longer than the genital segment in *P*. *flores* and *P*. *brevicauda* and shorter than the genital segment in *P*. *minor*. *Pupulina* sp. 1 can further be distinguished from all three described species by possessing antennae that are 3-segmented, mandibles with 11 teeth and rows of spinules lining the inner margins of the caudal rami while all three described species have 4-segmented antennae, mandibles with 12 teeth and no ornamentations on the inner margins of caudal rami. Therefore, the most distinguishing characteristics of *Pupulina* sp. 1 are the anterolateral corners of the genital segment that are slightly extending anteriorly and an abdomen that is as long as the genital segment.

Distribution statistics of species component population:

Pupulina sp. 1 exhibited a prevalence of 61.29% and 100% (Fig. 1), a mean intensity of 41 and 5 individuals per host and mean abundance of 3 (Fig. 2) and 5 (Fig. 3) individuals per host on *M*. *kuhlii* and *M*. *eregoodootenkee* respectively (Tables 1 &

2). Component populations on both hosts showed aggregated distribution patterns (Tables 1 & 2).

3.2.2.2 *Pupulina* sp. 2

Material collected: From *M. kuhlii* --- three ♀♀ from two hosts off Karridene (30.07S 30.37E) caught during January and November 2011. From *M. eregoodootenkee* --- one ♀ from one host off Richards Bay (28.48S 32.06E) caught during September 2004.

All collected specimens were found on the external skin surfaces and gill rakers of their hosts.

Adult female (Figs. 10-13):

Overall body length, including setae of caudal rami about 4.8 mm. Cephalothorax (Fig. 10a, b) composed of head fused with first three thoracic segments, posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 10a, b) circular, slightly wider than long, lined with minute papillae bearing thin, fine setules along ventral border, posterior margin ovally extended posteriorly and armed with scattered spinules dorsally. Fourth thoracic segment distinctly wider than long, covered with scattered spinules dorsally. Genital segment (Fig. 10a, b) square, without posterolateral and anterolateral lobes and with spinules dorsally and ventrally, spinules more abundant along lateral and distolateral margins. Abdomen (Fig. 10a, b) overall length 0.605 mm, indistinctly 2-segmented, highly spinulated dorsally and ventrally, longer than wide, almost as long as genital complex. Caudal rami (Fig. 12a) slender, as long as abdomen (excluding setae), armed with row of long stiff hairs along inner margins, minute spinules on dorsal and ventral surfaces and six (one distomedial and five terminal) naked setae.

Antennule (Fig. 10c) 2-segmented; segment 1 broad, with 27 pinnate setae anterodistally; segment 2 slender, moderately long, armed with one pinnate seta at mid-length and 11 (two sub-apical naked setae, seven terminal naked seta and two aesthetascs) setae. Antenna (Fig. 10d) 3-segmented; coxa (segment 1) small, armed with short, pointed inner process posteriorly; basis (segment 2) broad, square-like, unarmed; subchela (segment 3) strongly curved, with one naked seta proximally and one naked seta at mid-length; post-antennal process (Fig. 10e), curved tubercle armed with two papillae each bearing two long setules and an inner papilla with three long setules. Mouth tube (Fig. 12b) longer than wide, with intrabuccal stylet and strigil (Fig. 12c); mandible (Fig. 811a) armed with 11 teeth. Maxillule (Fig. 11b) biramous; palp a small basal papilla armed with three naked setae; endite sub-triangular, spiniform process; post-maxilluliary process (Fig. 11b) a small sub-triangular, dentiform process attached to the ventral surface of cephalothorax medial to tip of endite. Maxilla (Fig. 11d) branchiform, 2-segmented; syncoxa (lacertus) broad, with short blunt process at base and a flattened protruding pad distomedially; basis (branchium) slender, armed with a serrated membranous flabellum and flattened, setuled ridge distally and a short serrated canna with a longer serrated calamus terminally. Maxilliped (Fig. 11e) 2-segmented; protopod (corpus) broad, unarmed; subchela armed with a sharply pointed terminal claw and one thin naked seta proximally on claw; post-maxillipedal process (Fig. 11f), a small dentiform process attached to the ventral surface of cephalothorax medially to base of protopod.

Armature of rami of legs 1-4 (Figs. 12d, e; 13a, b, c) as follows (Roman numerals indicating spines and Arabic numerals setae):

	Exopod	Endopod
Leg 1	0-1; IV- 3	0-0; 0-3
Leg 2	II-1; I-1; II-6	0-1; 0-2; 0-6
Leg 3	I-1; I-1; III-4	0-0; 0-2; 0-4
Leg 4	I-0; I-1; III-1	absent

First three pairs of legs biramous with 3-segmented rami (except leg 1 with 2-segmented rami); sympods of legs 1 and 2 each with one inner and one outer pinnate setae, not observed on leg 3 sympod.

Leg 1 (Fig. 12d, e) exopod segment 1 with row of setules on inner margin and one small naked seta distolaterally; segment 2 with four (two serrated; one setuled; one naked with a bifurcate tip) spines terminally and three pinnate setae; endopod segment 1 unarmed; segment 2 small, with three pinnate setae and bearing fine setules on inner margin. Leg 2 (Fig. 13a) sympod armed with a striated membrane on inner margin; exopod segments 1 and 2 each armed with one serrated spine distolaterally (segment 1 spine with pectinate membrane and one smaller naked spine at base) and one inner pinnate seta; segment 3 small, with two spines (one moderately large, serrated on both sides and one smaller, unarmed spine) distally and six pinnate setae; endopod segments 1 and 2 each extended into semicircular lamina lined with rows of fine setules; segment 1 small, with one inner distal pinnate seta; segment 2 longer, with 2 inner pinnate setae distally; third segment small, armed with six pinnate setae. Leg 3 (Fig. 13b) exopod segments 1 and 2 each bearing one setuled outer spine and one inner pinnate seta distally (segment 2 with setules on lateral margin); segment 3 small, with three naked, distal spines and four pinnate setae; endopod segment 1 armed with extremely large semicircular lamina (covers entire surface of first two exopodal segments and half of endopod segment 2) lined with rows of fine setules; segment 2 extended laterally into smaller semicircular velum lined with fine setules on border and two large inner pinnate setae distally; segment 3 small, with four pinnate setae. Leg 4 (Fig. 13c) uniramous, 3-segmented; sympod broad, armed with short scattered spinules dorsally, a pectinate membrane at base of distolateral spinule and a row of long, stiff hairs on dorsal surface; segment 1 armed with one outer serrated spine with a pectinate membrane at base and short spinules on distolateral margin; segment 2 with one inner, pinnate seta and an outer serrated spine with pectinate membrane at base; segment 3 armed with one inner, pinnate seta with pectinate membrane at base and three serrated terminal spines. Leg 5 (Fig. 11g) small, papilla-like process armed with three pinnate setae. Leg 6 absent/not observed.

Remarks:

Pupulina sp. 2 is the second record of a *Pupulina* species infecting *M*. *kuhlii* and *M*. *eregoodootenkee* from across the world's oceans as well as from South African waters. *Pupulina* sp. 2 appears different from *Pupulina* sp. 1, *P*. *flores*, *P*. *minor* and *P*. *brevicauda* with particular reference to the appearance of the general habitus and the shape of the genital segment. The anterior margin of the genital segment in *Pupulina* sp. 2 is square-like and the genital segment lacks anterolateral and posterolateral expansions while the anterior margin is rounded in *P*. *flores*, *P*. *minor* and *P*. *brevicauda*, whereas the anterolateral corners slightly extend anteriorly in *Pupulina* sp. 1. *Pupulina* sp. 2 also possesses a 1-segmented abdomen while the abdomen is 2-segmented in *Pupulina* sp. 1, *P. minor* and *P. brevicauda* and 3-segmented in *P. flores*. The caudal rami of *Pupulina* sp. 2 are as long as the abdomen and are armed with rows of stiff hairs along the inner margins, while the caudal rami are shorter than the abdomen in *Pupulina* sp. 1, *P. flores* and *P. brevicauda* and longer than the abdomen in *P. minor* and all are armed with short spinules along the inner margins. *Pupulina* sp. 2 also possesses long, inner pinnate setae on segments 2 and 3 of leg 4 and a pectinate membrane basal to the outer distal spinule on the sympod of leg 4 while *Pupulina* sp. 1, *P. flores*, *P. minor* and *P. brevicauda* all have short spines on segments 2 and 3 of Leg 4 and the sympods are armed with minute spinules. Therefore, the most distinguishing characteristics of *Pupulina* sp. 2 are the caudal rami which are the same length as the abdomen and armed with rows of long stiff hairs along the inner margins as well as the lack of anterolateral and posterolateral expansions on the genital segment.

Distribution statistics of component population:

Pupulina sp. 2 exhibited a prevalence of 3.22% and 50% (Fig. 1); a mean intensity of one individual per host (Fig. 2) and a mean abundance of 0 and 1 (Fig. 3) individual per host on *M. kuhlii* and *M. eregoodootenkee* (Tables 1 & 2) respectively.

3.2.2.3 *Pupulina* sp. 3

Material collected: From *M. eregoodootenkee* --- one ♀ from one host off Richards Bay (28.48S 32.06E) caught during September 2004. The collected specimen was found attached to the gill rakers of the examined host.

Adult female (Figs. 14-17):

Overall body length, excluding setae of caudal rami about 7.74 mm. Cephalothorax composed of head fused with first three thoracic segments, posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 14a, b) nearly circular, almost as wide as long, posterior margin ovally extended, reaching fourth free thoracic segment. Fourth thoracic segment wider than long, unornamented. Genital segment (Fig. 14a, b) rectangular-like, longer than wide, posterolateral corners extending slightly posteriorly, without anterolateral expansions. Abdomen (Fig. 14a, b) overall length 2.15 mm; longer than genital segment, 3-segmented with segments decreasing in length. Caudal rami (Figs. 14a, b & 16a) less than ¼ length of the abdomen, sparsely spinulated, with six (one pinnate dorsally about mid-length, one pinnate sub-apically and four long, naked apical) setae.

Antennule (Fig. 14c) 2-segmented; segment 1 broad with 25 plumose setae on anterodistal surface; segment 2 slender, elongate, armed with 13 naked setae (one seta at mid-length, two sub-terminal setae, eight terminal setae and two aesthetascs). Antenna (Fig. 15a) apparently 3-segmented; coxa (segment 1) armed with short pointed posteromedial process; basis (segment 2) broad, with small, flat, serrated inner protruding pad; subchela (segment 3) long, strongly curved claw armed with one naked seta on protrusion proximally and one slender, naked seta at mid-length; post-antennal process (Fig. 15b), strongly curved tubercle armed with two papillae each bearing two long setules plus an inner papilla with two long setules. Mouth tube longer than wide with intrabuccal stylet and strigil (Fig. 17c); mandible (Fig. 17a, b) with 11 teeth. Maxillule (Fig. 15c) biramous; palp, a small papilla (not well pronounced) bearing two short and one long naked setae; endite a simple, sub-triangular spiniform process; post-maxilluliary process (Fig. 15d) a small sub-triangular, spine-like process attached to the ventral surface of cephalothorax medial to tip of endite. Maxilla (Fig. 15e) branchiform, 2-segmented; syncoxa (lacertus) long, armed with short, blunt process proximally; basis (branchium) slender, armed with a serrated membranous flabellum and a flat setuled ridge distally and a serrated longer calamus and shorter canna terminally. Maxilliped (Fig. 15f) 2-segmented; protopod (corpus) long, broad, unarmed; subchela short, armed with one naked seta proximally and a short, sharply curved terminal claw; post-maxillipedal process not observed.

Armature of rami of legs 1-4 (Figs. 16b, c, d; 17d, e) as follows (Roman numerals indicating spines and Arabic numerals setae):

	Exopod	Endopod
Leg 1	0-0; IV-3	0-0; 0-3
Leg 2	I-1; I-1; I-6	0-1; 0-2; 0-6
Leg 3	I-1; I-1; III-4	0-0; 0-2; 0-4
Leg 4	I-0; I-1; III-1	absent

First three pairs of legs biramous with 3-segmented rami (except leg 1 with 2-segmented rami); sympods of legs 1 and 2, 2-segmented, each with one inner pinnate seta and one outer pinnate seta, not observed on leg 3 sympod.

Leg 1 (Figs. 16b, c) exopod segment 1 with rows of setules on medial margin; segment 2 with four (two serrated; one setuled; one naked with a bifurcate tip) (Fig. 16c) spines terminally and three long pinnate setae; endopod segment 1 broad, tapering apically, unarmed; segment 2 small, curved inwards, armed with three long pinnate setae terminally and bearing setules on lateral margin. Leg 2 (Fig. 17d) sympod with striated membrane on inner margin; exopod segments 1 and 2 each armed with one serrated outer spine (extending the length of next segment) and one long inner pinnate seta; segment 3 small, with one distal spine and six pinnate setae; endopod segments 1 and 2 each armed with a semicircular lamina lined with rows of fine setules; segment 1 small, with one inner pinnate seta; segment 2 longer, with two large, inner pinnate setae distally; segment 3 small, armed with six (three long, three short) pinnate setae. Leg 3 (Fig. 16d) exopod segments 1 and 2 each bearing one outer spine and one long, inner pinnate seta; segment 3 small, armed with three, naked apical spines, four pinnate setae distally and lined with fine setules on outer lateral margin; endopod segment 1 armed with extremely large semicircular lamina (covers entire surface of first two exopodal segments and half of endopod segment 2) lined with rows of fine setules; segment 2 extended laterally into a smaller semicircular lamina lined with fine setules on border and two large distomedial pinnate setae; segment 3 small, armed with four pinnate setae. Leg 4 (Fig. 17e) uniramous, 3-segmented; sympod large, armed with scattered spinules and a row of scattered slender hairs dorsally and one inner pinnate seta; segments 1 and 2, each armed with one setuled spine distolaterally, each with a pectinate membrane at base (segment 1 with short spinules on dorsal margin and segment 2 with a short, inner spine); segment 3 armed with three setuled terminal spines and one, thin naked seta with pectinate membrane at base. Leg 5 (Fig. 17f) small, papilla-like process armed with three pinnate setae. Leg 6 absent/not observed.

Remarks:

Pupulina sp. 3 is the third record of a *Pupulina* species encountered on *M. eregoodootenkee* worldwide as well as the east coast of South Africa. The general habitus of *Pupulina* sp. 3 differs from the three nominal species as well as *Pupulina* sp. 1 and *Pupulina* sp. 2 encountered in the present study. *Pupulina* sp. 3, like *P*. *flores*, *P*. *minor* and *P*. *brevicauda* has a rounded anterior margin of the genital segment which lacks anterolateral expansions and the posterolateral corners are extending posteriorly while *Pupulina* sp. 1 and *Pupulina* sp. 2 both lack posterolateral expansions. *Pupulina* sp. 3, similar to *P*. *flores*, also possesses a distinctly 3-segmented abdomen while the abdomen is 2-segmented in *P*. *minor*, *P*. *brevicauda*, *Pupulina* sp. 1 and 1-segmented in *Pupulina* sp. 2. Additionally, the abdomen is longer than the genital segment in *Pupulina* sp. 3, *P*. *flores* and *P*. *brevicauda* while the abdomen is as long as the genital segment in *Pupulina* sp. 1 and shorter than the genital segment in both *P*. *minor* and *Pupulina* sp. 2. Though *Pupulina* sp. 3 shares most characteristics with *Pupulina* sp. 1 and *Pupulina* sp. 2, it can easily be distinguished from them by having no ornamentations on the inner margin of the caudal rami while *Pupulina* sp. 2 has long stiff hairs along the inner margins of the caudal rami and *Pupulina* sp. 1 has rows of short spinules lining the inner margins of the caudal rami. *Pupulina* sp. 3 can also be distinguished from *Pupulina* sp. 1, *Pupulina* sp. 2, *P*. *flores*, *P*. *minor* and *P*. *brevicauda* by measuring the length of the caudal rami relative to the length of the abdomen. Thus, the caudal rami are less than a ¼ the length of the abdomen in *Pupulina* sp. 3; about $^{2}/_{3}$ the length of the abdomen in *Pupulina* sp. 1; the same length as the abdomen in *Pupulina* sp. 2; about ¾ the length of the abdomen in *P*. *flores*; about $^{1}/_{6}$ the length of the abdomen in *P*. *brevicauda* and longer than the abdomen in *P*. *minor*. The most distinguishing characteristics in *Pupulina* sp. 3 therefore, are an abdomen which is extremely longer than the genital segment and the caudal rami which are less than ¼ the length of the abdomen.

Distribution statistics of component population:

Pupulina sp. 3 exhibited a prevalence of 50%, a mean intensity of 1 individual per host and a mean abundance of 1 individual per host on *M*. *eregoodootenkee* (Table 2; Figs. 1, 2, 3).

3.2.2.4 Key to species of *Pupulina*

1. a. Leg 4 sympod with long stiff hairs along the inner margin………………….2

   b. Leg 4 sympod without long stiff hairs along inner margin………………...4

2. a. Abdomen 3-segmented; caudal rami shorter than abdomen and without long stiff hairs along inner central margin……………………………..…………3

   b. Abdomen 1-segmented; caudal rami as long as abdomen and armed with long stiff hairs along inner central margin……..……………………*Pupulina* sp. 2

3. a. Genital segment more than half as wide as cephalothorax; caudal rami less than a ¼ the length of the abdomen………...……………*Pupulina flores*

   b. Genital segment half as wide as cephalothorax; caudal rami about ¾ the length of the abdomen……………………………………………*Pupulina* sp. 3

4. a. Genital segment posterolateral corners extending posteriorly and without anterolateral expansions…………………..……………………………………..5

   b. Genital segment posterolateral corners not extending posteriorly and anterolateral corners extending anteriorly; abdomen as long as genital segment……………………………………………………………*Pupulina* sp. 1

5. a. Genital segment posterolateral extensions almost reaching segment 1 of the abdomen; caudal rami longer than the abdomen the abdomen…………………………………………………………..*Pupulina minor*

   b. Genital segment posterolateral corners extending well beyond the caudal rami; caudal rami about $^1/_6$ the length of the abdomen……………………………………………………*Pupulina brevicauda*

3.2.2.5 Remarks

Pupulina species have only been reported from mobulid rays (see Table 4); this therefore leads to the conclusion that these species are specific to these particular hosts (Wilson 1935; Wilson 1952; Dojiri 1983). Previous host records (see Table 4) for the three nominal species show a distribution across the world's oceans. *Pupulina flores* was reported from *Manta* sp. caught off Azores (Atlantic Ocean), *M.*

birostris caught off the Galapagos Islands (Pacific Ocean) and *M. hypostoma* caught off Lemon Bay Florida (Atlantic Ocean); *P. minor* and *P. brevicauda* were reported from *M. lucasana* caught off Santa Catalina, California in the Pacific Ocean, and *M. mobular* caught off Trivandrum India (Indian Ocean) (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964; Dojiri 1983). *Pupulina* sp. 1, *Pupulina* sp. 2 and *Pupulina* sp. 3 mark the first records of *Pupulina* species reported from *M. kuhlii* and *M. eregoodootenkee* frequenting South African waters as well as from the rest of the host's distribution (from the east coast of Africa to Indonesia).

Though the three nominal species of *Pupulina* are similar, they can be distinguished from each other by the shape and posterolateral expansions of the genital segment as well as its length relative to the length of the cephalothorax. *Pupulina brevicauda* has a genital segment that is longer than the cephalothorax and posterolateral processes that reach well beyond the tips of the caudal rami while *P. minor* has a genital segment that is slightly shorter than the cephalothorax and posterolateral corners which are slightly extending posteriorly and *P. flores* has a genital segments that is ½ as long as the cephalothorax and posterolateral corners that are slightly extending posteriorly (see Table 4 and key to species), (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964). The length of the caudal rami relative to the length of the abdomen can further be used to distinguish between the three described species as well as the three *Pupulina* species collected in the present study. According to previous descriptions and illustrations (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964), the caudal rami of *P. minor* are slightly longer than the abdomen; those of *P. brevicauda* are $^{1}/_{6}$ the length of the abdomen and those of *P. flores* are ¾ the length of the abdomen. Similarly, the main distinguishing feature between the three *Pupulina* species encountered in the present study is also the size and length of the caudal rami relative to the length of the abdomen. *Pupulina* sp. 1 has caudal rami that are a little over $^{2}/_{3}$ the length of the abdomen while the caudal rami of *Pupulina* sp. 2 are as long as the abdomen and those of *Pupulina* sp. 3 are less than a ¼ the length of the abdomen. The three *Pupulina* species encountered in the present study can also be distinguished from the three nominal species by having spinulated dorsal and ventral surfaces of the abdomen, 3-segmented antennae, mandibles with 11 teeth and basis of maxillae, each armed with a patch of fine setules on a ridge along the posterior margins (Table

4), while the three nominal species have naked ventral and dorsal surfaces of the abdomen, 4-segmented antennae and mandibles with 12 teeth (see Table 4).

Based on the calculated values of prevalence per host (Table 1; Fig. 1), *Pupulina* sp. 1 and *Pupulina* sp. 2 appear to have a higher preference for *M. eregoodootenkee* than they do for *M. kuhlii*. However, the mean intensity of infection was higher on *M. kuhlii* than on *M. ergoodootenkee* for *Pupulina* sp. 1 (with 41 and 5 individuals per host on *M. kuhlii* and *M. eregoodootenkee* respectively) and equal for *Pupulina* sp. 2, each with one individual per host, on both *M. kuhlii* and *M. eregoodootenkee* (Tables 1 & 2; Fig. 2). Furthermore, only two hosts of *M. eregoodootenkee* were examined in the current study compared to 31 hosts of *M. kuhlii* (Tables 1 & 2). More data is needed to properly compare intensity of infection and degree of preference by *Pupulina* species encountered on both *M. kuhlii* and *M. eregoodootenkee*, since they co-occur on the same host individuals. The coefficients of dispersion values (s^2) of *Pupulina* sp. 1 were higher than the mean ($\bar{x}$) on both *M. kuhlii* ($s^2 = 10.19$; $\bar{x} = 2.51$; $s^2 > \bar{x}$) and *M. eregoodootenkee* ($s^2 = 25$; $\bar{x} = 4.5$; $s^2 > \bar{x}$). Thus, the aggregated distribution pattern, typical for most parasite populations in a natural environment (Bush *et al*. 2001), indicates a degree of interaction by *Pupulina* sp. 1 individuals possibly due to a need for mating purposes, corporative feeding and mutual defense, as is the case with most aggregated parasite populations (Bush *et al*. 2001).

Because of their moderately large size (compared to other siphonostomatoids), caligids (in particular members of *Pupulina*) appear to need more space and thus are unable to infect a host in large numbers. This was previously noted by Dojiri (1983) where it was stated that *Pupulina* species tend to exhibit a low parasite load relative to each host. Previous encounters of the three nominal species have yielded relatively low numbers of species on each type of mobulid host. Thus, the highest number of *Pupulina* species encountered on mobulid hosts is 12 females of *P. flores* on *Manta birostris* (Wilson 1935). However, even though Wilson (1935) did not specify the number of *M. birostris* hosts that were infected with *P. flores*, it can be concluded that the present study provides the highest number of *Pupulina* species encountered on a single host with a mean intensity of 41 individuals of *Pupulina* sp. 1 on *M. kuhlii* (Table 1; Fig. 2). The current study also provides the lowest numbers of *Pupulina* species encountered on each host type with *Pupulina* sp. 2 and *Pupulina* sp. 3 both exhibiting mean intensities of one individual per host (Tables 1 & 2; Fig.

2). According to Williams and Williams (1996) and Vinoth *et al*. (2010), copepods and most external parasites tend to leave, be knocked off or washed off the host when it is caught. In the current study, most of the examined hosts were caught, washed and frozen for some time before examination, thereby causing some ectoparasitic specimens to be lost in the process. The current study therefore partly agrees with Dojiri's statement that *Pupulina* species commonly exhibit low parasite loads relative to each type of host. However, a deeper investigation on the intensity and preference of infection on each host will provide conclusive evidence on the host parasite relationships of these species on their mobulid hosts.

3.2.3 Unidentified species

3.2.3.1 Unidentified sp. 1

Material collected: From *Manta alfredi* --- two ♀♀ from one host caught off Margate (30.52S 30.21E) during February 2009. Both specimens were collected from the gill rakers of the examined host.

Adult female (Figs. 18-21):

Total body length including setae of caudal rami 24.15 mm. Cephalothorax (Fig. 18a, b) composed of head fused with first three thoracic segments, posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 18a, b) sub-orbicular, almost as wide as long, posterior margin ovally extended to cover most of third thoracic segment, frontal plates distinct without lunules. Fourth free thoracic segment hexagon shaped, wider than long, without dorsal plates. Genital segment (Fig. 18a, b) almost square, as wide as long, with short spinules lining the lateral borders and posterior corners slightly extending posteriorly. Abdomen (Fig. 18a, b) overall length about 4.46 mm, 3-segmented with segments decreasing in size. Caudal rami (Fig. 20a) slightly more than ½ the length of abdomen (1.92 mm excluding setae), with scattered spinules dorsally, armed with six (one small, distolaterally, one small distomedially and four longer, terminally) pinnate setae.

Antennule (Fig. 19a) 3-segmented; segment 1 with 20 pinnate setae; segment 2 elongate, armed with one pinnate seta distally; segment 3 armed with nine naked setae, one pinnate seta at mid-length and two aesthetascs distally. Antenna (Fig. 19b) 2-segmented; segment 1 broad and unarmed; segment 2 long, strongly curved claw, armed with one naked seta on a protrusion proximally, claw armed with one naked seta at mid-length; post-antennal process (Fig. 19c), strongly curved tubercle armed with two papillae each bearing two long setules plus an inner papilla with two long setules. Mouth tube longer than wide with strigil (Fig. 18e); mandible (Fig. 18d, e) with 12 teeth. Maxillule (Fig. 19d) biramous; palp small, elongated papilla armed with three naked setae; endite a dentiform process with extremely long and pointed tip, unarmed. Maxilla (Figs. 19e, f) branchiform, 2-segmented; syncoxa (lacertus) elongated, unarmed; basis (branchium) elongated, armed with rows of long fine setules along a line from the dorsal to the ventral margins, a short serrated canna and a longer calamus with serrations coiled around tip. Maxilliped (Fig. 20b) robust, large, 2-segmented; protopod (corpus) broad, unarmed; subchela short, armed with one naked seta and a long curved terminal claw. Sternal furca absent.

Armature of rami of legs 1-4 (Figs. 20c, d; 21a, b) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	0-1; IV-3	0-0; 0-3
Leg 2	I-1; I-1; I-6	0-1; 0-2; 0-6
Leg 3	I-1; I-1; III-5	0-1; 0-2; 0-4
Leg 4	I-1; I-1; III-2	Absent

First three pairs of legs biramous; leg 1 with 2-segmented rami, legs 2 and 3 with 3-segmented rami. Sympods of legs 1 and 2 each armed with one inner pinnate seta, almost at mid-length and one outer pinnate seta.

Leg 1 (Fig. 20c) sympod with cobblestone-like patches on lateral surface; exopod segment 1 broad, with row of picket fence-like extensions along inner margin and one naked seta distolaterally; segment 2 short, armed with four (two serrated, one naked with a bifurcate tip and one pinnate) spines and three longer, inner pinnate setae distally; endopod segment 1 broad and unarmed; segment 2 small, with three pinnate setae and bearing setules on lateral margin. Leg 2 (Fig. 21a) sympod armed with a striated membrane proximally on posterior margin; exopod segments 1 and 2 each armed with one serrated outer spine (spine of segment 1 with spinulated membrane at base) and one inner pinnate seta; segment 3 armed with one serrated spine and six pinnate setae; endopod segments 1 and 2 each armed with a large lateral semicircular lamina on lateral margins lined with rows of fine setules ; segment 1 armed with one medial, pinnate seta and segment 2 with two pinnate setae medially; segment 3 small, armed with six pinnate setae. Leg 3 (Fig. 21b) exopod segments 1 and 2 each armed with one inner pinnate seta and one outer serrated spine with spinulated membrane at base (segment 1 with a row of short spinules on lateral margin); segment 3 armed with three serrated spines, five pinnate setae and a setuled membrane on lateral margin; endopod segments 1 and 2 each bearing large semicircular lamina lined with fine setules on lateral margins; segment 1 with one very small outer naked seta distolaterally and segment 2 with two pinnate setae on inner margin; segment 3 small, armed with four pinnate setae. Leg 4 (Fig. 20d) uniramous, 3-segmented; sympod large, armed with short spinules on surface; segment 1 elongated, armed with short spinules on surface, one serrated spine distolaterally with spinulated membrane at base and one small, inner naked spine; segment 2 small, with one serrated spine and one small, inner naked spine, each with a setuled membrane at base, and lined with row of fine setules on lateral margin; segment 3 smaller, armed with row of fine setules on lateral margin, one short naked seta with setuled membrane at base, near base of longest serrated spine distally, one short, inner terminal seta and three serrated spines distally. Leg 5 (Fig. 20e) a small papilla-like process armed with three pinnate setae. Leg 6 not observed.

Remarks:

Unidentified sp. 1 exhibits similar morphological features as the species of *Pupulina*. These features include frontal lobes of the cephalothorax without lunules; the lack of dorsal plates on fourth free thoracic segment; the absence of a sternal furca; the presence of posterolateral expansions on the genital segment; well-developed endopods of leg 1 and the presence of semi-circular lamina on segments 1 and 2 of the endopods of legs 2 and 3. The current species can however, be distinguished from *Pupulina* species by possessing 3-segmented antennules; 2-segmented antennae; lack of a posteriorly directed spine-like process on segment 1 of the antennae; more pronounced palps of the maxillules; the absence of post-maxilluliary processes; the armature of the maxilla (no membranous flabellum on basis and calamus armed with coiled serrations around tip); medium sized laminae on segment 1 of leg 3 exopods and short, naked setae on the outer margins of the first endopodal segments of leg 3, while *Pupulina* species, have 2-segmented antennules; 4-segmented (three nominal species) and 3-segmented (three species encountered in the present study) antennae; posteriorly pointed spine-like processes on the antennae; flattened palps of the maxillules; the presence of post-maxilluliary processes; a membranous flabellum and bilaterally serrated calamus on the basis of the maxillae; an enlarged velum on segment 1 of the exopod of legs 3 and the elongated, pinnate setae on the first endopodal segments of legs 3. Unidentified sp. 1 is therefore not a *Pupulina* species. Possible distinguishing features of Unidentified sp. 1 are the caudal rami which are slightly less than ½ the length of the abdomen, the anterior margin of the genital segment is square-like and posterolateral corners are slightly extending posteriorly.

<u>Distribution statistics of species component populations:</u>

Unidentified sp. 1 exhibited a prevalence of 14.28%, a mean intensity of 2 individuals per host and a mean abundance of 0 individuals per host on *M. alfredi* (Table 3; Figs. 1, 2 & 3).

3.2.3.2 Unidentified sp. 2

<u>Material collected:</u> From *M. alfredi* --- two ♀♀ from one host caught off Margate (30.52S 30.21E) during February 2009. All specimens were collected from the gill rakers of the examined hosts.

<u>Adult female (Figs. 22-25):</u>

Total body length, including caudal rami 11.75 mm. Cephalothorax (Fig. 22a, b) composed of head fused with first three thoracic segments, posteriorly bearing fourth thoracic segment. Cephalothoracic shield (Figs. 22a, b) sub-orbicular, slightly wider than long, posterior margin ovally extended to cover most of third thoracic segment. Fourth free thoracic segment almost square-like. Genital segment (Figs. 22a, b) almost rectangular, longer than wide, about ⅔ the length of cephalothorax and armed with scattered spinules ventrally, without anterolateral and posterolateral expansions. Abdomen (Figs. 22a, b) overall length about 3.35 mm, elongated, distinctly 3-segmented; segment 1 oval shaped; segment 2 elongated and segment

3 short. Caudal rami (Fig. 24a) longer than wide, less than a ¼ the length of the abdomen (0.62 mm excluding setae), armed with scattered spinules dorsally and six very short, naked spine-like setae distally.

Antennule (Fig. 23a) 3-segmented; segment 1 broad, armed with 29 pinnate setae; segment 2 elongate with one naked terminal seta; segment 3 with nine naked setae and two aesthetascs distally. Antenna (Fig. 23b) 2-segmented; segment 1 broad, with small striated, protruding pad distomedially; segment 2 long, strongly curved claw armed with one broad naked seta near base; post-antennal process (Fig. 23c), strongly curved tubercle armed with two papillae each bearing two long setules plus an inner papilla with two long setules. Mouth tube longer than wide, intrabuccal stylet and strigil not observed. Mandible (Fig. 24b) with 12 teeth. Maxillule (Fig. 24c) biramous; palp small, elongated papilla bearing one long and one short seta; endite slightly bended dentiform process. Maxilla (Fig. 23d) branchiform, 2-segmented; syncoxa (lacertus) short and unarmed; basis (branchium) elongated, armed with rows of fine setules extending from the dorsal to the ventral surfaces, a shorter serrated canna (Fig. 23e) and a longer calamus (Fig. 23e) with serrations coiled around tip. Maxilliped (Fig. 24d) 2-segmented, extremely large, robust; protopod (corpus) broad, armed with short, inner naked seta at myxal area; subchela elongate, armed with a long, sharply curved terminal claw and one naked seta proximally on claw.

Armature of rami of legs 1-4 (Figs. 24e; 25a, b, c) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	0-0; IV-3	0-0; 0-3
Leg 2	I-1; I-1; I-6	0-1; 0-2; 0-6
Leg 3	I-1; I-1; III-4	I-0; 0-2; 0-4
Leg 4	I-1; I-1; III-2	Absent

First three pairs of legs biramous; leg 1 with 2-segmented rami and legs 2 and 3 with 3-segmented rami; sympods of legs 1 and 2 each with one inner pinnate seta, cobblestone-like patches and one outer pinnate seta.

Leg 1 (Fig. 24e) exopod segment 1 with rows of short spinules on inner margin; segment 2 small, armed with four short (two serrated, one setuled and one naked with a bifurcate tip) spines terminally and three longer pinnate setae distally; endopod segment 1 unarmed; segment 2 small, armed with three pinnate setae terminally and lined with fine setules on lateral margin. Leg 2 (Fig. 25a) sympod with a striated membrane proximally; exopod segments 1 and 2 each armed with one larger outer spine (spine of segment 1 serrated both sides with spinulated membrane lining base) and one inner pinnate seta; segment 3 small, armed with one naked spine and six pinnate setae; endopod segments 1 and 2 each extended into large lateral semicircular lamina lined with rows of fine setules and one inner pinnate seta (segment 2 with two inner pinnate setae); segment 3 small, armed with six pinnate setae. Leg 3 (Fig. 25b) exopod segment 1 with spinules on lateral margin and one outer serrated spine with spinulated membrane at base; segment 2 with one inner pinnate seta, one short serrated spine with spinulated membranes at base distally, lateral margin armed with membrane lined with setules; segment 3 armed with four pinnate setae and three serrated spines with a setuled membrane on lateral margin; endopod segments 1 and 2 each armed with large semicircular lamina lined with fine setules; segment 1 bearing one small, naked seta on inner margin; segment 2 armed with two inner pinnate setae distally; segment 3 small, armed with four pinnate setae. Leg 4 (Fig. 25c) uniramous, 3 segmented; sympod broad, armed with short spinules along dorsoventral surface and one small, outer naked seta distally; segment 1 elongated, armed with spinules on lateral surface and one outer serrated spine with setule bearing membrane at base and one short, inner naked seta distally; segment 2 armed with one serrated spine distolaterally with a setule bearing membrane at base, as well as a very small spinule, one short, inner naked seta with setule bearing membrane at base and setules along lateral margin; segment 3 lined with row of setules on lateral margin, armed with three serrated spines, two short naked setae and a spinulated membrane at base of longest serrated spine. Legs 5 and 6 not observed.

Remarks:

Unidentified sp. 2 is morphologically similar to Unidentified sp. 1; however, its general habitus is more slender than that of Unidentified sp. 1. Additionally, Unidentified sp. 2 possesses a fourth free thoracic segment that is as wide as long while the fourth free thoracic segment is wider than long in Unidentified sp. 1. The genital segment is almost rectangular, a little longer than wide and lacks posterolateral expansions in Unidentified sp. 2; while Unidentified sp. 1 has a genital segment that is almost square, as wide as it is long and has posterolateral corners that are slightly extending posteriorly. Unidentified sp. 2 can also be distinguished from Unidentified sp. 1 by measuring the length of the caudal rami relative to length of the abdomen. Thus the caudal rami are less than ¼ the length of the abdomen and armed with short, spine-like naked setae in Unidentified sp. 2 and slightly less than ½ the length of the abdomen and armed with slender, elongated pinnate setae in Unidentified sp. 1. Furthermore, the abdomen is longer than the genital segment and the first exopodal segments of leg 1 are armed with rows of short spinules along the inner margins in Unidentified sp. 2 while Unidentified sp. 1 possesses an abdomen that is as long as the genital segment and the first exopodal segments of leg 1 that are lined with rows of picket fence-like extensions along the inner margins. Therefore, the most distinguishing characteristics of Unidentified sp. 2 are the caudal rami that are less than a ¼ the total length of abdomen and armed with six short, naked spine-like setae terminally as well as the first exopodal segment armed with rows of short spinules along the inner margins.

Distribution statistics of species component populations:

Unidentified sp. 2 exhibited a prevalence of 14.28%, a mean intensity of two individuals per host and a mean abundance of 0 individuals per host on *M. alfredi* (Table 3; Figs. 1, 2, 3).

3.2.3.3 Unidentified sp. 3

Material collected: From *M. alfredi* --- one ♀ from one host caught off Margate (30.52S 30.21E) during February 2009. The specimen was collected from the gill rakers of the examined host.

Adult female (Figs. 26-28):

Total body length including caudal rami about 7 mm. Cephalothorax composed of head, fused with first three thoracic segments, posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 26a, b) sub-orbicular, as wide as long, posterior margin ovally extended to cover most of third free thoracic segment. Fourth free segment oval shaped, longer than wide. Genital segment (Fig. 26a, b) rectangular, wider than long with posterolateral corners slightly extending posteriorly, anterior corners not extended. Abdomen (Fig. 26a, b) overall length 1.42 mm, elongate, almost as long as fourth free thoracic segment and genital segment combined, 3-segmented with last segment oval shaped and longer than preceding segment. Caudal rami (Fig. 26c) short, about a ¼ the length of abdomen (0.42 mm excluding setae), armed with scattered spinules dorsally and five (three terminal and two distolateral) pinnate setae with probably another distomedially (not observed).

Antennule (Fig. 27a) 3-segmented; segment 1 broad, armed with 30 pinnate setae; segment 2 elongated, slender, armed with one naked seta terminally; segment 3 small, armed with 12 (10 naked and two aesthetascs) setae distally. Antenna (Fig. 28a) 2-segmented; segment 1 broad, unarmed; segment 2 elongate, strongly curved claw, armed with one broad, naked seta on a protrusion near base and one naked seta on a protrusion at mid-length; post-antennal process (Fig. 27b) strongly curved tubercle armed with papillae, each bearing two long slender setules. Mouth tube longer than wide, with strigil (Fig. 26e); mandible (Fig. 26d, e) with 12 teeth. Maxillule (Fig. 27c) biramous; palp a small, elongated papilla armed with three naked setae; endite large, elongated dentiform process, unarmed; post-maxilluliary process (Fig. 27c) small, mammiform, attached to the ventral surface of the cephalothorax inner to tip of the endite. Maxilla (Fig. 27d) branchiform, 2-segmented; syncoxa (lacertus) elongate, unarmed; basis (branchium) elongated, armed with rows of fine setules extending from the dorsal to the ventral surface, a short serrated canna and a longer calamus with serrations coiled around tip. Maxilliped (Fig. 27e) 2-segmented, extremely large, robust; protopod (corpus) broad, armed with one thin, inner naked seta at myxal area; subchela short, armed with a short curved terminal claw and one naked seta proximally on claw.

Armature of rami of legs 1-4 (Figs. 27f; 28b, c, d) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	0-0; IV-3	0-0; 0-3
Leg 2	I-1; I-1; I-6	0-1; 0-2; 0-6

Leg 3	I-1; I-1; III-4	0-1; 0-2; 0-4
Leg 4	I-1; I-1; III-2	Absent

First three pairs of legs biramous; leg 1 with 2-segmented rami and legs 2 and 3 with 3-segmented rami; sympods of legs 1 and 2 with spinulated patches on dorsoventral surface, not observed on leg 3 sympod.

Leg 1 (Fig. 28b) sympod with one small, inner naked seta, about mid-length; exopod segment 1 elongate, with rows of setules along inner margin; segment 2 small, armed with four (two serrated, one setuled and one naked with bifurcate tip) spines and three pinnate setae; endopod segment 1 unarmed; segment 2 small, armed with three pinnate setae terminally and lined with fine setules on lateral margin. Leg 2 (Fig. 28c) sympod armed with one long, inner pinnate seta, one short, outer pinnate seta distally and a striated membrane proximally; exopod segments 1 and 2 each armed with one setuled outer spine and one inner pinnate seta; segment 3 small, armed with one spine and six pinnate setae; endopod segments 1 and 2 each extended into large semicircular lamina laterally lined with rows of fine setules; segment 1 armed with one inner pinnate seta; segment 2 armed with two, inner pinnate setae; segment 3 small, armed with six pinnate setae. Leg 3 (Fig. 27f) exopod segments 1 and 2 each armed with one, inner pinnate seta and one outer serrated spine with a spinulated membrane at base (segment 1 also armed with rows of short spinules on lateral margin); segment 3 armed with three naked spines and four pinnate setae distally; endopod segments 1 and 2 each armed with a large semicircular lamina laterally lined with fine setules on border; segment 1 with one small naked seta distomedially, segment 2 armed with two pinnate setae on outer margin; segment 3 small, armed with four pinnate setae. Leg 4 (Fig. 28d) uniramous, 3-segmented; sympod broad, armed with short spinules, a short blunt process on inner distal margin, two spinulated membranes dorsally and one slender, pinnate seta on outer distal margin; segment 1 armed with short spinules on dorsal surface; segments 1 and 2 each armed with one naked, inner seta and one serrated outer spine with spinulated membrane at base; segment 3 armed with one naked inner seta, three setuled spines apically, a slender terminal pinnate seta and a spinulated membrane. Leg 5 (Fig. 28e) a small, papilla-like process armed with three pinnate setae. Leg 6 not observed/absent.

Remarks:

The outward appearance of the general habitus clearly separates this species from Unidentified sp. 1 and Unidentified sp. 2. Thus the habitus of Unidentified sp. 3 is shorter than the general habitus of both Unidentified sp. 1 and Unidentified sp. 2 and the shape and size of the genital segments of all three species are very different from each other. Additionally Unidentified sp. 3 is distinguishable from its congeners by possessing an oval shaped fourth free thoracic segment that is slightly longer than wide; while the fourth free thoracic segment is wider than long in Unidentified sp. 1 and as wide as long in Unidentified sp. 2; the fourth free thoracic segments are rectangular-like in both Unidentified sp. 1 and Unidentified sp. 2. Unidentified sp. 3, also possesses a genital segment that is rectangular, wider than long and with posterolateral corners slightly extending posteriorly while Unidentified sp. 1's genital segment is square-like, as wide as long and also has posterolateral corners that are slightly extending posteriorly, and Unidentified sp. 2's genital segment is almost rectangular, a little longer than wide and lacks posterolateral expansions. Unidentified sp. 3 can further be distinguished from its congeners by the measurement of the length of the caudal rami relative to the length of the abdomen, which are about a ¼ the length of the abdomen in Unidentified sp. 3; slightly less than ½ the length of the abdomen in Unidentified sp. 1 and less than a ¼ the length of the abdomen in Unidentified sp. 2. Unidentified sp. 3, like Unidentified sp. 2 has an abdomen that is longer than the genital segment while the abdomen of Unidentified sp. 1 is as long as the genital segment. Furthermore, Unidentified sp. 3 possesses post-maxilluliary processes while its congeners lack them.

The first exopodal segments of legs 1 are lined with rows of fine setules along the inner margins in Unidentified sp. 3; with rows of picket fence-like extensions along the inner margins in Unidentified sp. 1 and lined with rows of short spinules on the inner margins in Unidentified sp. 2. Additionally, the sympods of the fourth legs are each armed with short spinules, a short blunt process on the inner distal margin and two spinulated membranes dorsally, while both Unidentified sp. 1 and Unidentified sp. 2's fourth legs sympods are armed with short spinules on the dorsal surfaces. Therefore, the most distinguishing characteristics of Unidentified sp. 3 are the caudal rami which are about a ¼ the length of the abdomen; the presence of post-maxilluliary processes and the ornamentations of the inner margins of the first exopodal segments of legs 1 and the sympods of legs 4.

Distribution statistics of species component populations:

Unidentified sp. 3 exhibited a prevalence of 28.57%, a mean intensity of 4 individuals per host and a mean abundance of 1 individual per host (Table 3; Figs. 1, 2, 3) on *M. alfredi*.

3.2.3.4 Unidentified sp. Male

Material collected: From *M. alfredi* seven ♂♂ --- six ♂♂ from one host off Margate (30.52S 30.21E) caught during February 2009 and one ♂ from one host off San Lameer (30.57S 29.20E) caught during August 2010. All specimens were collected from the gill rakers of the examined hosts.

Adult male (Figs. 29-31):

Total body length (excluding setae of caudal rami) about 6.71 mm. Cephalothorax (Fig. 29a, b) composed of head, fused together with first three thoracic segments and posteriorly bearing fourth free thoracic segment. Cephalothoracic shield (Fig. 29a, b) orbicular, as wide as long, posterior margin ovally extended to beginning of fourth free thoracic segment; frontal plates distinct without lunules. Fourth free thoracic segment (Fig. 29b) rectangular-like, longer than wide, without dorsal plates. Genital complex (Fig. 29a, b) oval shaped, longer than wide, without posterolateral or anterolateral expansions. Abdomen (Fig. 29a, b) overall length 1.23 mm, 2-segmented, elongated, with segment 2 twice the size of segment 1, armed with scattered spinules on ventral margin. Caudal rami (Fig. 30a) overall length (excluding setae) 0.65 mm, elongated, almost as long as second segment of the abdomen, a little over $^{3}/_{5}$ the length of the abdomen, armed with scattered spinules on ventral margin and five short, pinnate setae with probably another distomedially (not observed).

Antennule (Fig. 29c) indistinctly 3-segmented; segment 1 broad, armed with 26 (some pinnate, some appear naked) setae; segment 2 slender, armed with one naked seta distally; segment 3 slender, armed with 13 (11 naked, two aesthetascs) setae distally. Antenna (Fig. 30b) 3-segmented; segments 1 and 2 broad, unarmed;

segment 3 short, armed with a slightly curved claw and two naked setae; post-antennal process (Fig. 30b) slightly curved tubercle, armed with two papillae each bearing three long slender setules and one inner papilla bearing three setules. Mouth tube typical of caligids, longer than wide, with strigil (Fig. 29e); mandible (Fig. 29d, e) with 12 teeth. Maxillule (Fig. 30d) biramous; palp an elongated papilla armed with three naked setae terminally; endite an enlarged, dentiform process with blunt tip, unarmed; post-maxilluliary process not observed. Maxilla (Fig. 30e) branchiform, 2-segmented; syncoxa (lacertus) elongated, unarmed; basis (branchium) armed with a patch of fine setules along anterior and posterior margins, a short serrated canna, a longer calamus with serrations coiled around tip and a patch of setules along the lateral margin next to base of calamus. Maxilliped (Fig. 30f) robust, 2-segmented; protopod (corpus) broad, armed with one naked seta at about myxal area on inner margin and one short, naked seta distomedially; subchela short, armed with an elongated, strongly curved terminal claw and one naked seta about mid-length; post-maxillipedal process, a short dentiform process attached to ventral surface of cephalothorax inner to base of protopod. Sternal furca absent.

Armature of rami of legs 1-4 (Fig. 31a, b, c, d, e) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	I-0; IV-3	0-0; 0-3
Leg 2	I-0; I-2; I-6	0-1; 0-2; 0-5
Leg 3	I-1; 0-1; IV-4	0-1; 0-2; 0-4
Leg 4	I-1; I-1; III-1	absent

First three pairs of legs biramous; leg 1 with 2-segmented rami; legs 2 and 3 with 3-segmented rami; sympods of legs 1 and 2 each armed with one short inner pinnate seta and one outer pinnate seta, not observed on sympod of leg 3.

Leg 1 (Fig. 31a, b), sympod setae pinnate; exopod segment 1 elongate, armed with rows of setules on inner margin and a short, outer spine distally; segment 2 small, armed with four (two serrated, one setuled and one naked with a bifurcate tip) terminal spines and three pinnate setae; endopod segment 1 unarmed; segment 2 small, armed with three pinnate setae terminally and lined with row of fine setules along inner margin. Leg 2 (Fig. 31c) sympod armed with a striated membrane proximally and a spinulated patch on anterior margin; exopod segments 1 and 2 each armed with one setuled outer spine (segment 2 with an additional small spine) and one inner elongated seta; segment 3 small, armed with one setuled spine and six pinnate setae; endopod segments 1 and 2 each extended into semicircular lamina, laterally lined with rows of fine setules; segment 1 armed with one, outer pinnate seta; segment 2 armed with two outer pinnate setae distally; segment 3 small, armed with five pinnate setae. Leg 3 (Fig. 31d) sympod armed with one pinnate seta; exopod segments 1 and 2 each armed with one inner pinnate seta; segment 1 with two short spinules and one serrated spine with picket fence-like membrane at base; segment 2 armed with a row of elongated setules on lateral margin and a spinulated membrane distally; segment 3 armed with four serrated spines and four pinnate setae terminally; endopod segments 1 and 2 each bearing semicircular lamina, laterally lined with fine setules; segment 1 armed with one small, distomedial pinnate seta; segment 2 with two distomedial pinnate setae; segment 3 armed with four pinnate setae. Leg 4 (Fig. 31e) uniramous, 3-segmented; sympod broad, armed with short spinules, a short setuled membrane dorsally and one slender naked seta distolaterally; segment 1 armed with row of short spines; segments 1 and 2 each armed with one naked inner spine-like seta and one serrated distolateral spine, with setuled membranes near base; segment 2 armed with rows of setules along lateral margin; segment 3 armed with one inner, naked spinule, three serrated spines and one slender terminal, naked seta with a setuled membrane at base. Leg 5 (Fig. 31f) a small papilla-like process armed with three pinnate setae. Leg 6 (Fig. 30g) a small papilla, armed with short spinules and three naked setae.

Remarks:

Similar to the three Unidentified sp. females, the current male specimen is similar to *Pupulina* male species, especially in the slender appearance of the general habitus, the wider than long free thoracic segment, a genital segment that is approximately the same length as the abdomen, the absence of lateral expansions on the genital segment, the presence of post-maxillipedal processes, the well-developed endopods of the first legs as well as the presence of semi-circular velums on the exopodal segments of legs 2 and 3. However, Unidentified sp. male can be distinguished from the known *Pupulina* males by possessing an abdomen that is 2-segmented, antennae that lack posteriorly pointed spine-like processes on the second segments, the absence of post-maxilluliary processes, elongated palps of the maxillules, patches of fine setules along the posterior margins of the branchiums of the maxillae, the calamus of the maxillae that have coiled serrations around the tips and the exopods of the third legs that have medium sized velums; while the *Pupulina* males have 3-segmented abdomens, the presence of post-maxilluliary processes, more flattened palps of the maxillae, branchiums with membranous flabellums on the posterior margins of the maxillae, calamus of maxillae that are bilaterally serrated and the first exopodal segments of the first legs are armed with enlarged semi-circular velums while the second segments have medium sized velums. Therefore, Unidentified sp. male is not *Pupulina*.

The current male species is similar to the three Unidentified sp. female species in most of the features, but appears to be more similar to the Unidentified sp. 3 female by possessing a relatively smaller sized general habitus, more elongated palps of the maxillules, rows of fine setules along the inner margins of the first exopodal segments of legs 1, cobblestone-like patches on the lateral margins of the sympods of legs 2 as well as short setuled membranes on the dorsal margins of the sympods of legs 4; while Unidentified sp. 1 and Unidentified sp. 2 both possess a more elongated general habitus, rows of picket fence-like processes and short spinules along the inner margins of the first exopodal segments respectively and lack cobblestone-like patches on the lateral margins and setuled membranes on the dorsal margins of the sympods of legs 2 and legs 4 respectively. Therefore the Unidentified sp. male species is more similar to the Unidentified sp. 3 females than it is to the females of Unidentified sp. 1 and Unidentified sp. 2. Additionally, the current male species is distinguished from all three female species by possessing post-maxillipedal processes that are attached to the ventral surface of the cephalothorax inner to base of the protopods as well as in the shape, size and armature of the caudal rami. The Unidentified sp. male, similar to all three females, may also be identified by measuring the length of the caudal rami relative to the length of the abdomen, with the caudal rami a little more than $^{3}/_{5}$ the length of the abdomen.

3.2.3.5 Remarks

The unidentified specimens encountered in the present study represent the third record of a caligid copepod infecting *Manta alfredi* from South African waters and possibly the host's distribution in various other localities. However, since *Manta birostris* was thought to be a monotypic species occurring in the Indian Ocean (Marshall *et al*. 2009; Marshall *et al*. 2011; Romanov 2010), previous reports of caligids infecting *Manta birostris* may have been wrongly documented, as the hosts could have been misidentified. According to Marshall *et al*. (2009 & 2011) there are indeed two different manta species and a possible third (A.D. Marshall pers. comm.), occurring in the Indian Ocean, with *M*. *alfredi* examined in the present study. The unidentified species encountered in the share morphological characteristics with *Pupulina* species; such as the frontal lobes of the cephalothorax that lack lunules; the lack of dorsal plates on the fourth free thoracic segments; the absence of a sternal furca; well-developed endopods of legs 1 as well as the presence of lamina on the first and second segments of the endopods of legs 2 and 3 (see Table 4). However, the three Unidentified sp. female species can be distinguished from members of *Pupulina* by possessing 3-segmented antennules; the 2-segmented antennae; the lack of posteriorly directed spine-like processes on the second segments of the antennae; more pronounced and elongated palps of the maxillules; the absence of post-maxillipedal processes; branchium of maxillae that are armed with rows of fine setules extending from the posterior margins to the base of the calamus; a calamus with coiled serrations around the tip; the shape, size and armature of the terminal spines on the third segments of the exopods of legs 1 where the spines are not serrated but setuled along the lateral margins; the velums of the first segments of leg 3 endopods which are the same medium size as the velums of the endopods the second segments as well as the relatively short sized, naked setae of the first segments of the endopods of legs 3 (see Table 4). In contrast, *Pupulina* species have 2-segmented antennules; 3-segmented (in three species encountered in the present study) and 4-segmented (in three nominal species) antennae; the presence of a posteriorly directed spine-like process on the second segments of the antennae; more flattened palps of the maxillules; the presence of post-maxilluliary processes attached to the ventral surface of cephalothorax inner to tips of the endites of the maxillules; the branchiums of the maxillae that are armed with membranous flabellums and setuled ridges on the posterior margins, calamus armed with bilateral serrations; terminal spines of the third segments of leg 1 exopods that are more serrated than setuled; the velums the first endopodal segments of leg 3 are enlarged, covering both the exopod and endopod segments as well as more elongated pinnate setae on the first endopodal segments of the third legs (see Table 4 & key to species).

It therefore, seems that the Unidentified sp. specimens encountered in the present study represent a genus independent of *Pupulina* within the siphonostomatoid family Caligidae. However, more specimens from more mobulid hosts need to be closely scrutinized in order to provide adequate evidence to validate these species. The three Unidentified sp. female species can further be distinguished from each other by the shape and size of the fourth free thoracic segments, the length of the abdomen relative to the length of the genital segment as well as the armature of the first segments of the exopods of legs 1. However, the main feature that distinguishes between the three species is the length of the caudal rami relative to the length of the abdomen. On Unidentified sp. 1, the caudal rami are slightly more than ½ the length of the abdomen in; about a ¼ the length of the abdomen in Unidentified sp. 2 and less than a ¼ the length of the abdomen in Unidentified sp. 3.

Male specimens are usually found within the vicinity of females of the same species (Kabata 1979; Benz 1994). Thus it is highly unusual to encounter females of one species with males that belong to another species on the same host. The unidentified males encountered are morphologically similar to all three Unidentified sp. female species found on the same host. However, the male specimens are more similar to the Unidentified sp. 3 female than they are to the other two females, especially since they possess rows of fine setules lining the inner margins of the first segments of legs 1 exopods; the cobblestone-like patches on the sympods of legs 2 and the short setuled membranes on the dorsal margins of the sympods of leg 4. Therefore, the male species encountered on *M. alfredi* more probably represent Unidentified sp. 3. However, further findings of these female species, with males on one host will assist with identification of the male specimens. Unidentified sp. 3 (male and female specimens included), exhibited the highest prevalence of 28.6% of all the Unidentified sp. species encountered on *M. alfredi* (Table 3; Fig. 1) while both Unidentified sp. 1 and Unidentified sp. 2 exhibited a prevalence of 14.28% on their examined hosts (Table 3; Fig. 1). Thus Unidentified sp. 3 is more prevalent on *M. alfredi* that it's two congeners. The mean intensity of infection was also the highest for Unidentified sp. 3 with four individuals per host while Unidentified sp. 1 and Unidentified sp. 2 both have two individuals per host (Table 3; Fig. 2). Therefore more encounters of these species will provide more information on the host parasite relationships they have with their hosts.

3.3 Phylogenetic analysis

3.3.1 Introduction

Phylogenetic trees allow thinking about an individual of interest in terms of its relationship to other individuals. This in turn allows one to draw conclusions about the biological function of that particular group of individuals (Martin & Davis 2001). One method used to determine the phylogenetic relationships between organisms is phylogenetic analysis using parsimony (Holmes 2003). The principle of parsimony states that the phylogram with the smallest number of inferred character transformations (the shortest tree length) is taken as the chosen hypothesis for reconstructing phylogenetic relationships (Page & Holmes 1998). Thus, parsimony searches for the phylogram with the least number of character transformations along its branches and uses it for explaining the resulting data (Holmes 2003). In order to determine character polarity of the in-group taxa, the out-group taxon (a more distantly related taxon) is defined (Bryant 1992; Page & Holmes 1998). Character polarity refers to the cladistic relationship among character states within taxa, thus it identifies the synapomorphies that unite members of monophyletic groupings in a phylogram (Bryant 1992; Hills *et al*. 1996; Page & Holmes 1998).The robustness and fit of a phylogram is measured by quantifying the consistency index (CI); retention index (RI); rescaled consistency index (RCI) and homoplasy index (HI), (Givnish & Sytsma 1997; Page & Holmes 1998). The consistency index measures the degree to which the inferred state of a particular character is not homoplasiously repeated on a given phylogram (Hills *et al.* 1996; Givnish & Sytsma 1997; Page & Holmes 1998). According to Page and Holmes (1998), a consistency index of 1.0 indicates no homoplasy while decreasing values show increased homoplasious repetition. As such, the homoplasy index (HI = 1-CI) explains the level of homoplasy for a given character (Sang 1995). The retention index measures the extent of synapomorphies by all characters on a given phylogram, where a value of 1.0 indicates synapomorphic character transformations while a value of 0 is scored by autapomorphies that show maximum levels of homoplasy (Hills *et al*. 1996; Page & Holmes 1998).

Fit and robustness of a given branching node in a phylogram can further be measured using bootstrap support values (Page & Holmes 1998; Holmes 2003; Wiesemull & Rothe 2006). Bootstrap support values (expressed in percentage), are the frequencies with which some branching nodes are maintained in a set of phylogenetic trees (Page & Holmes 1998). Bootstrap tests (bootstrapping) involve the random sampling and resampling of data from a given character matrix and generates numerous replicate matrices of the same size (Bryant 1992). High bootstrap values indicate support for branching nodes of a given phylogram, thus confidence can be placed in the monophyletic grouping deduced from that particular branching node (Bryant 1992; Page & Holmes 1998; Holmes 2003). According to Wiesemull and Rothe (2006), bootstrap values of less than 95% indicate that those particular branching nodes are not well supported by the data, thus less confidence is inferred in the clades formed by the nodes. Bootstrap values however, do not measure or test the accuracy of the tree, but gives information about the stability of the branching order of the tree (Holmes 2003; Wiesemull & Rothe 2006). Thus bootstrap values help assess whether morphological data analyzed is adequate enough to validate the branching pattern of the tree (Holmes 2003).

3.3.2 Material and Methods

A parsimony analysis using PAUP* version 4.0b10 (Swofford 2002) was done in an effort to estimate the phylogenetic relationships of nine (six *Pupulina* species and three unidentified species) selected female species of the family Caligidae. In an attempt to determine character polarity of the in-group taxa, *C. grandifer* was used as an out-group taxon. A character matrix consisting of 40 morphological characters of adult females of the selected species using previous descriptions and descriptions formulated in the present study was compiled (Appendices I & II). All the characters were treated and analyzed as unordered and un-weighted. An exhaustive search with random starting trees was employed in PAUP* using tree-bisection reconnection (TBR) branch swapping in conjunction with the random stepwise addition feature.

Robustness of the most parsimonious tree were evaluated using the consistency index (CI), retention index (RI), rescaled consistency index (RCI) and homoplasy index (HI). Bootstrap analysis of 1000 replicates with random addition searches was performed to determine the nodal support for each of the clades on the resulting most parsimonious tree. From the resulting trees, the 50% majority rule consensus was calculated.

3.3.3 Results

Out of the 40 morphological characters analysed (Appendix I), 33 were parsimoniously informative while only seven were parsimoniously uninformative. The exhaustive search retained a single most parsimonious tree with a tree length (TL) = 85; consistency index (CI) = 0.7; retention index (RI) = 0.7; homoplasy index (HI) = 0.3 and a rescaled consistency index (RCI) = 0.5. Bootstrap support for the estimated clades was mostly low (most values less than 95%) with only the monophyletic grouping of the in-group taxa and the monophyly of the three Unidentified sp. species well supported by 100% bootstrap values and the sister-group of Unidentified sp. 1 and Unidentified sp. 2 supported by a bootstrap value of 98%. On the estimated topology (Fig. 32), the in-group taxa are monophyletic due to the following synapomorphies: the abdomen (character 12) is transformed from being short to elongate; mouth tube from a short blunt siphon (character 24) to a longer one; the absence of a sternal furca (character 28); the absence of lunules (character 29) and the development of semicircular lamina on the first segments of endopods of legs 3 (character 32). The topology (Fig. 32) exhibits the monophyletic grouping of the six *Pupulina* species that have retained some of the pleisiomorphic character states. These shared pleisiomorphic character states (Fig. 32; Appendix I) include the 2-segmented antennules (character 20); the short blunt, posteromedial processes of segments 2 of the antennae (character 22); the flattened palps of the maxillules (character 26); a calamus with parallel serrated membranes (character 31) and leg 1 sympods that lack cobblestone-like patches (character 38). Additionally the monophyletic grouping of the three new *Pupulina* species underwent four character transformations from the ancestral characters that grouped them together with the three nominal species. These characters include the development of spinules on the dorsal and ventral surfaces of the abdomen (characters 14 & 15); the mandible with 11 teeth (character 25) and segments 2 of the maxillae that are armed with patches of fine setules from the posterior to the anterior surfaces at the base of the calamus (character 30).

Pupulina sp. 1 was the first to diverge from the *Pupulina* sp. group by developing anterolateral corners of the genital segment that are slightly extending upwards (character 8); an abdomen that is as long as the genital segment (character 16); rows of short spinules lining the inner margins of the caudal rami (character 34) and the absence of post-maxillipedal processes (character 35). The sister group of *Pupulina* sp. 2 and *Pupulina* sp. 3 developed a row of minute spinules along the posterior edges of their fourth free thoracic segments (character 6); posterolateral corners of the genital segment that are slightly extending posteriorly (character 9) and the long stiff hairs lining the inner margins of legs 4 sympods (character 33). *Pupulina* sp. 2 further developed long slender hairs along the ventral borders of the cephalothorax (character 2); square-like anterior margins of the genital segment (character 8); an abdomen that is shorter than the genital segment (character 16); caudal rami that are as long as the abdomen (character 17), armed with long stiff hairs along the inner margins (character 34) and a pectinate membrane at base of outer distal spinule on the sympods legs 4 (character 37); while *Pupulina* sp. 3 kept most of the pleisiomorphic characters and additionally developed a genital segment which is more than half as wide as the cephalothorax (character 7) and 3-segmented antennae (character 13). The branching nodes leading to the groupings of *Pupulina* sp. 1; *Pupulina* sp. 2 and *Pupulina* sp. 3 however, are not well supported with relatively low bootstrap values of 60% and 61% respectively.

The clade formed by the three nominal species of *Pupulina* with *P*. *flores* as the basal species is also not well supported with a bootstrap value of 53%. *Pupulina flores* is separated from the sister grouping of *P*. *minor* and *P*. *brevicauda* by the absence of long slender hairs along the ventral borders of the cephalothorax (character 2); the 3-segmented antennae (character 13); the caudal rami that lack scattered spinules on the dorsal and the ventral surfaces (character 18) and sympods of legs 4 that are armed with long stiff hairs along inner margins (character 33). The sister group of *P. minor* and *P. brevicauda* (node 11) developed long slender hairs along ventral borders of the cephalothorax (character 2) and a row of short spinules along the lateral edges of the genital segment’s dorsal margin (character 10). *Pupulina minor* further diverged from *P*. *brevicauda* by developing an abdomen that is shorter than the genital segment (character 16) and caudal rami that are longer than the abdomen (character 17); while *P. brevicauda* developed a fourth free thoracic segment that is as wide as it is long (character 4); posterolateral corners of genital segment which are extending well beyond the caudal rami (character 9) as well as caudal rami which are armed with six terminal setae (character 19).

The topology further identifies the three Unidentified sp. species as a monophyletic group (100% bootstrap support) with Unidentified sp. 1 and sp. 2 as a sister group of Unidentified sp. 3 (98% bootstrap support). The clade diverged from the monophyletic grouping of the 6 *Pupulina* species by developing a longer than wide cephalothorax (character 1); square-like anterior margins of the genital segments (character 8); 3-segmented abdomens (character 13); 3-segmented antennules (character 20); 2-segmented antennae (character 21); antennae that lack the posteriorly pointed, blunt tipped processes (character 22); more elongated palps of the maxillules (character 26); branchiums of the maxillae that are armed with rows of long setules extending from the dorsal to the ventral surfaces, near the bases of the calamus (character 30); calamus of the maxillae that are armed with coiled serrations around the tips (character 31) and velums of the endopods of the first segments of legs 3 which are the same size as those of the second segments (character 32). Unidentified sp. 1 diverges from the sister grouping of Unidentified sp. 2 and Unidentified sp. 3 by developing cobblestone-like patches on the sympods of legs 2 (character 40); while the sister grouping developed square-like anterior margins of the genital segments (character 8); rows of spinules along the lateral edges of the genital segment’s dorsal surface (character 10) and rows of short spinules on the medial margins of the first exopodal segments of legs 1 (character 39). Unidentified sp. 2 retains all characteristics shared by the monophyletic grouping and undergoes no further character transformations. Unidentified sp. 3 further develops a longer than wide genital segment (character 4) and posterolateral corners that are slightly extended posteriorly (character 9); a sub-triangular post-maxilluliary process (character 7); leg 4 sympod armed with a short blunt process on the inner distal margin and two spinulated membranes on the dorsal surface (character 37) and lacks cobblestone-like patches on sympod of leg 1 (character 38).

3.3.4 Remarks

Members of the family Caligidae are generally very similar, with only a few distinct morphological characteristics separating them (Kabata 1979; Dojiri 1983; Benz 1994). The structural outlines of the genus *Caligus* are consistent in all members within the family (Kabata 1979; Dojiri 1983; Pillai 1985; Benz 1994) and both *Pupulina* and Unidentified sp. species are no exceptions. The estimated phylogeny (Fig. 32) recognizes that the clade of the six *Pupulina* species has retained some of the pleisiomorphic characters that define the out-group taxon. Characters 20, 22, 26, 31 and 38 (Appendices I & II) represent the retained pleisiomorphic characters while characters 7, 23, 27, 32 and 35 are all synapomorphies that define the *Pupulina* clade consisting of the sister groups formed by the three known species and the three new species encountered. The separation of the three new *Pupulina* species from the three nominal species on the estimated cladogram indicates that the three *Pupulina* species encountered on *M. kuhlii* and *M. eregoodootenkee* in South African waters are more closely related to each other than they are to the three nominal species. The separation of the two groups can be attributed to nine synapomorphies which include characters 1, 9, 19, 21 and 35 (Appendices I & II) which define the known *Pupulina* group and characters 14, 15, 25 and 30 (Appendices I & II) which define the new *Pupulina* group. The separation of the two groupings can also be attributed to the geographic location of the examined hosts as well as the host species. Thus, the three known species have never been reported from either *M. kuhlii* or *M. eregoodootenkee* or from South African waters (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964; Dojiri 1983), while the three new species represent the first record of *Pupulina* species from *M. kuhlii* and *M. eregoodootenkee* from South African waters (see Table 4). Therefore, the separation of the two groupings may also be a result of their specificity to certain species of hosts or hosts that frequent a particular locality. However, a deeper examination of *M. kuhlii* and *M. eregoodootenkee* from other localities is needed to validate this statement.

Furthermore, the estimated phylogeny (Fig. 32) identifies the separation of the *Pupulina* group and the Unidentified sp. species group, where the Unidentified sp. clade represents the most evolved lineage of the two groups defined by thirteen synapomorphies. The synapomorphies include characters 1, 4, 6, 8, 13, 20, 21, 22, 26, 30, 31, 32 and 38 (Appendices I & II). The Unidentified sp. species in the group were all encountered on *M*. *alfredi* (see Table 4). It is interesting to note that the genus *Manta* also represents the most recent lineage while the genus *Mobula* represents the oldest lineage within the family Mobulidae (Nishida 1990; Lovejoy 1996; Kashiwagi *et al*. 2012). Additionally, within the genus *Manta*, *M*. *alfredi* is considered to be the most recent line while *M*. *birostris* represents the oldest (Kashiwagi *et al*. 2012). Therefore, as an adaptation mechanism to their newly evolved host or as a way to increase their attachment efficiency, the Unidentified sp. species have developed or modified most of the characteristics that define *Pupulina* species in general. However, *P*. *flores* has also been reported from *Manta* sp. and *M*. *birostris* (Wilson 1935; Wilson 1952; Dojiri 1983) and is more related to the other five *Pupulina* species than to the Unidentified sp. species (Fig. 38). Also, since *M*. *birostris* was previously thought to be a monotypic species (Marshall *et al*. 2009; Romanov 2010; Marshall *et al*. 2011), any previous reports of *Pupulina* species or any other siphonostomatoids encountered on them may have been wrongly documented, as the hosts could have been misidentified. Therefore, in order to understand the phylogenetic distinction between the two groups, a deeper scrutiny of more hosts from different localities for infection by *Pupulina* species and Unidentified sp. species is needed. However, it seems that the three Unidentified sp. species are quite different to *Pupulina*, thus the species may represent a different genus within the family Caligidae. However, a thorough examination of more specimens still needs to be done to help with correct identification of these specimens. The phylogenetic hypothesis of the nine caligid species presented in the current study was derived from a phylogenetic analysis of the information for adult females from previous descriptions and those formulated in the present study, and therefore not intended to be a definitive theory. It should however, be treated as a testable hypothesis than can be further analyzed using more data generated from scrutinizing more specimens.

Table 1: Ecological statistics of *Eudactylina oliveri* Laubier, 1968; *Caligus chrysophrysi* Pillai, 1985; *Pupulina* sp. 1; *Pupulina* sp. 2; *Kroeyerina mobulae* Deets, 1987 and *Entepherus laminipes* Bere, 1936 collected from *Mobula kuhlii (*Müller & Henle, 1841).

Copepod species	*Eudactylina oliveri*	*Caligus chrysophrysi*	*Pupulina* sp. 1	*Pupulina* sp. 2	*Kroeyerina mobulae*	*Entepherus laminipes*
Number of hosts examined	31	31	31	31	17	31
Number of hosts infected by specific copepod	27	11	19	1	2	1
Prevalence (%)	87.1	35.34	61.29	3.22	-	3.22
Mean intensity (individuals per host)	42	5	41	1	-	1
Mean abundance (individuals per host)	37	2	3	0	-	0
Mean ($\bar{x}$)	36.7	1.58	2.51	0.32	-	0.32
Variance s^2	2452.34	-	10.19	-	-	-
Distribution pattern of component population	Aggregated	-	Aggregated	-	-	-

Table 2: Ecological statistics of *Eudactylina oliveri* Laubier, 1968; *Pupulina* sp. 1; *Pupulina* sp. 2 and *Pupulina* sp. 3 collected from *Mobula ergoodootenkee* (Bleeker, 1859).

Copepod species	*Eudactylina oliveri*	*Pupulina* sp. 1	*Pupulina* sp. 2	*Pupulina* sp. 3
Number of hosts examined	2	2	2	2
Number of infected hosts	2	2	1	1
Prevalence (%)	100	100	50	50
Mean intensity (per host)	130	5	1	1
Mean abundance (per host)	130	5	1	1
Mean ($\bar{x}$)	129.5	4.5	0.5	0.5
Variance s^2	21424.5	25	-	-
Distribution pattern of component population	Aggregated	Aggregated	-	-

Table 3: Ecological statistics of *Eudactylina diabolophila* Deets, 1994; *Nemesis* sp.; *Caligus chrysophrysi* Pillai, 1985; *Entepherus laminipes* Bere, 1936 and *Anthosoma crassum* (Abildgaard, 1794) collected from *Manta alfredii* (Krefft, 1868).

Copepod species	*Eudactylina diabolophila*	*Nemesis* sp.	*Caligus chrysophrysi*	*Entepherus laminipes*	*Anthosoma crassum*	Unidentified sp. 1	Unidentified sp. 2	Unidentified sp. 3
Number of hosts examined	7	7	7	7	7	7	7	7
Number of infected hosts	2	1	2	3	1	1	1	2
Prevalence (%)	28.57	14.28	28.57	42.85	14.28	14.28	14.28	28.57
Mean intensity (per host)	5	5	6	2	6	2	2	4
Mean abundance (per host)	1	1	2	1	2	0	0	1
Mean ($\bar{x}$)	1.42	0.71	1.57	0.71	1.57	0.28	0.28	1.14
Variance s^2	-	-	-	-	-	-	-	-

Table 4: Reported hosts and distribution and the distinguishing features of six *Pupulina* van Beneden, 1892 (three nominal species and three species encountered in the present study) species and three Unidentified sp. species (Wilson 1935; Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964; Dojiri 1983).

	Pupulina flores Van Beneden, 1892	*Pupulina minor* Wilson, 1952	*Pupulina brevicauda* Wilson, 1952	*Pupulina* sp. 1	*Pupulina* sp. 2	*Pupulina* sp. 3	Unidentified sp. 1	Unidentified sp. 2	Unidentified sp. 3
Hosts	- *Manta birostris* Walbaum, 1792. - *Manta* sp. - *Mobula hypostoma* (Bancroft, 1831).	-*Mobula lucasana* Beebe and Teevan, 1938. - *Mobula diabolos* Smith, 1943.	-*Mobula lucasana* Beebe and Teevan, 1938. -*Mobula diabolos* Smith, 1943.	-*Mobula kuhlii* (Valenciennes *in* Müller & Henle, 1841).	-*Mobula kuhlii* (Valenciennes *in* Müller & Henle, 1841).	-*Mobula kuhlii* (Valenciennes *in* Müller & Henle, 1841).	*Manta alfredi* (Krefft, 1868).	*Manta alfredi* (Krefft, 1868).	*Manta alfredi* (Krefft, 1868).
Reported distribution.	- Atlantic ocean (Azores, Lemon Bay	- Pacific ocean (Santa Catalina,	- Pacific ocean (Santa Catalina,	- Indian Ocean (East coast South Africa).	- Indian Ocean (East coast South Africa).	- Indian Ocean (East coast South Africa).	- Indian Ocean (East coast South Africa).	- Indian Ocean (East coast South Africa).	- Indian Ocean (East coast South Africa).

	Florida). - Pacific (Galapagos islands).	California). -Indian Ocean (Trivandrum, India).	California). - Indian ocean (Trivandrum, India).						
Cephalothorax	- Orbicular, as wide as long.	- Orbicular, as wide as long.	- Circular, wider than long.	- Nearly circular, longer than wide.	- Circular, wider than long,	- Nearly circular, almost as wide as long.	- Sub-orbicular, almost as wide as long.	- Sub-orbicular, slightly wider than long.	- Sub-orbicular, as wide as long.
Free thoracic segment	- Wider than long with no short spinules posteriorly.	- Wider than long and spiny.	- As wide as long and without short spinules posteriorly.	- Short and broad, without short spinules posteriorly.	- Wider than long, with patch of spinules posteromedially.	- Wider than long, without short spinules dorsally.	- Wider than long, without short spinules dorsally.	- As wide as long, without short spinules dorsally.	- Longer than wide, without short spinules dorsally.
Genital segment	- Anterior margin rounded without anterolateral expansions. - Posterior margin with	- Anterior margin rounded and without anterolateral expansions. - Posterior margin with	- Anterior margin rounded and without anterolateral expansions. - Posterior margin with	- Anterior margin with corners slightly extending anteriorly. - Posterior margin	- Anterior margin square-like and without posterolateral expansions. - Posterior margin	- Anterior margin square-like and without anterolateral expansions. - Posterior margin with	- Anterior margin square-like and without anterolateral expansions. - Posterior margin with	- Anterior margin square-like and without anterolateral expansions. - Posterior margin	- Anterior margin square-like and without anterolateral expansions. - Posterior margin with

	posterolateral corners slightly extended posteriorly.	posterolateral corners slightly extended posteriorly.	posterolateral corners well beyond the tips of the caudal rami.	square-like and without posterolateral expansions.	square-like and without posterolateral expansions.	posterolateral corners slightly extended posteriorly.	posterolateral corners slightly extended posteriorly.	square-like and without posterolateral expansions.	posterolateral corners slightly extended posteriorly.
Abdomen	- 3 segmented slender, longer than genital segment.	- 2 segmented spiny, narrow, and as long as genital segment.	- 2 segmented slender and only slightly shorter than the genital segment.	- 2 segmented Slender and spinulated and longer than genital segment.	- 1 segmented sparsely spinulated, almost as long as genital complex.	- 3 segmented and longer than genital segment.	- 3 segmented and almost as long as genital segment.	- 3 segmented and longer than genital segment.	- 3 segmented and longer than genital segment.
Caudal rami	- Extremely slender and slightly shorter than the abdomen and without short spinules on surface.	- Slender with scattered spinules, slender, a little longer than abdomen.	- Slender and shorter than the abdomen and without short spinules on the surface.	- Slender, almost as long as the abdomen, heavily spinulated.	- Slender, as long as abdomen armed with row of long stiff hairs along central margin.	- Slender, almost as long as smallest segment of abdomen, sparsely spinulated.	- Slender, slightly more than half the length of abdomen and without short spinules.	- Slender, longer than wide, about a quarter the length of the abdomen armed with scattered spinules dorsally.	- Slender, shorter than the abdomen and armed with scattered spinules dorsally.
Antennules	- 2-segmented.	-2 -segmented.	- 2-segmented.	- 2-segmented.	- 2-segmented.	- 2-segmented.	- 3-segmented.	- 3-segmented.	- 3-segmented.

Antennae	- 4-segmented; - Posteriorly directed spine-like process found on the second segment.	- 4-segmented; - Posteriorly directed spine-like process found on the second segment.	- 4-segmented; - Posteriorly directed spine-like process found on the first segment.	- 3-segmented; - Posteriorly directed spine-like process found on the first segment.	- 3-segmented; - Posteriorly directed spine-like process found on the first segment.	- 3-segmented; - Posteriorly directed spine-like process found on the first segment.	- 2-segmented. - Posteriorly directed spine-like process absent.	- 2-segmented. - Posteriorly directed spine-like process absent.	- 2-segmented. - Posteriorly directed spine-like process absent.
Post-antennal process	- Small rounded protrusion with slender tine.	- Small rounded protrusion with slender tine.	- Small rounded protrusion with slender tine.	- Small rounded protrusion with slender tine.	- Small rounded protrusion with slender tine.	- Small rounded protrusion with slender tine.	- Small rounded protrusion with more robust tine.	- Small rounded protrusion with more robust tine.	- Small rounded protrusion with more robust tine.
Mouth tube	- Longer than wide.	- Short and blunt.	- Short and blunt.	- Longer than wide.	- Longer than wide.	- Longer than wide.	- Longer than wide.	- Longer than wide.	- Longer than wide.
Intrabuccal stylet and strigil	- Located on both sides of inner surface of front labri.	- Located on both sides of inner surface of front labri.	- Not observed	- Located on both sides of inner surface of front labri.	- Located on both sides of inner surface of front labri.	- Located on both sides of inner surface of front labri.	- Located on both sides of inner surface of front labri.	- Not observed	- Located on both sides of inner surface of front labri.
Mandibles	- With12 teeth on the inner margin.	- With12 teeth on the inner margin.	- With 12 teeth on the inner margin.	- With 11 teeth on the inner margin.	- With 11 teeth on the inner margin.	- With 11 teeth on the inner margin.	- With 12 teeth on the inner margin.	- With 12 teeth on the inner margin.	- With 12 teeth on the inner margin.

Maxillules	- Biramous; - Palp more flattened and armed with three setae - Post-maxilluliary process present.	- Biramous; - Palp more flattened and armed with three setae. - Post-maxilluliary process present.	- Biramous; - Palp more flattened and armed with three setae. - Post-maxilluliary process present.	- Biramous; - Palp more flattened and armed with three setae. - Post-maxilluliary process present.	- Biramous; - Palp more flattened and armed with three setae. - Post-maxilluliary process present.	- Biramous; - Palp more flattened and armed with three setae. - Post maxilluliary process present.	- Biramous; - Palp elongated and armed with three setae. - Post-maxilluliary process absent.	- Biramous; - Palp elongated and armed with three setae. - Post-maxilluliary process absent.	- Biramous; - Palp elongated and armed with three setae. - Post-maxilluliary process present.
Maxilla	- Branchiform, 2-segmented. - Calamus with parallel serrated membranes. - Basis bears serrated	- Branchiform, 2-segmented. - Calamus with parallel serrated membranes. - Basis bears serrated	- Branchiform 2-segmented - Calamus with parallel serrated membranes. - Basis with no serrated	- Branchiform, 2-segmented. - Calamus with parallel serrated membranes. - Basis armed with	- Branchiform 2-segmented. - Calamus with parallel serrated membranes. - Basis with serrated	- Branchiform 2-segmented. - Calamus with parallel serrated membranes. - Basis slender with	- Branchiform, 2-segmented. - Calamus with coiled serrations and twice the size of canna. - Basis short, armed with	- Branchiform, 2-segmented. - Calamus with coiled serrations and twice the size of canna. - Basis short, armed with	- Branchiform, 2-segmented. - Calamus with coiled serrations and twice the size of canna. - Basis short, armed with

	membranous flabellum.	membranous flabellum.	membranous flabellum.	serrated membranous flabellum and a flat setuled ridge sub-terminally.	membranous flabellum and a flattened, setuled ridge sub-terminally.	a serrated membranous flabellum and a flat setuled sub-terminally.	rows of fine setules along the posterior and anterior margins.	rows of fine setules along the posterior and anterior margin.	rows of fine setules along the posterior and anterior margin.
Maxilliped	- 2-segmented. - Protopod moderately slender and unarmed. - Post-maxillipedal process present.	- 2-segmented. - Protopod long, broad and unarmed. - Post-maxillipedal process present.	- 2-segmented. - Protopod long broad and unarmed. - Post-maxillipedal process present.	- 2-segmented. - Protopod slender and unarmed. - Post-maxillipedal process present.	- 2-segmented. - Protopod broadly long and unarmed. - Post-maxillipedal process absent.	- 2-segmented. - Protopod long, broad and unarmed. - Post-maxillipedal process absent.	- 2-segmented. - Protopod broad, long and unarmed. - Post-maxillipedal process absent.	- 2-segmented. - Protopod broad, armed with short, inner naked seta about mid-length. - Post-maxillipedal process absent.	- 2-segmented. - Protopod broad, armed with short, naked seta about mid-length. - Post-maxillipedal process absent.
Sternal furca	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.

Leg 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segmented rami. - Exopod segment 1	- Biramous with 2-segemnted rami. - Exopod segment 1	- Biramous with 2-segemnted rami. - Exopod segment 1	- Biramous with 2-segemnted rami. - Exopod segment 1
	with distolateral spine and row of setules along inner margin.	with distolateral spine and row of setules along inner margin.	with outer spine located distally and row of setules along inner margin.	with row of setules along inner margin.	with row of setules along inner margin and one small, terminal outer seta.	with rows of setules along inner margin.	broad, with row of picket fence-like extensions along inner margin and one naked seta distally.	with rows of short spinules along inner margin.	elongate with rows of hair-like setules along inner margin.
Leg 2	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. -Sympod without	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. - Sympod with	- Biramous with 3-segmented rami. - Sympod without	- Biramous with 3-segmented rami. - Sympod without

	patches proximally.	patches proximally.	patches proximally.	patches proximally.	patches proximally.	patches proximally.	cobblestone-like patches proximally.	patches proximally.	patches proximally.
Leg 3	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 with an extremely larger semi-circular lamina.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 with an extremely large semi-circular lamina.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 with an extremely large semi-circular lamina.	- Biramous with 3-segmented rami. - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 with an extremely large semi-circular lamina.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 with an extremely large semi-circular lamina.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 without row of short spinules on outer lateral margin. - Endopod segment 1 armed with extremely large semi-circular lamina.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 with row of short spinules on outer lateral margin. - Endopod segments 1 and 2 each bearing medium sized semi-circular lamina on inner margin.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 with row of short spinules on outer lateral margin. - Endopod segments 1 and 2 each bearing medium sized semi-circular lamina on inner margin.	- Biramous with 3-segmented rami; - Exopod segments 1 and 2 with row of short spinules on outer lateral margin. - Endopod segments 1 and 2 each bearing medium sized semi-circular lamina on inner margin.

Leg 4	- Uniramous, 3-segmented; - Sympod robust, carrying numerous spinules and small distal plumose seta.	- Uniramous, 3-segmented; - Sympod broad, and armed with scattered spinules on dorsal and ventral surface.	- Uniramous, 3-segmented; - Sympod segment narrow, with short spines posteriorly situated.	- Uniramous, 3-segmented; - Sympod broadly large, bearing scattered minute spinules and a pinnate seta distolaterally.	- Uniramous, 3-segmented; - Sympod armed marginally with spinules lining dorsal edge plus pectinate membrane basal to distolateral spinule, a row of long, stiff hairs along medial margin.	- Uniramous, 3-segmented; - Sympod armed with scattered spinules and a row of scattered slender hairs dorsally and one pinnate seta anterodistally.	- Uniramous, 3-segmented; - Sympod armed with short spinules on posterior margin.	- Uniramous, 3-segmented; - Sympod armed with short spinules along posterior margin and a small, naked seta distolaterally.	- Uniramous, 3-segmented; - Sympod armed with short spinules, a short blunt process distomedially, two spinulated membranes dorsally and one slender, pinnate seta terminally.
Leg 5	- A small, papilla-like process bearing three pinnate setae.	- Vestigial.	- Vestigial.	- Small, papilla-like process armed with three pinnate setae.	- Small, papilla-like process armed with three pinnate setae.	- Small, papilla-like process armed with three pinnate setae.	- Small, papilla-like process armed with three pinnate setae.	- Not observed.	- Small, papilla-like process armed with three pinnate setae.
Leg 6	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.	- Absent.

Fig. 1: Prevalence of *Caligus chrysophrysi* Pillai, 1985; *Pupulina* sp. 1; *Pupulina* sp. 2; *Pupulina* sp. 3; Unidentified sp. 1; Unidentified sp. 2; Unidentified sp. 3; *Eudactylina oliveri* Laubier, 1968; *Eudactylina diabolophila* Deets, 1994; *Nemesis* sp.; *Kroeyerina mobulae* Deets, 1987; *Entepherus laminipes* Bere, 1936 and *Anthosoma crassum* (Abildgaard, 1794) on *M*obula *kuhlii* (Müller & Henle, 1841); *Mobula eregoodootenkee* (Bleeker, 1859) and *Manta alfredi* (Krefft, 1868).

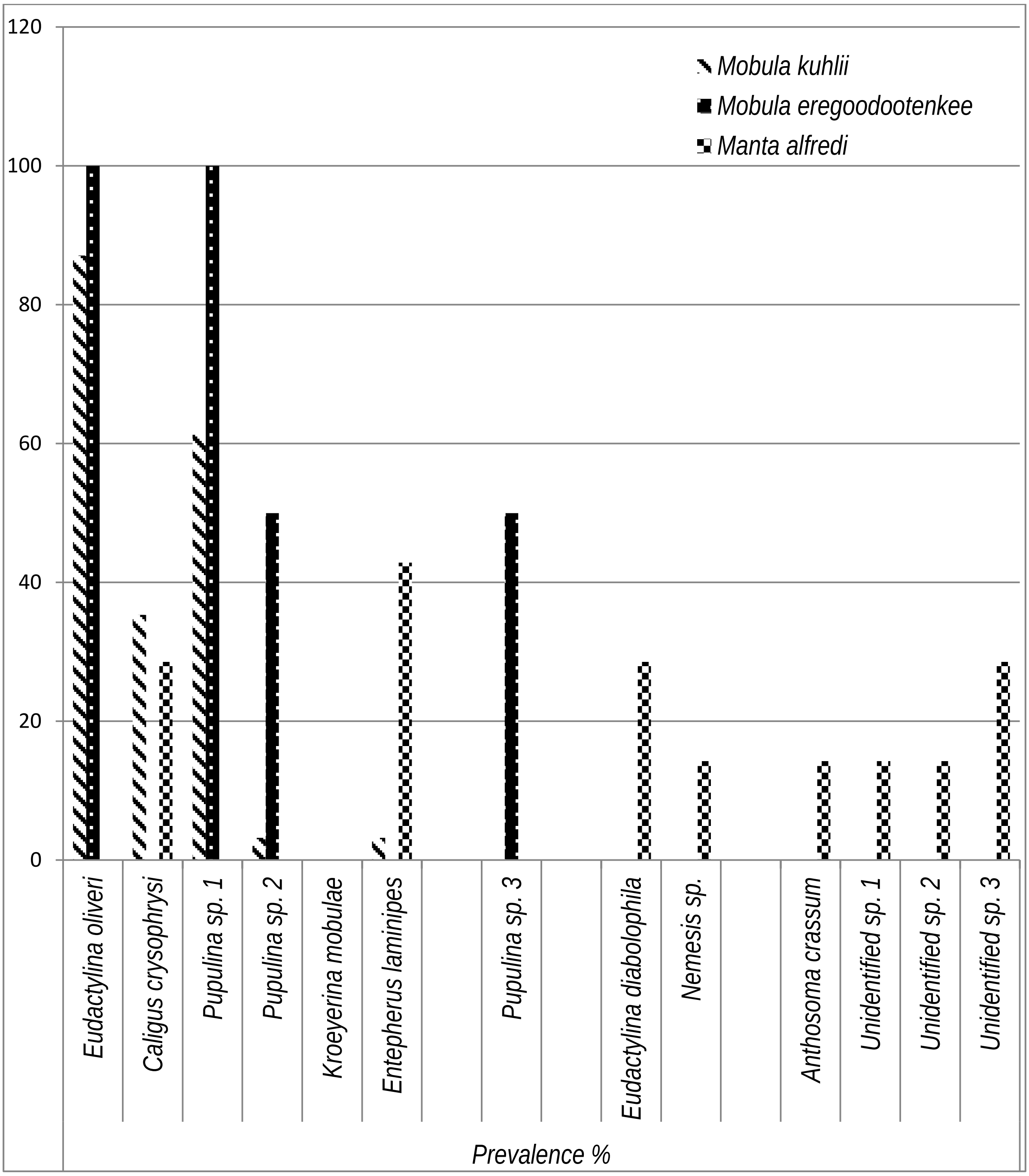


Fig. 2: Mean Intensity of infection by *Caligus chrysophrysi* Pillai, 1985; *Pupulina* sp. 1; *Pupulina* sp. 2; *Pupulina* sp. 3; Unidentified sp. 1; Unidentified sp. 2; Unidentified sp. 3; *Eudactylina oliveri* Laubier, 1968; *Eudactylina diabolophila* Deets, 1994; *Nemesis* sp.; *Kroeyerina mobulae* Deets, 1987; *Entepherus laminipes* Bere, 1936 and *Anthosoma crassum* (Abildgaard, 1794) on *M*obula *kuhlii* (Müller & Henle, 1841); *Mobula eregoodootenkee* (Bleeker, 1859) and *Manta alfredi* (Krefft, 1868).

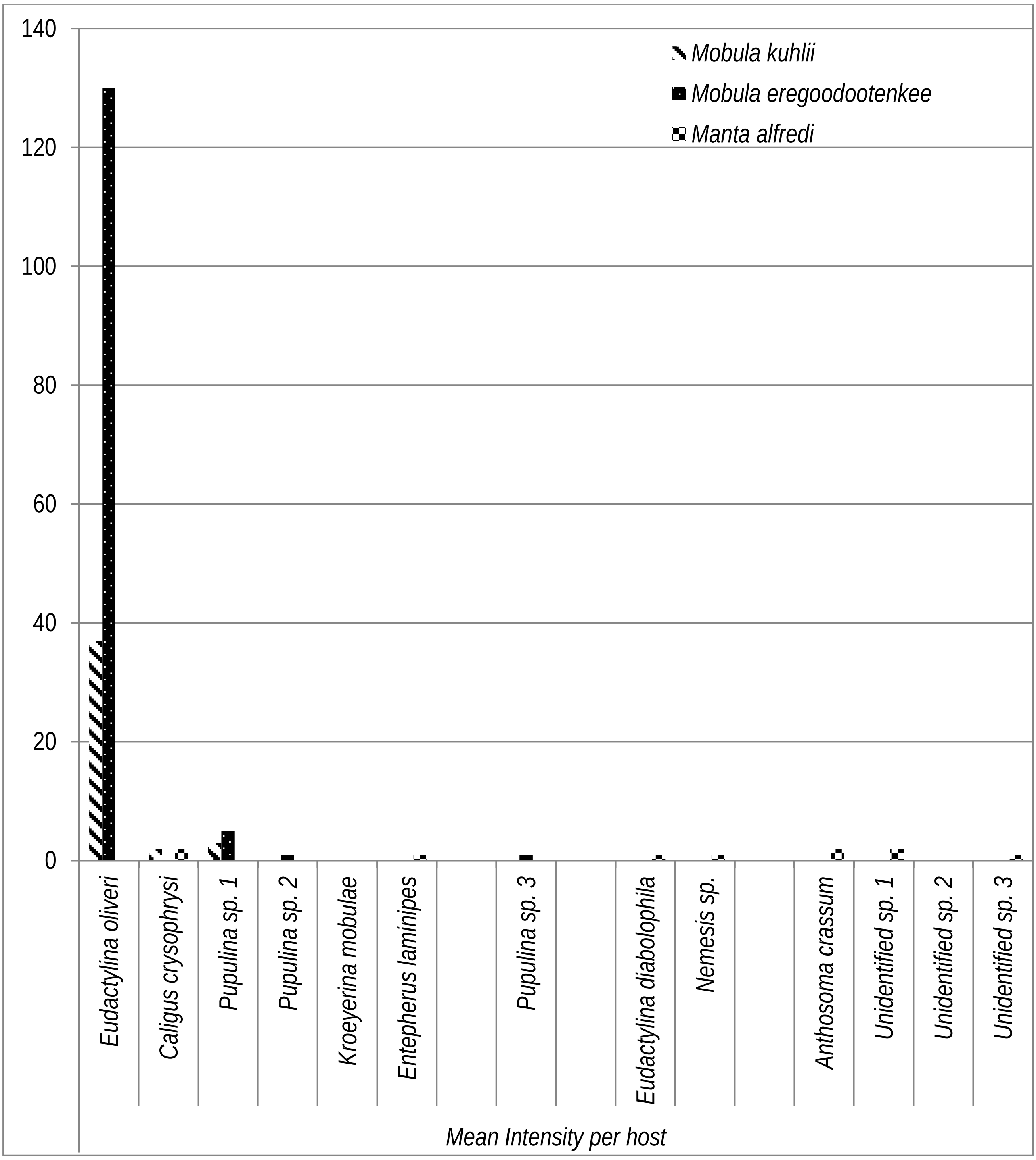


Fig. 3: Mean Abundance of *Caligus chrysophrysi* Pillai, 1985; *Pupulina* sp. 1; *Pupulina* sp. 2; *Pupulina* sp. 3; Unidentified sp. 1; Unidentified sp. 2; Unidentified sp. 3; *Eudactylina oliveri* Laubier, 1968; *Eudactylina diabolophila* Deets, 1994; *Nemesis* sp.; *Kroeyerina mobulae* Deets, 1987; *Entepherus laminipes* Bere, 1936 and *Anthosoma crassum* (Abildgaard, 1794) on *M*obula *kuhlii* (Müller & Henle, 1841); *Mobula eregoodootenkee* (Bleeker, 1859) and *Manta alfredi* (Krefft, 1868).

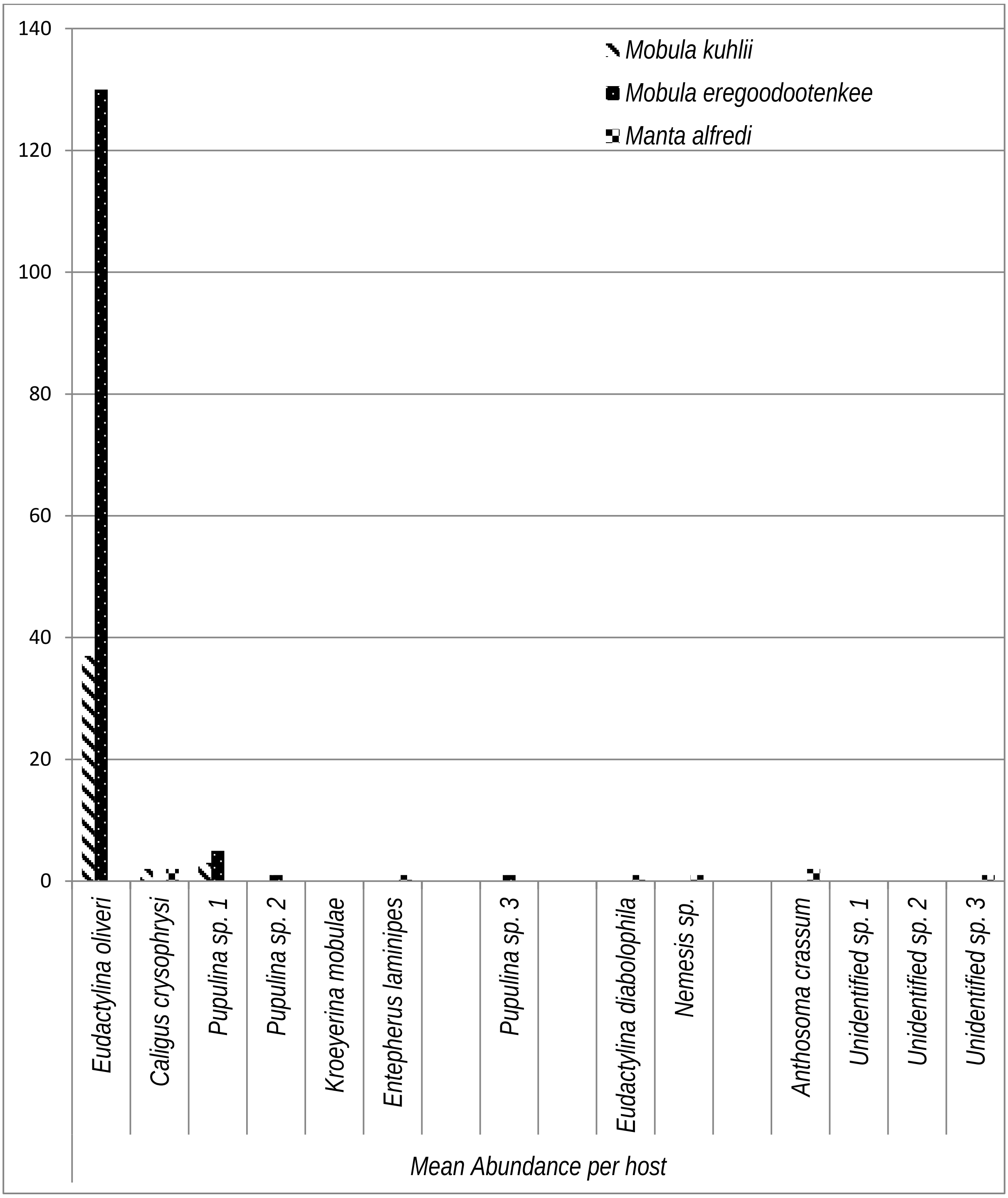


Fig. 4: Adult female *Pupulina* sp. 1

a. Habitus, ventral view; b. Habitus, dorsal view; c. Antennule; d. Antenna; e. Post-antennal process. Scale bars: a, b, 0.5 mm; c-d, 50 µm.

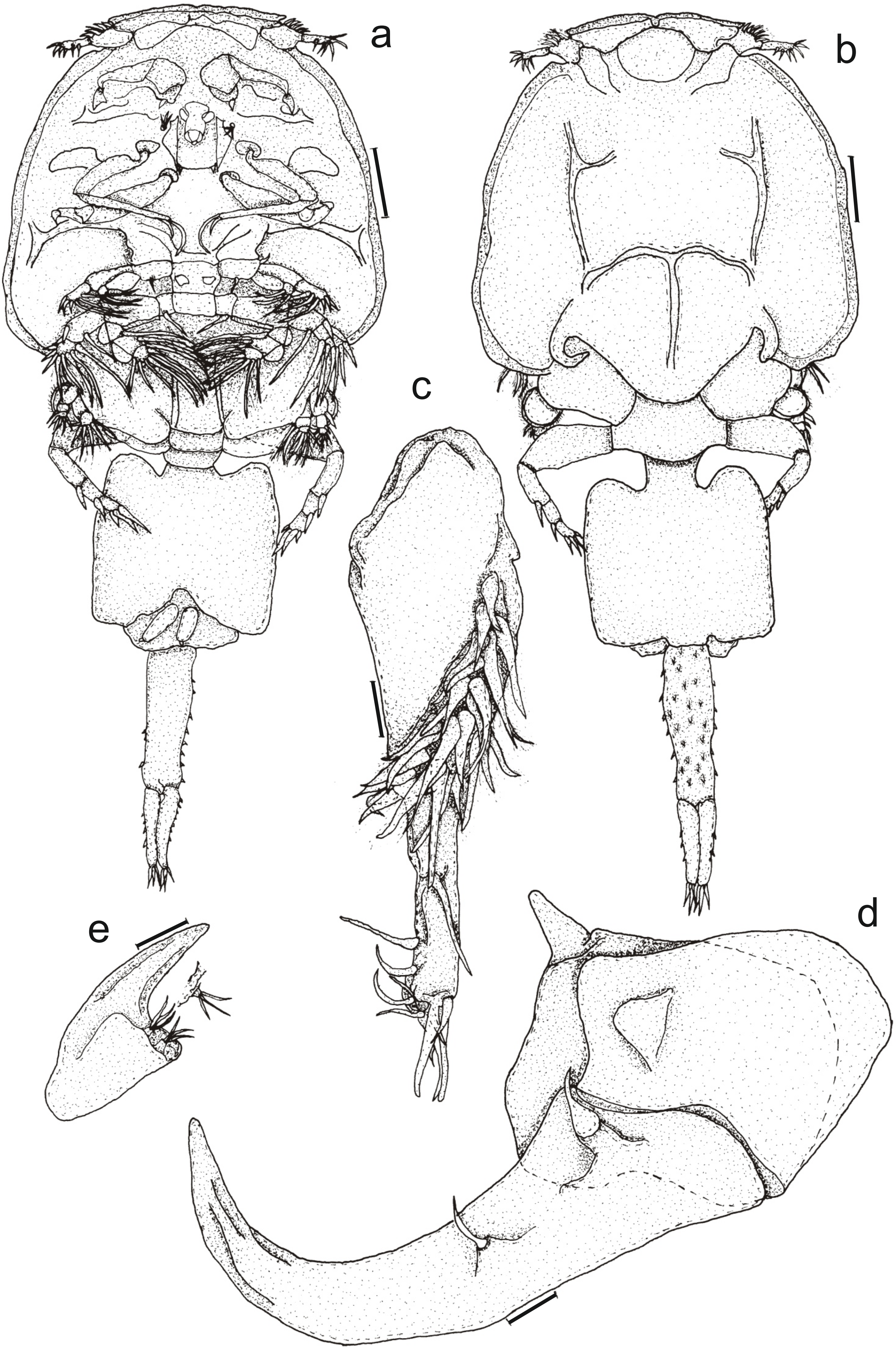

a
b
c
d
e

Fig. 5: Adult female *Pupulina* sp. 1

a. Caudal ramus; b. Mandible; c. Tip of mandible; d. Mandibles & Strigil; e. Maxillule & Post-maxilluliary process; f. Maxilla; g. Maxilliped. Scale bars: a, e, g, 50 μm; b-d, 20 μm; f, 100 μm.

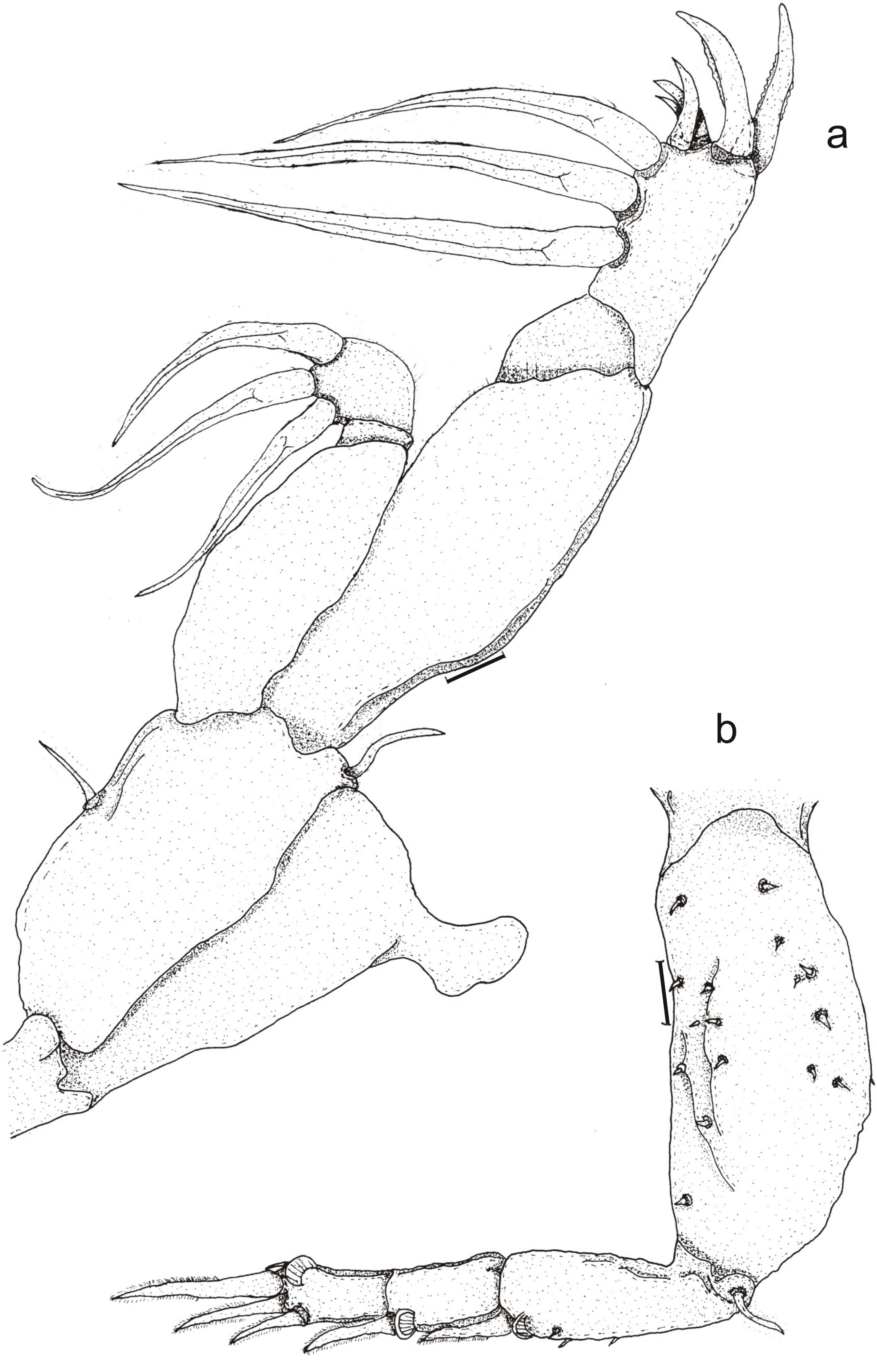

a
b

Fig. 6: Adult female *Pupulina* sp. 1

a. Leg 1; b. Leg 4. Scale bars: a, 50 µm; b, 100 µm.

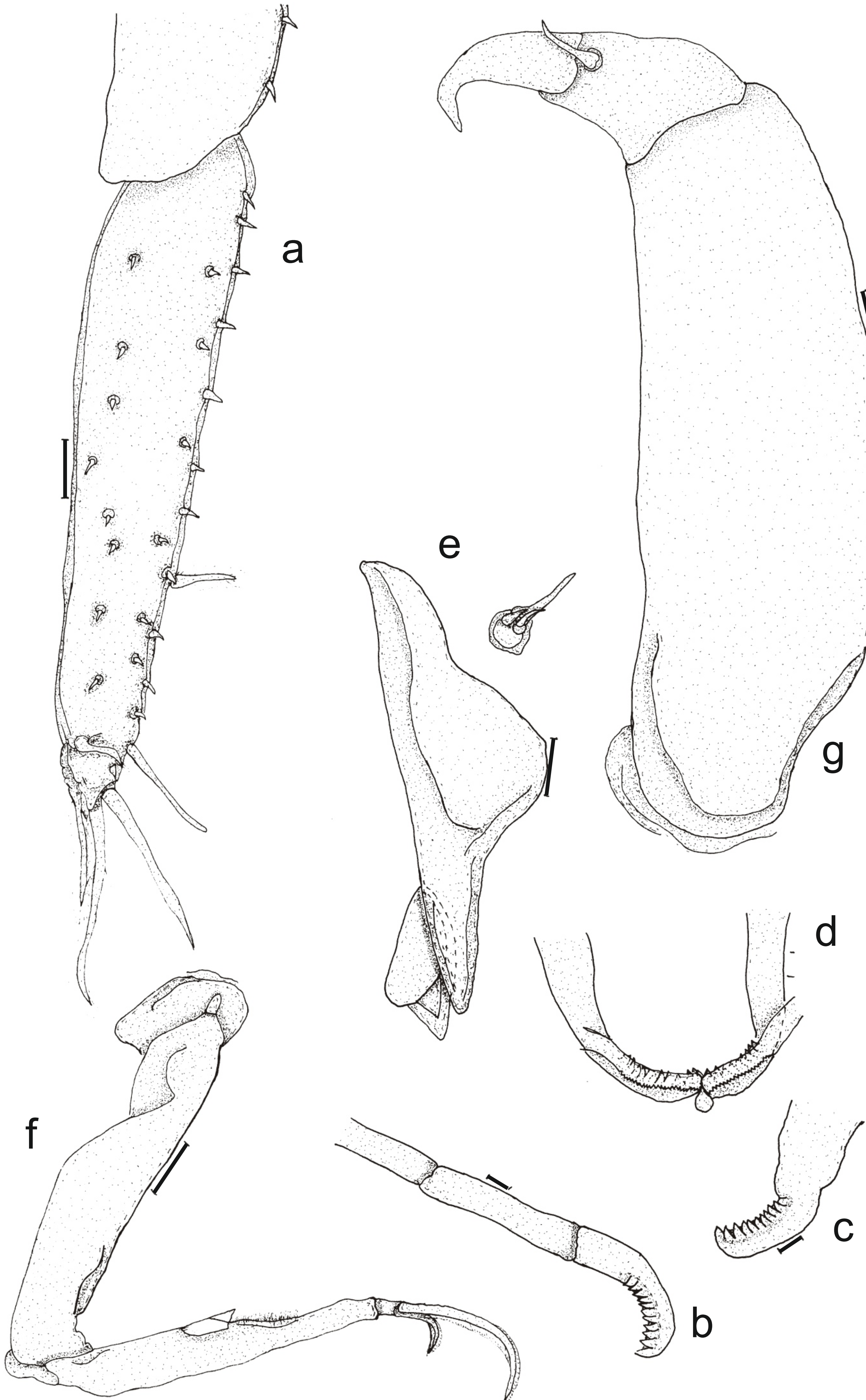

a
b
c
d
e
f
g

Fig. 7: Adult female *Pupulina* sp. 1

a. Leg 2; b. Leg 3; c. Leg 5. Scale bars: a, 100 µm; b, 50 µm; c, 20 µm.

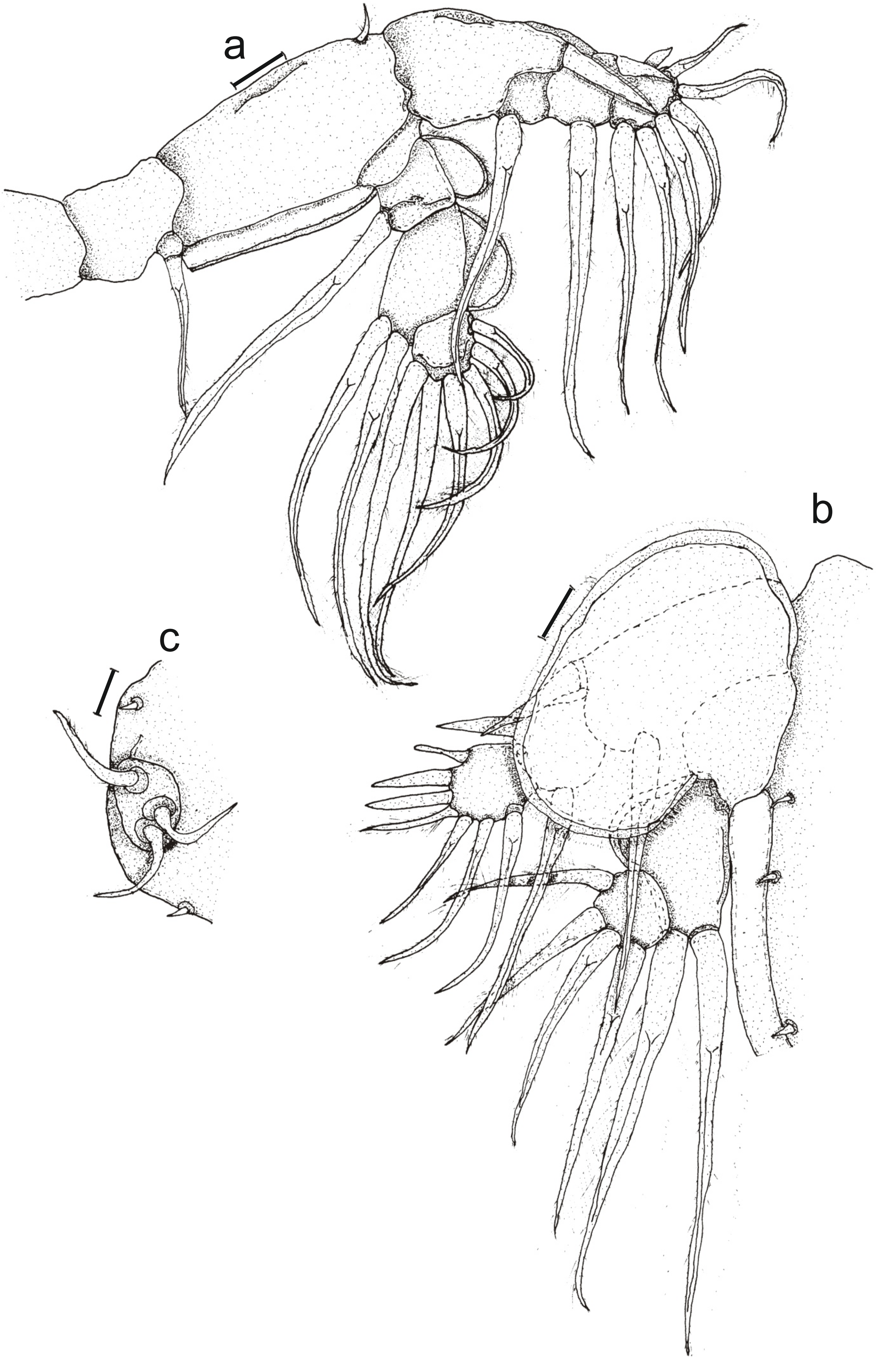
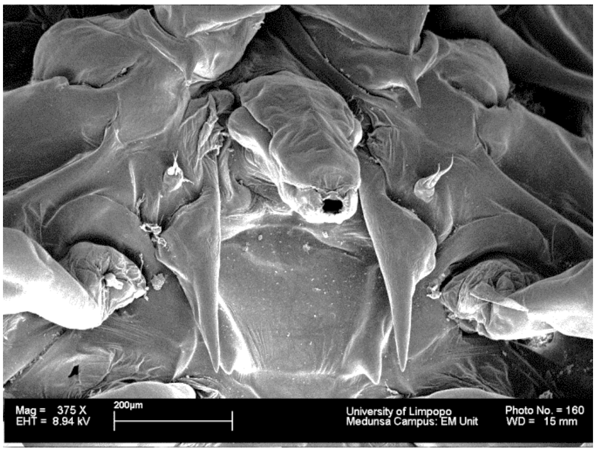
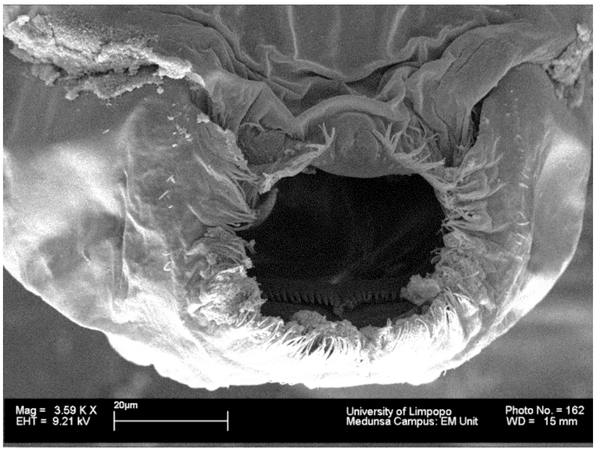

a
b
c

Fig. 8: SEM micrographs of adult female *Pupulina* sp. 1

a. Mouth tube & Maxillule; b. Mouth tube opening & Mandibles; c. Antenna; d. Tip of antenna; e. Maxillule & Post-maxilluliary process; f. Mouth tube, Maxillules, Maxilla & Maxilliped.


Mag = 375 X
EHT = 8.94 kV
200µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 160
WD = 15 mm

a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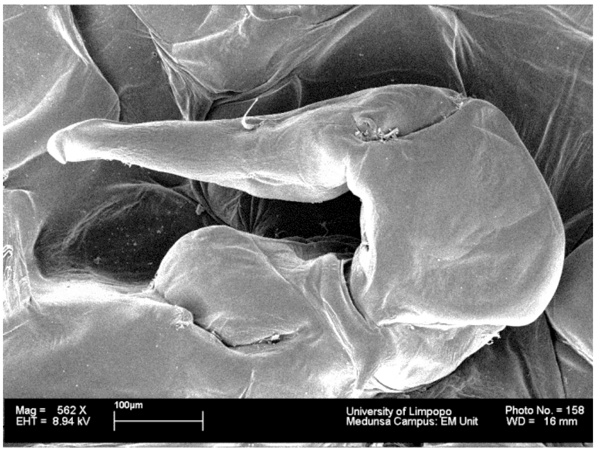
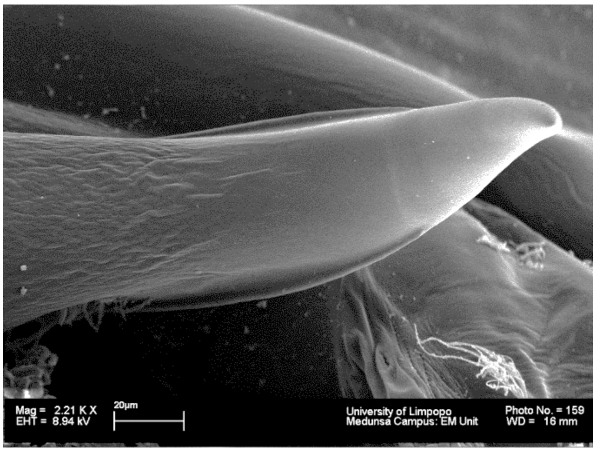
EHT = 9.21 kV
20µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 162
WD = 15 mm

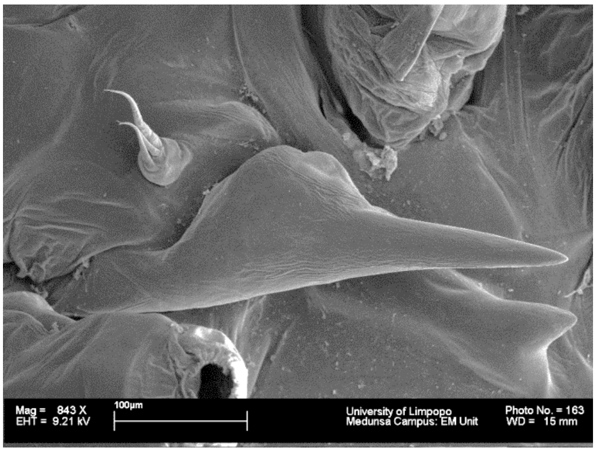
b


Mag = 562 X
EHT = 8.94 kV
100µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 158
WD = 16 mm

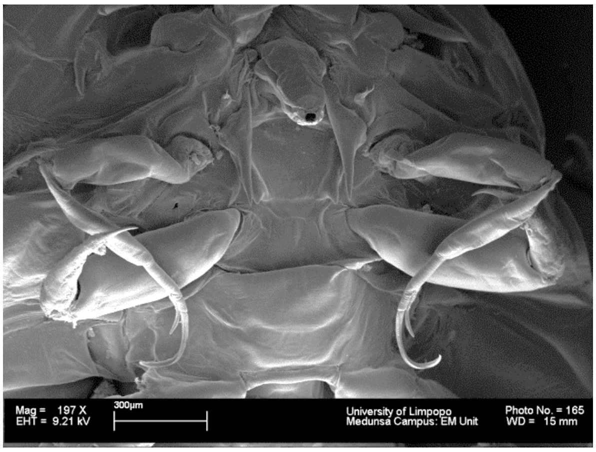
c


Mag = 2.21 K X
EHT = 8.94 kV
20µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 159
WD = 16 mm

d


Mag = 843 X
EHT = 9.21 kV
100µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 163
WD = 15 mm

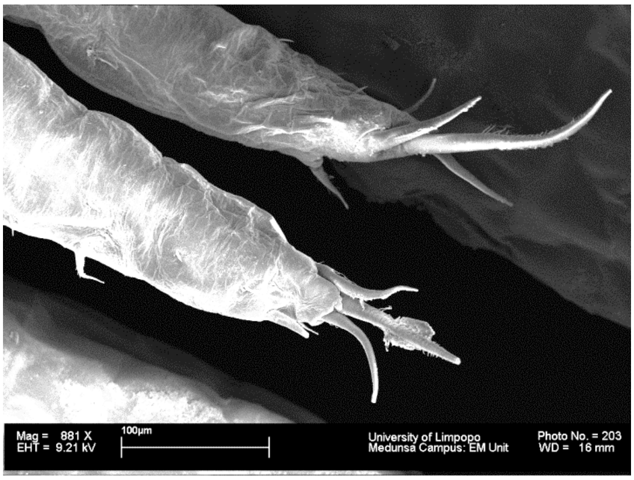
e


Mag = 197 X
EHT = 9.21 kV
300µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 165
WD = 15 mm

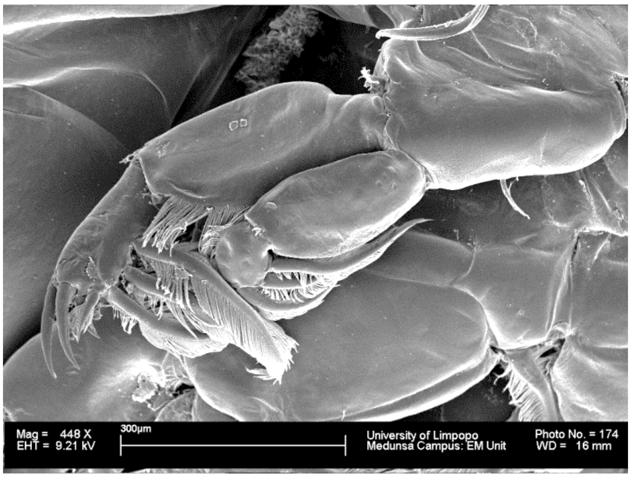
f

Fig. 9: SEM micrographs of adult female *Pupulina* sp. 1

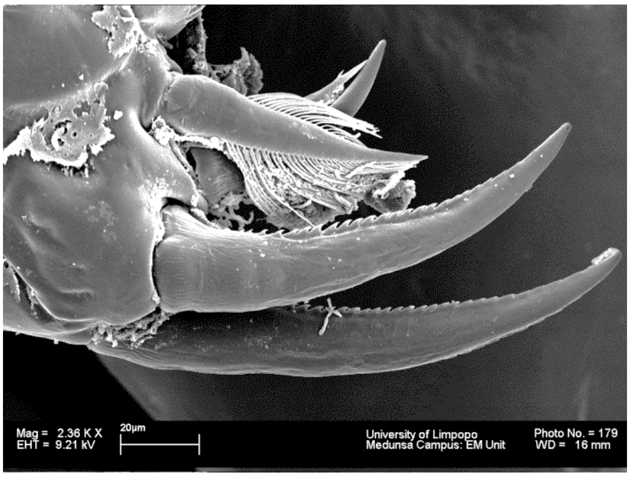
a. Tips of caudal rami; b. Leg 1; c. Tip of segment 2 of leg 1 exopod; d. Leg 2; e. Leg 3; f. Leg 4.


Mag = 881 X
EHT = 9.21 kV
100µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 203
WD = 16 mm

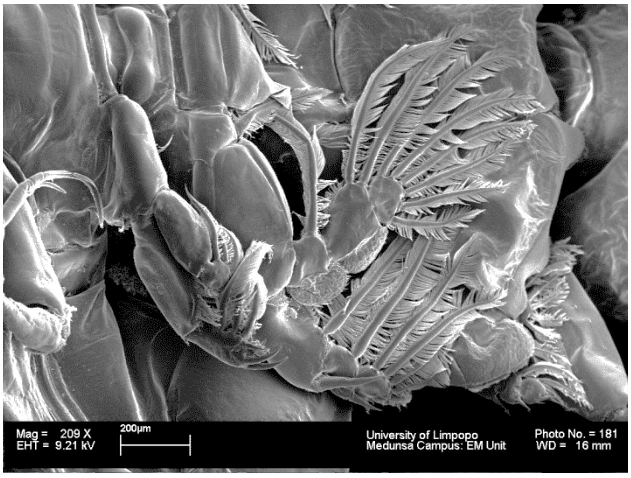
a


Mag = 448 X
EHT = 9.21 kV
300µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 174
WD = 16 mm

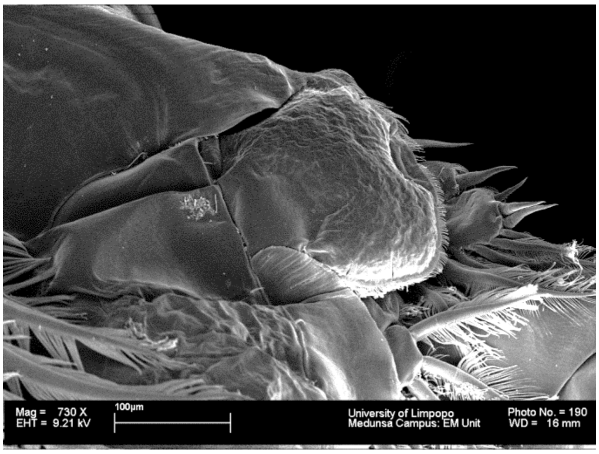
b


Mag = 2.36 K X
EHT = 9.21 kV
20µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 179
WD = 16 mm

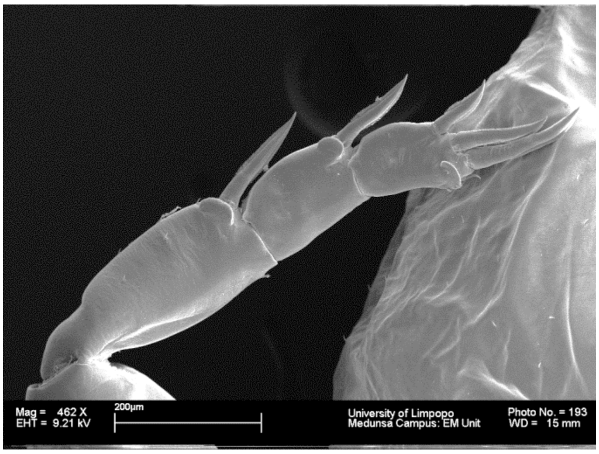
c


Mag = 209 X
EHT = 9.21 kV
200µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 181
WD = 16 mm

d


Mag = 730 X
EHT = 9.21 kV
100µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 190
WD = 16 mm

e


Mag = 462 X
EHT = 9.21 kV
200µm
University of Limpopo
Medunsa Campus: EM Unit
Photo No. = 193
WD = 15 mm

f

Fig. 10: Adult female *Pupulina* sp. 2

a. Habitus, ventral view; b. Habitus, dorsal view; c. Antennule; d. Antenna; e. Post-antennal process. Scale bars: a, b, 0.5 mm; c, d, 50 μm; e, 20 μm.

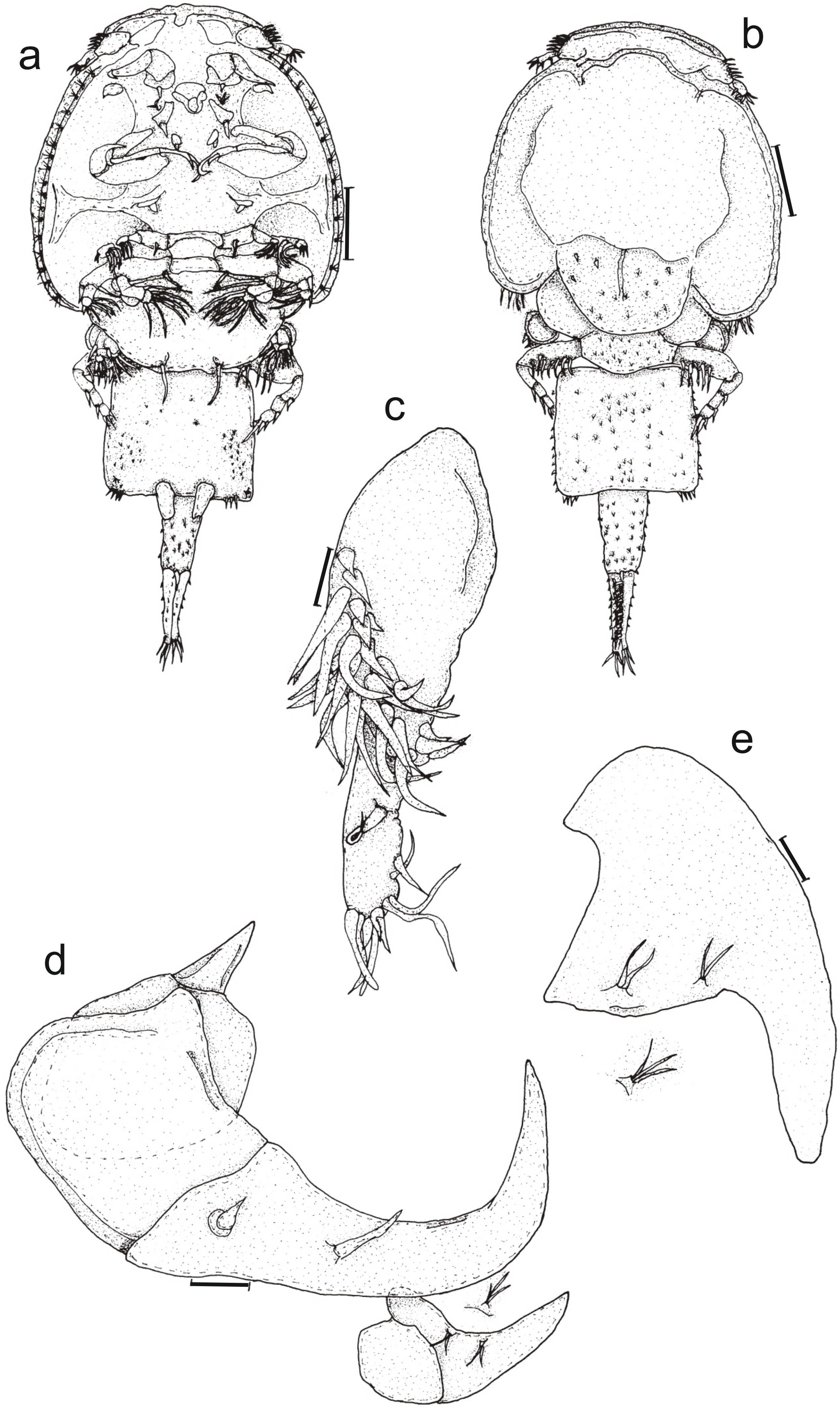

a
b
c
d
e

Fig. 11: Adult female *Pupulina* sp. 2

a. Tip of mandible; b. Maxillule; c. Post-maxilluliary process; d. Maxilla; e. Maxilliped; f. Post-maxillipedal process; g. Leg 5. Scale bars: a, 10 µm; b, d, e, 50 µm; f, g, 20 µm.

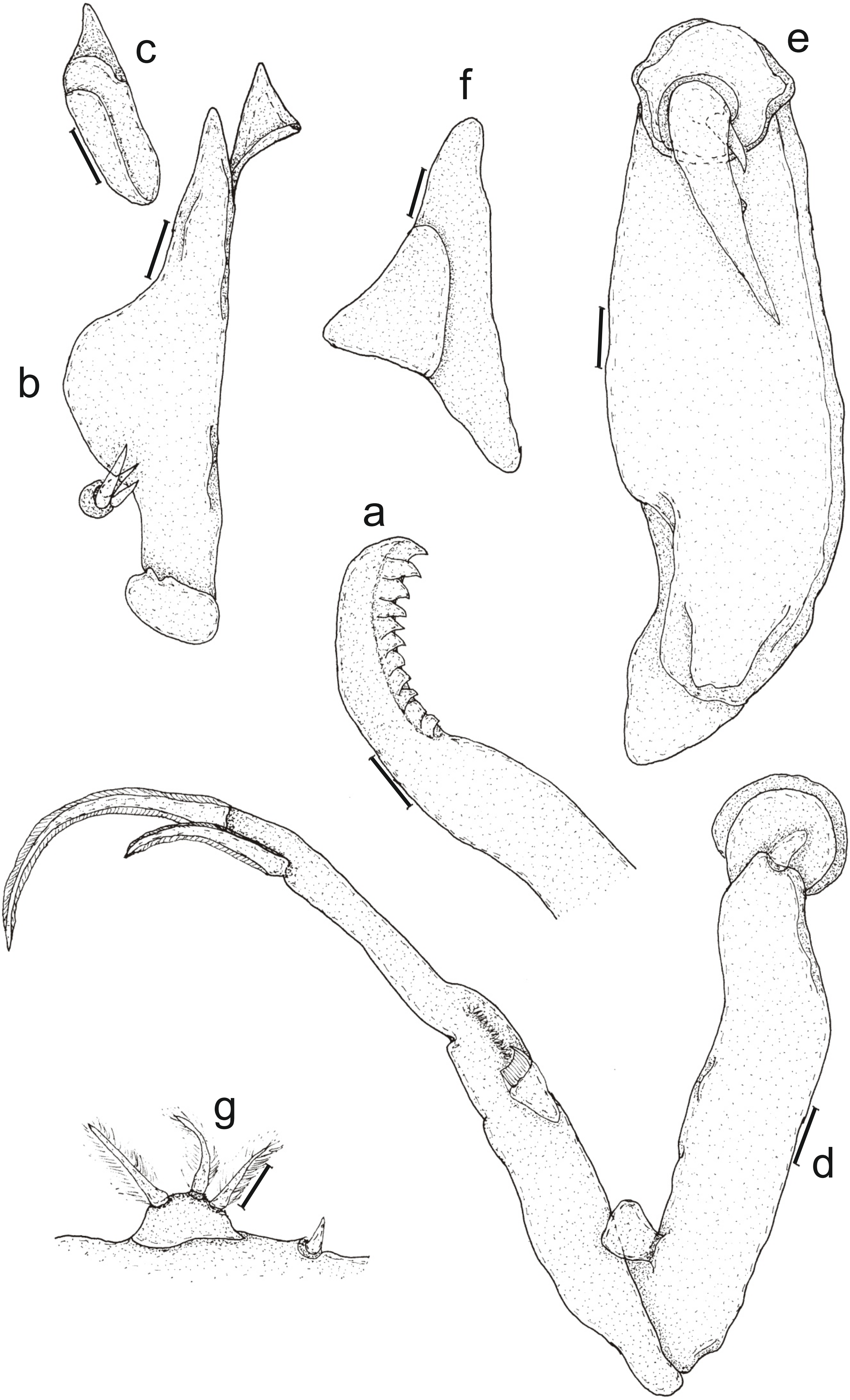

c
e
f
b
a
g
d

Fig. 12: Adult female *Pupulina* sp. 2

a. Caudal ramus; b. Mouth tube with mandibles & Strigil; c. Mandible & Strigil; d. Leg 1; e. Tip of segment 2 of leg 1 exopod. Scale bars: a, d, e, 50 µm; b, 20 µm; c, 10 µm.

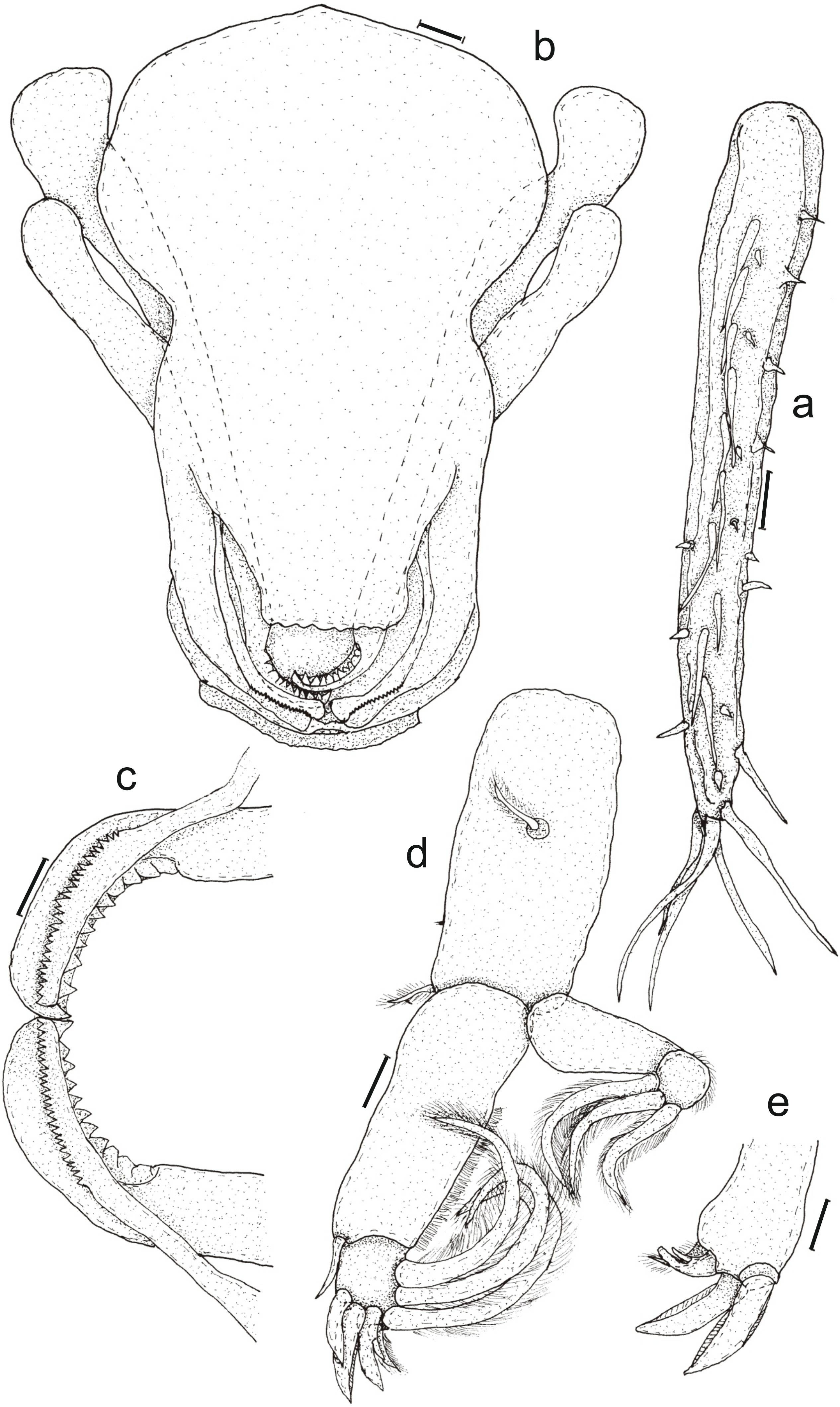

b
a
c
d
e

Fig. 13: Adult female *Pupulina* sp. 2

a. Leg 2; b. Leg 3; c. Leg 4. Scale bars: a-c, 50 µm.

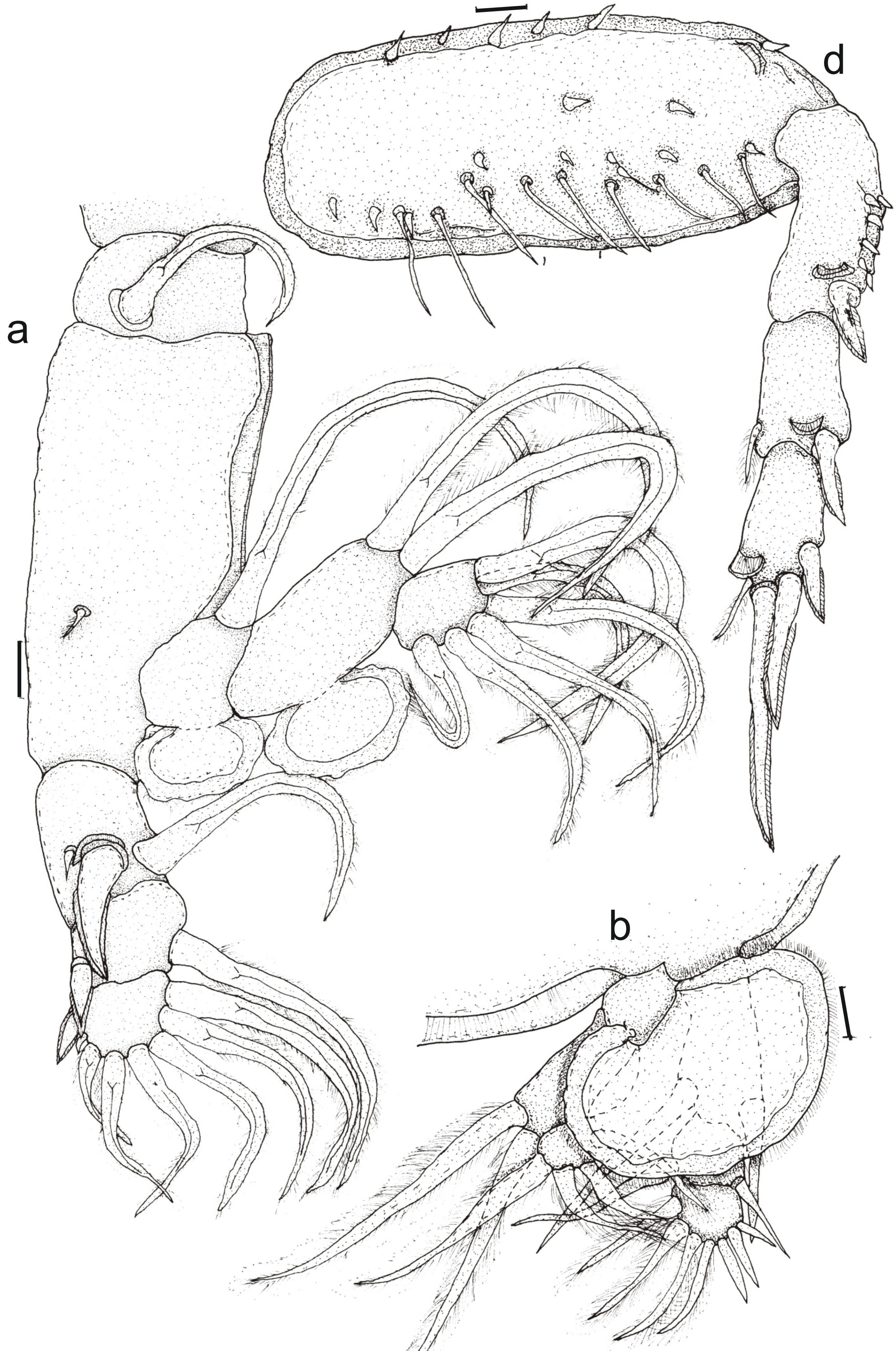

a
b
d

Fig. 14: Adult female *Pupulina* sp. 3

a. Habitus, ventral view; b. Habitus, dorsal view; c. Antennule. Scale bars: a, b, 0,5 mm; c, 50 µm.

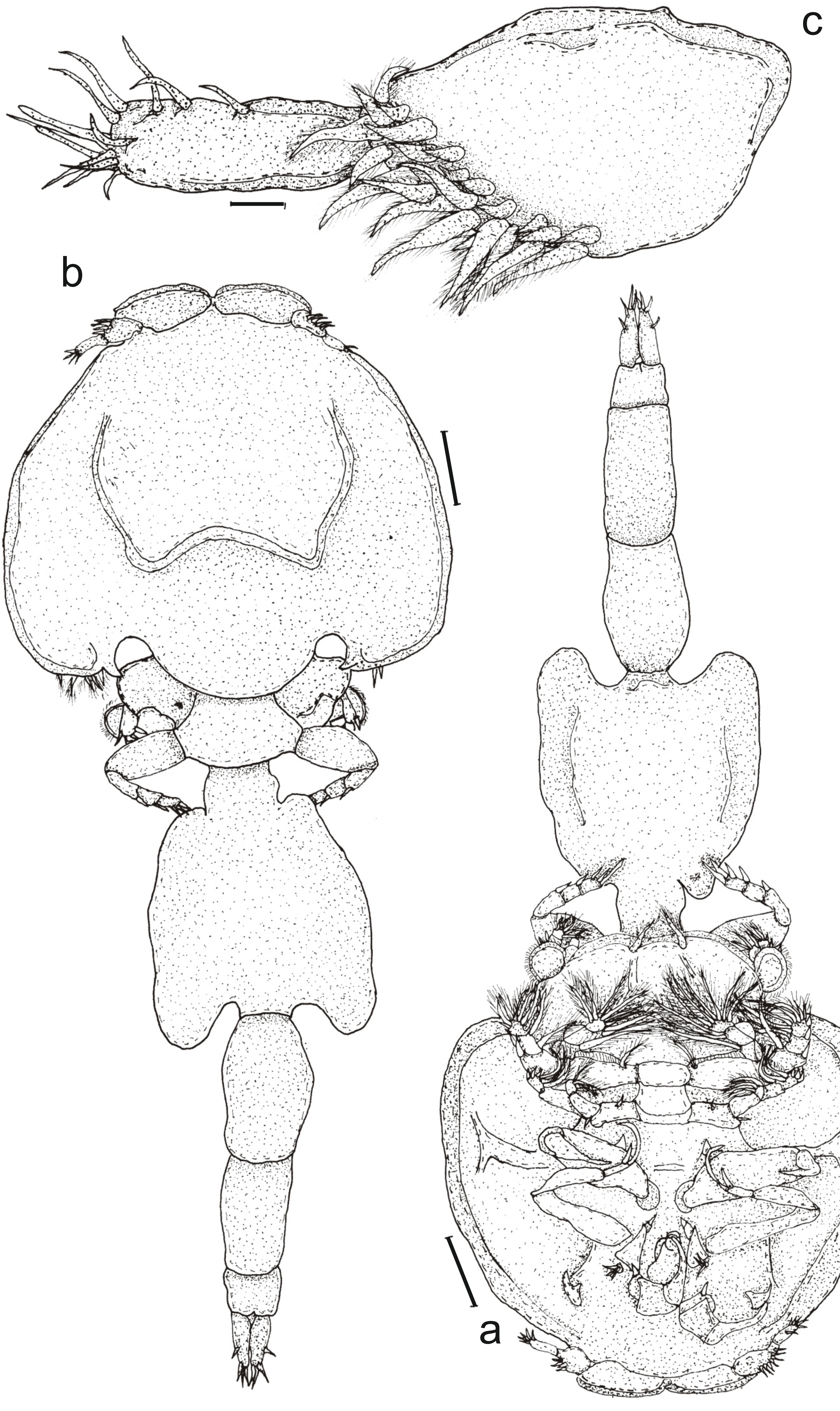

c
b
a

Fig. 15: Adult female *Pupulina* sp. 3

a. Antenna; b. Post-antennal process; c. Maxillule; d. Post-maxilluliary process; e. Maxilla; f. Maxilliped. Scale bars: a, b, 50 µm; c, d, f, 100 µm; d, 20 µm.

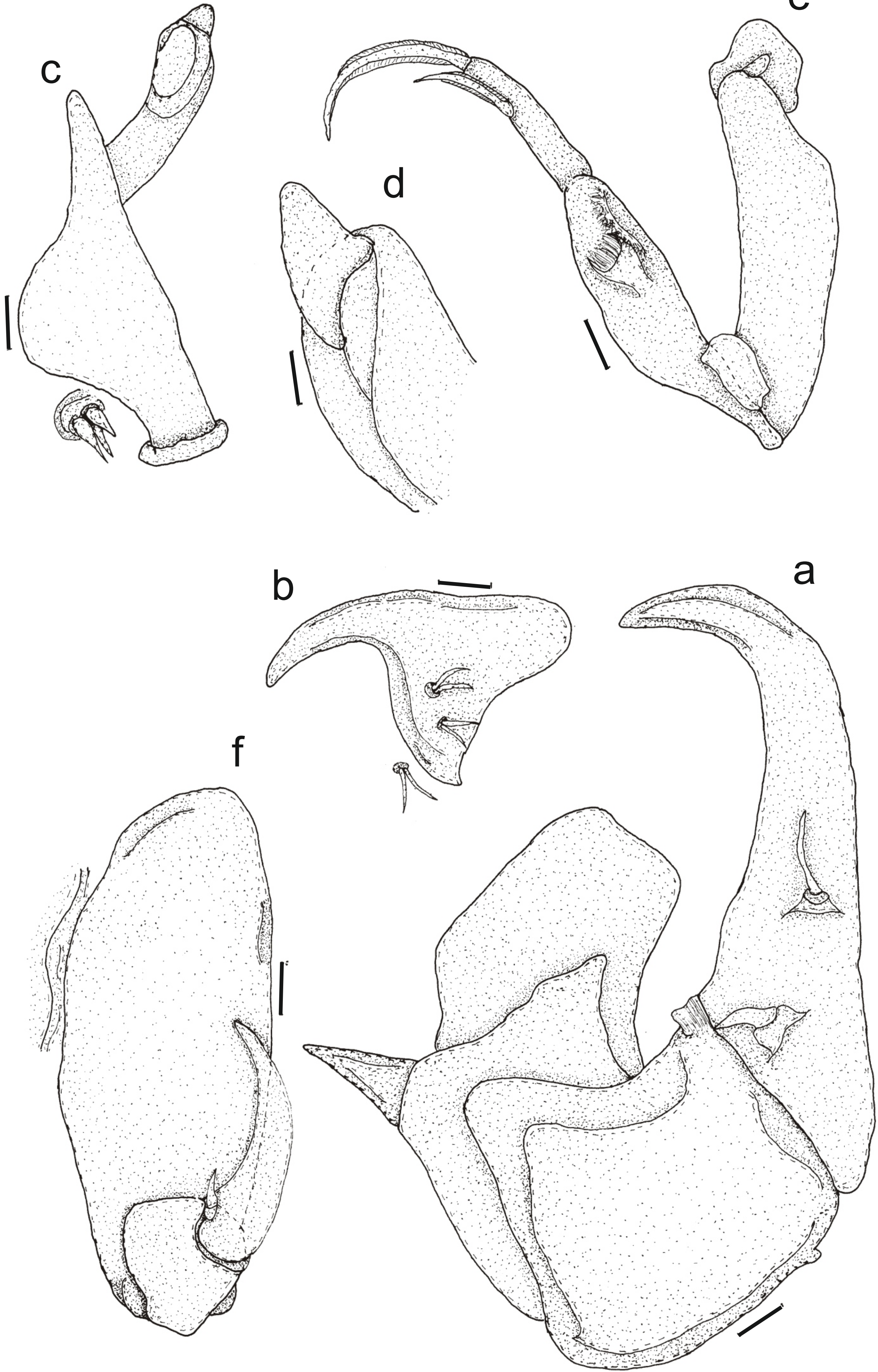

c
d
e
b
a
f

Fig. 16: Adult female *Pupulina* sp. 3

a. Caudal ramus; b. Leg 1; c. Tip of segment 2 of leg 1 exopod; d. Leg 3. Scale bars: a, b, d, 50 μm; c, 20 μm.

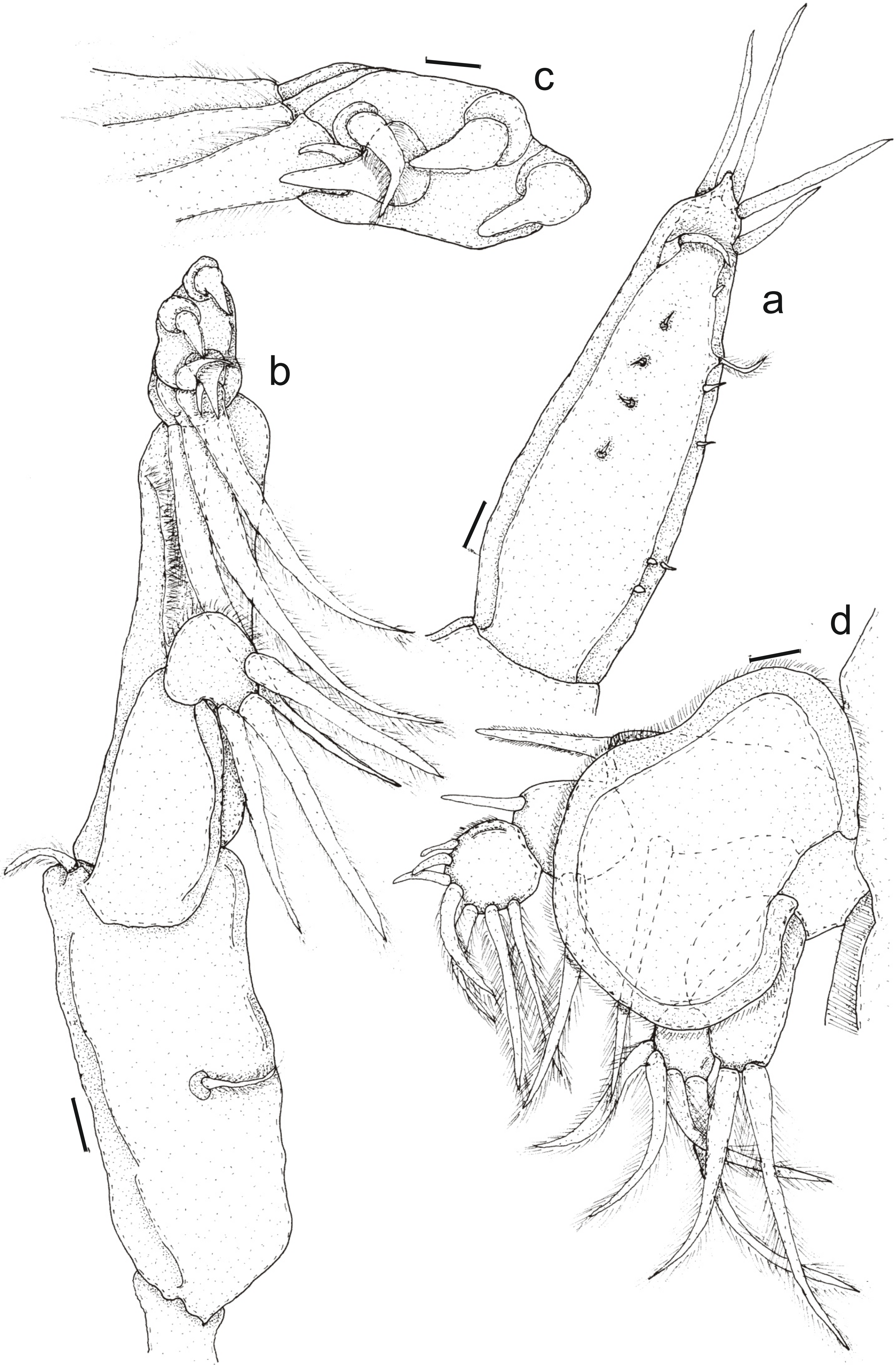

c
a
b
d

Fig. 17: Adult female *Pupulina* sp. 3

a. Tip of mandible; b. Tip of mandible; c. Strigil; d. Leg 2; e. Leg 4; f. Leg 5. Scale bars: a-c, f, 20 µm; d, 100 µm.

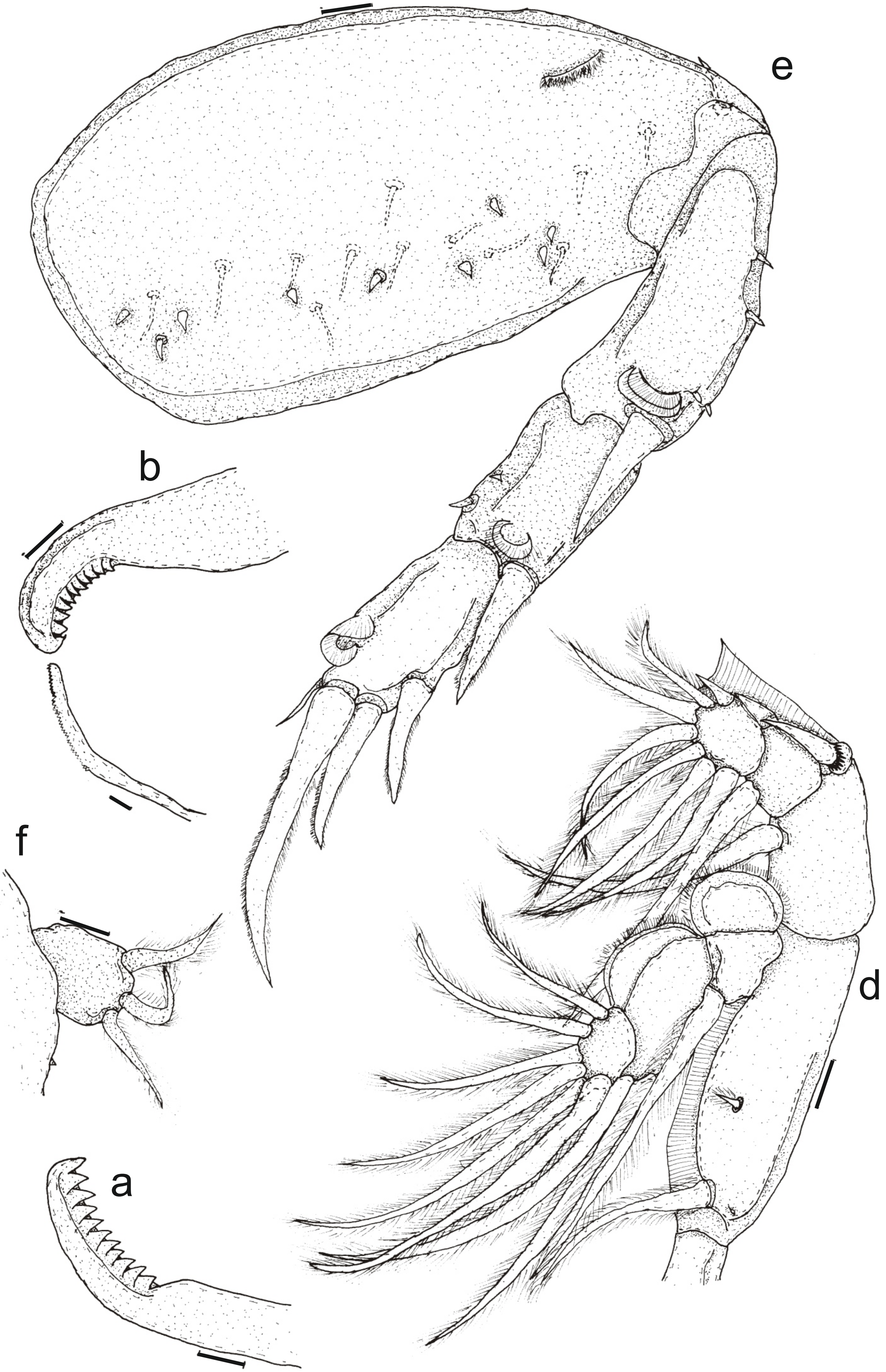

e
b
f
d
a

Fig. 18: Adult female Unidentified sp. 1

a. Habitus, ventral view; b. Habitus, dorsal view; c. Tip of mandible; d. Mandible; e. Mandibles & Strigil. Scale bars: a, b, 2 mm; c, e, 20 µm; d, 50 µm.

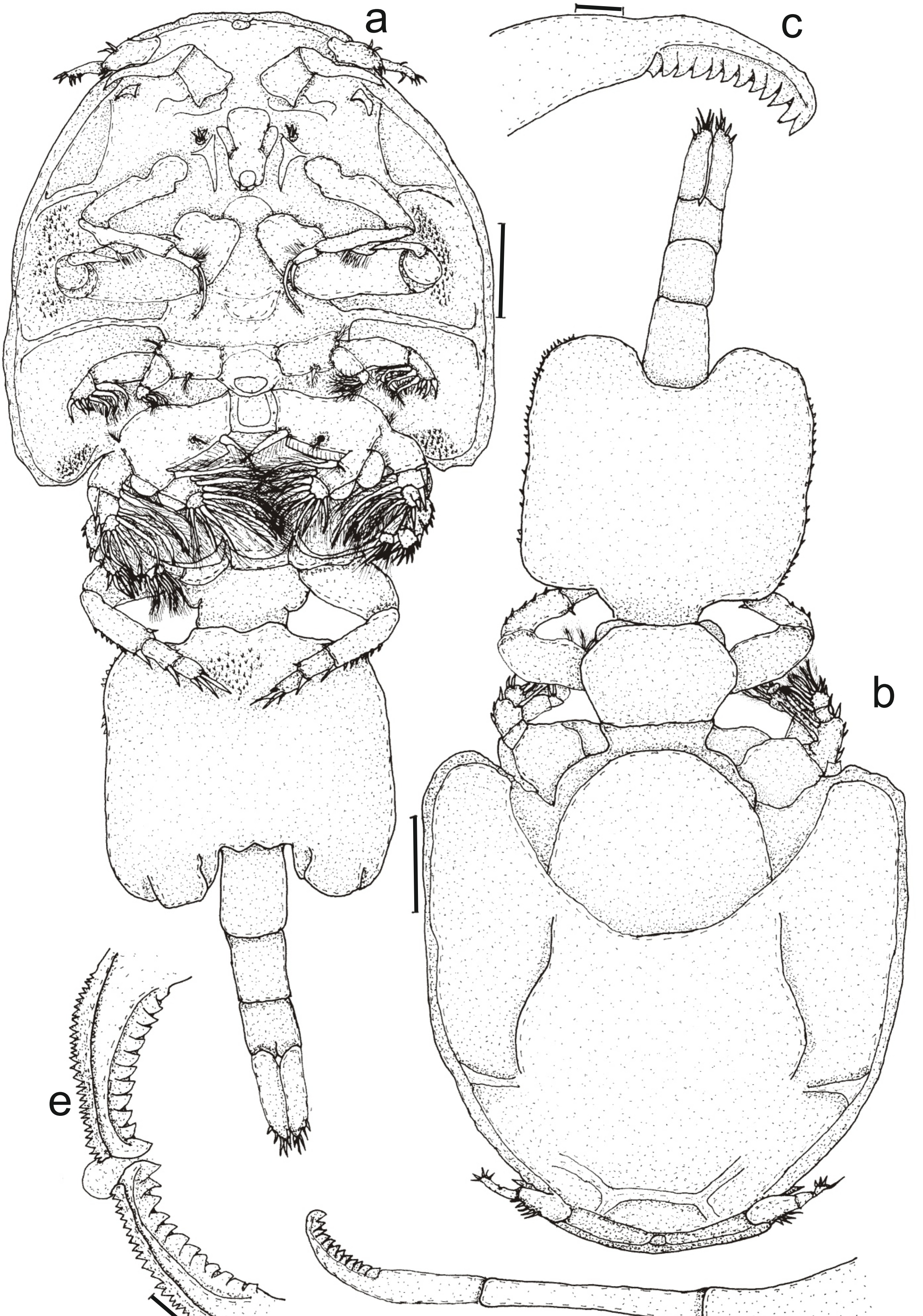

a
c
b
e
d

Fig. 19: Adult female Unidentified sp. 1

a. Antennule; b. Antenna; c. Post-antennal process; d. Maxillule; e. Maxilla; f. Tip of maxilla. Scale bars: a, c, d, f, 50 µm; b, e, 100 µm.

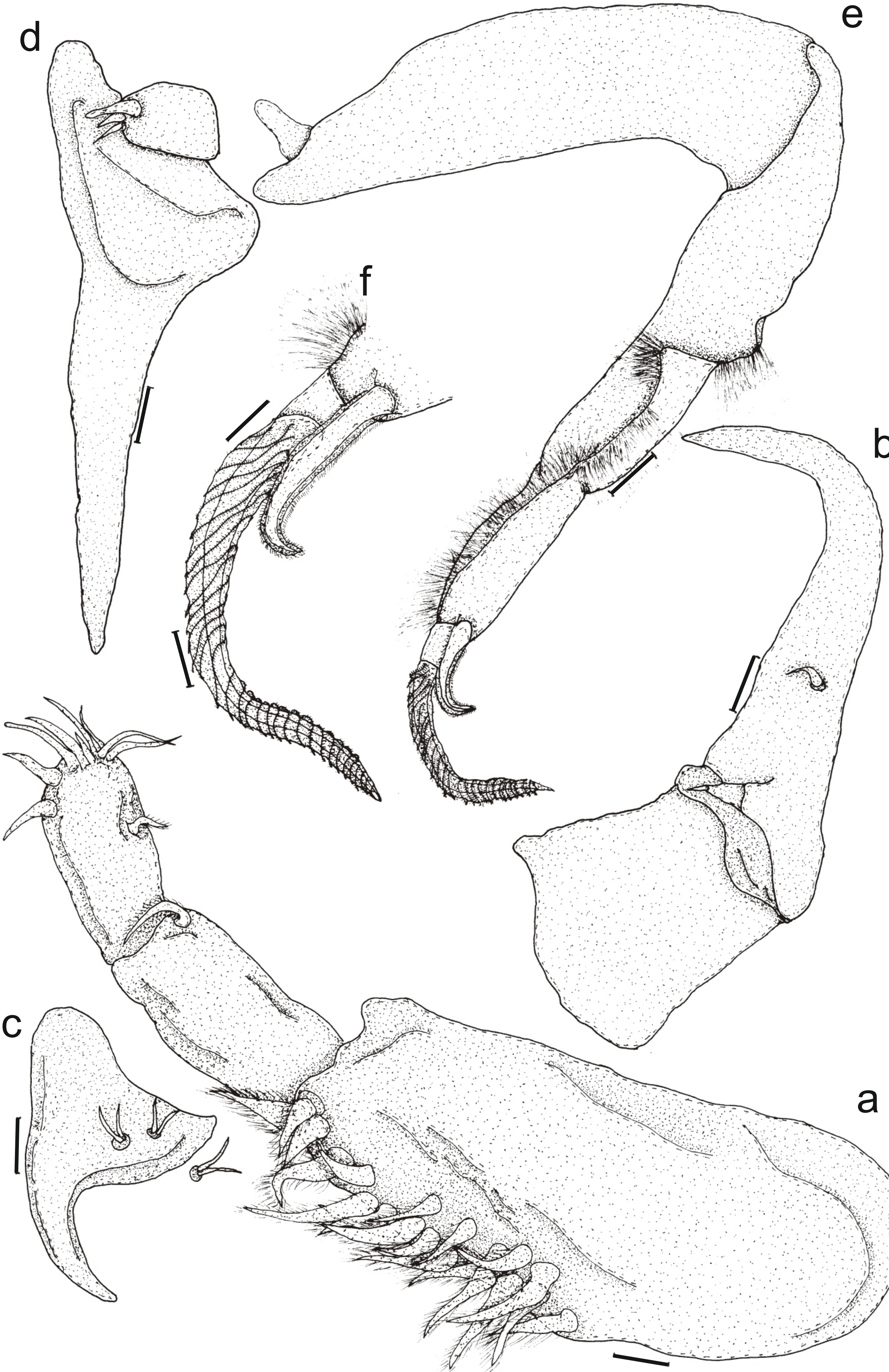

d
e
f
b
c
a

Fig. 20: Adult female Unidentified sp. 1

a. Caudal ramus; b. Maxilliped; c. Leg 1; d. Leg 4; e. Leg 5. Scale bars: a-d, 100 µm; e, 20 µm.

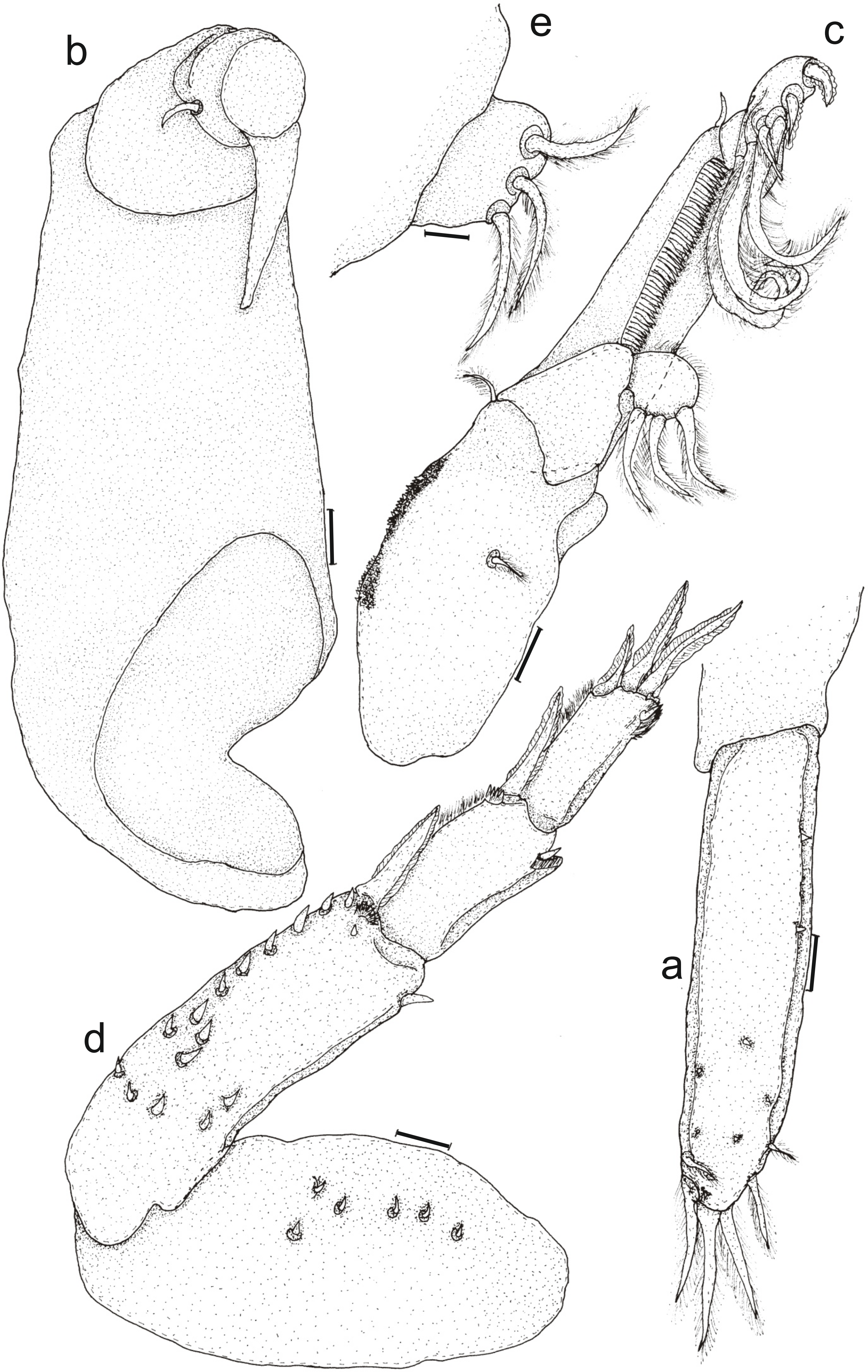

b
e
c
a
d

Fig. 21: Adult female Unidentified sp. 1

a. Leg 2; b. Leg 3. Scale bars: a, b, 100 μm.

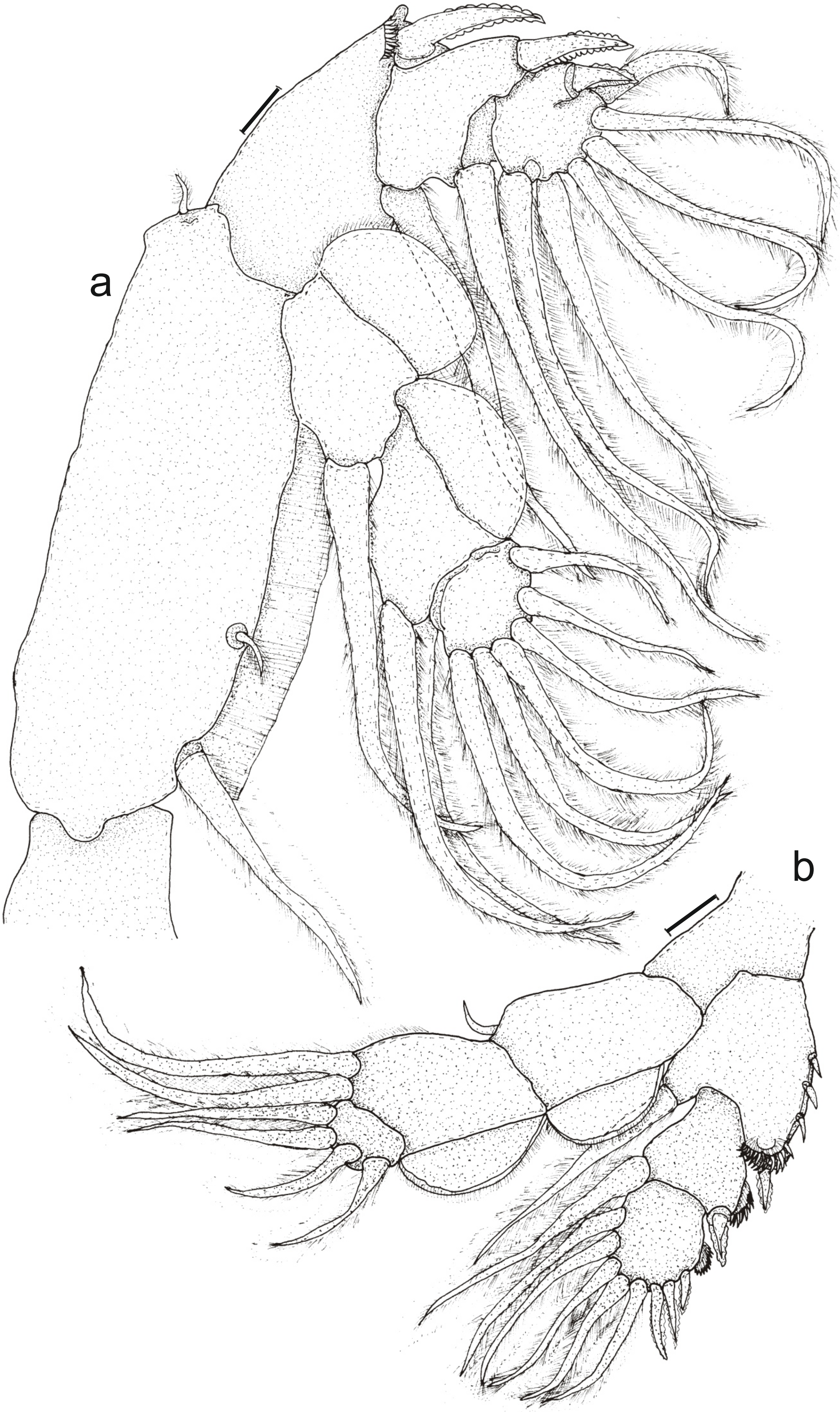

a
b

Fig. 22: Adult female Unidentified sp. 2

a. Habitus, ventral view; b. Habitus, dorsal view. Scale bars: a, b, 1 mm.

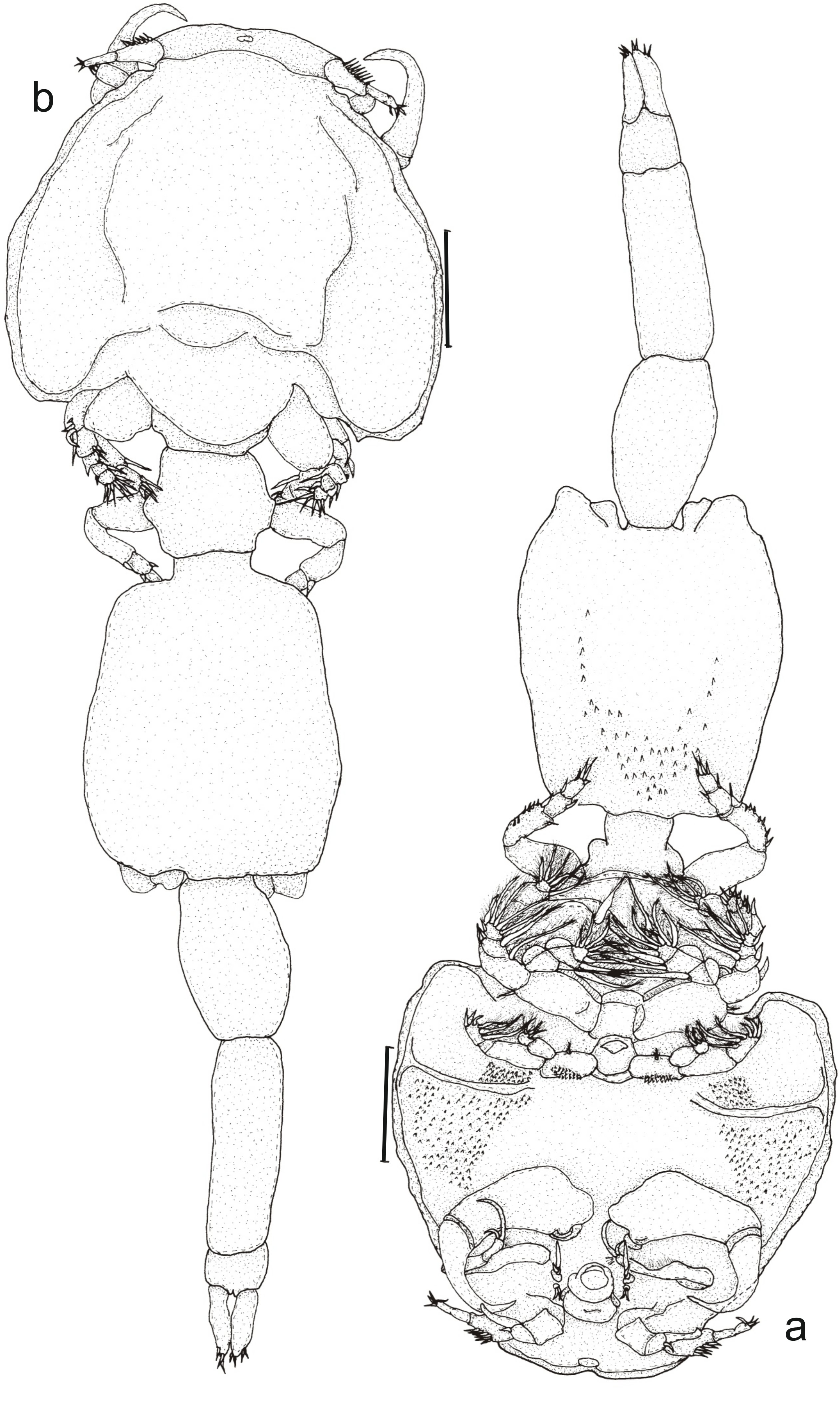

b
a

Fig. 23: Adult female Unidentified sp. 2

a. Antennule; b. Antenna; c. Post-antennal process; d. Maxilla; e. Tip of maxilla. Scale bars: a-c, e, 50 µm; d, 100 µm.

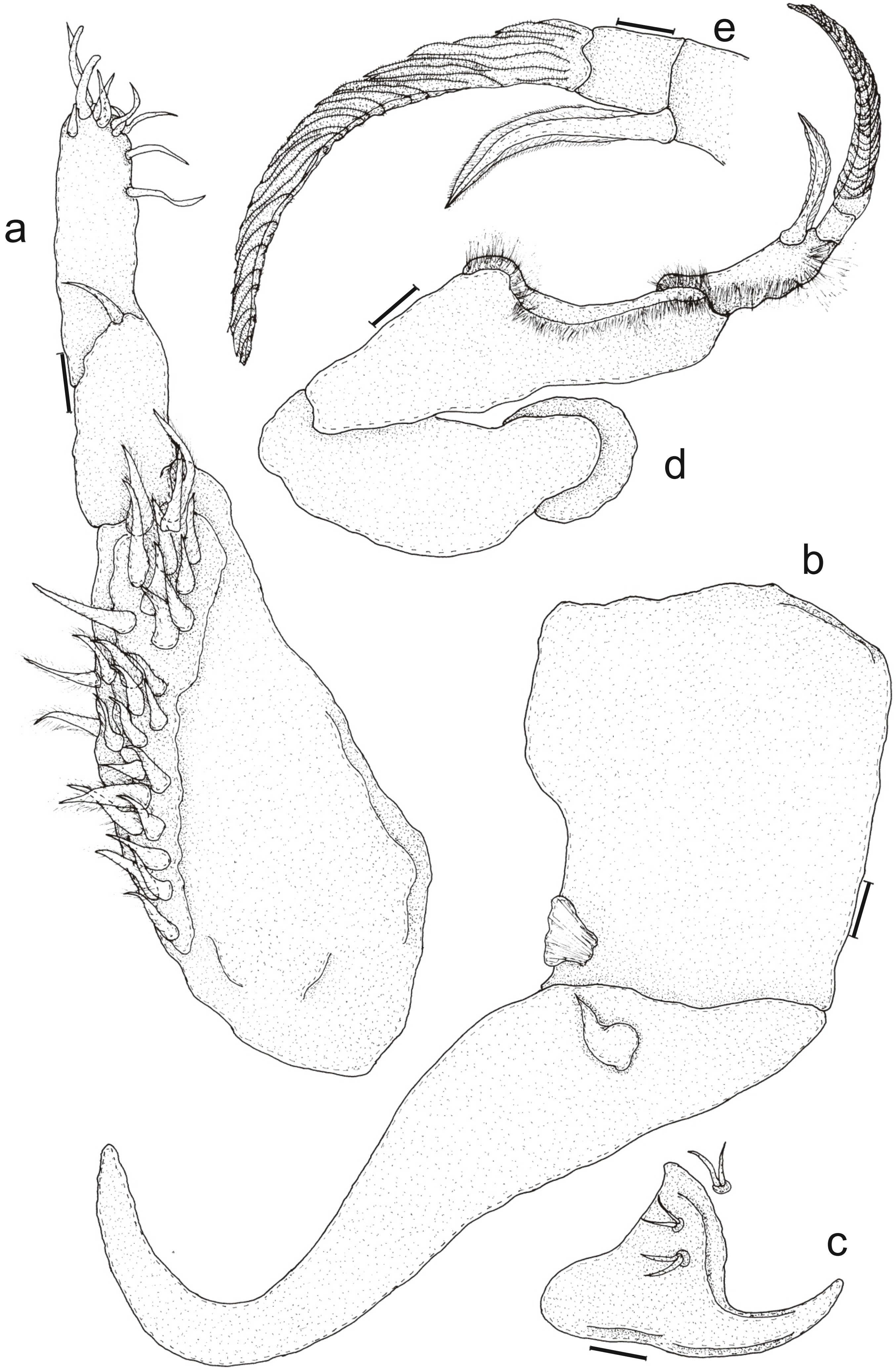

e
a
d
b
c

Fig. 24: Adult female Unidentified sp. 2

a. Caudal ramus; b. Mandible; c. Maxillule; d. Maxilliped; e. Leg 1. Scale bars: a, d, e, 100 μm; b, 10 μm; c, 50 μm.

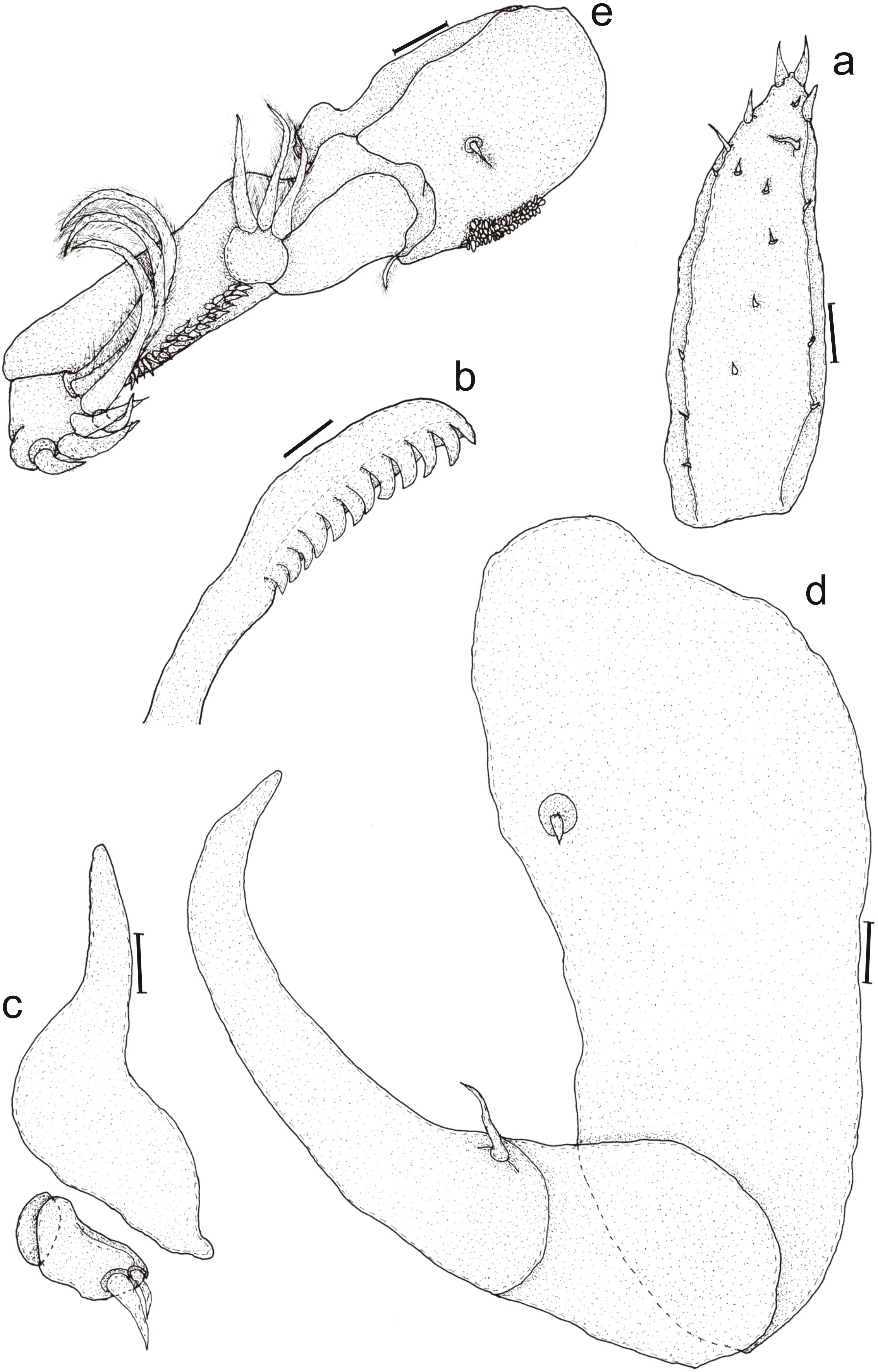

e
a
b
d
c

Fig. 25: Adult female Unidentified sp. 2

a. Leg 2; b. Leg 3; c. Leg 4. Scale bars: a-c, 100 µm.

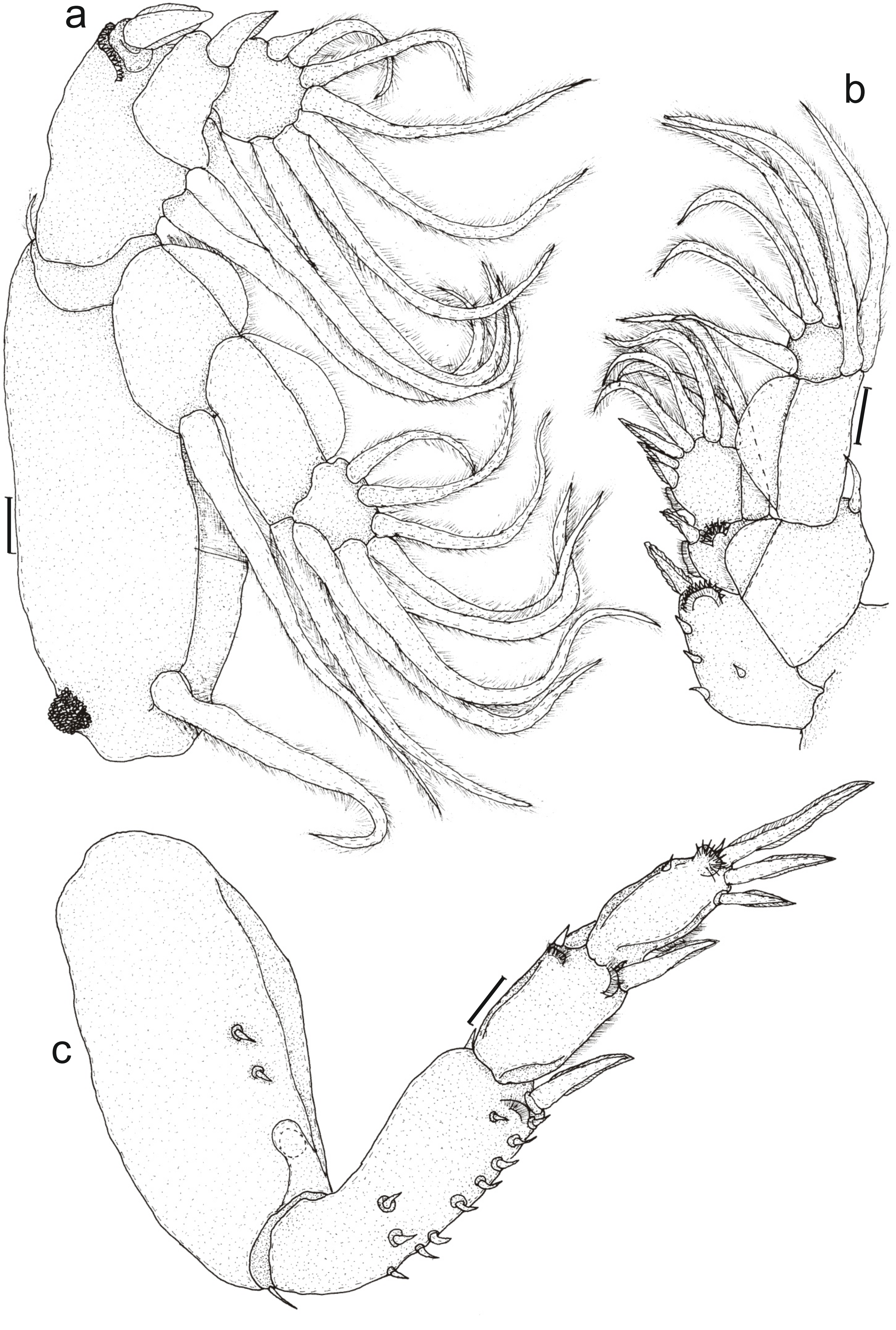

a
b
c

Fig. 26: adult female Unidentified sp. 3

a. Habitus, ventral view; b. Habitus, dorsal view; c. Caudal rami; d. Mandible; e. Mandible & Strigil. Scale bars: a, b, 0.5 mm; c, 50 μm; d, e, 20 μm.

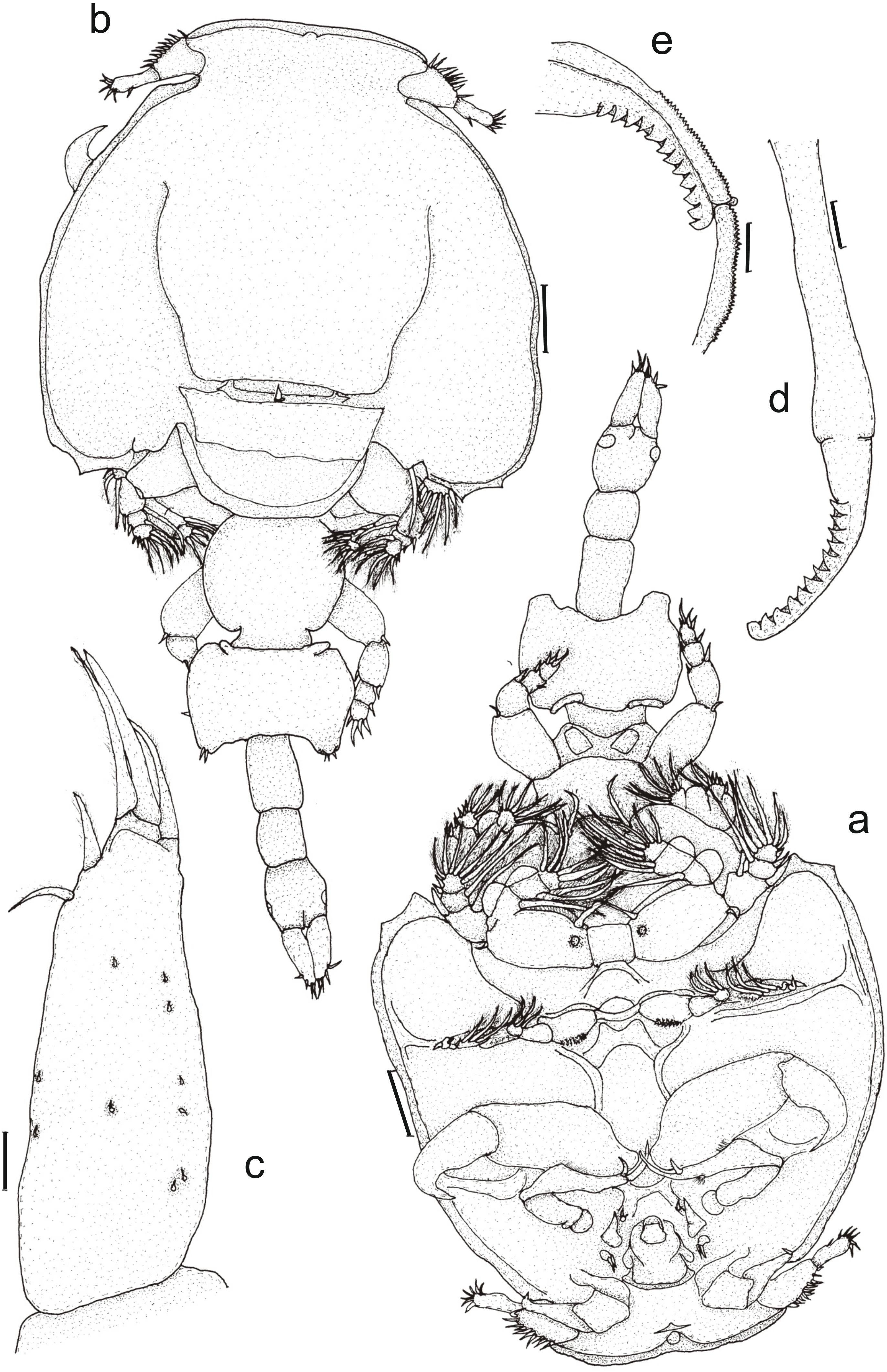

b
e
d
a
c

Fig. 27: Adult female Unidentified sp. 3

a. Antennule; b. Post-antennal process; c. Maxillule & Post-maxilluliary process; d. Maxilla; e. Maxilliped; f. Leg 3. Scale bars: a, 50 μm; b, c, 20 μm; d-f, 100 μm.

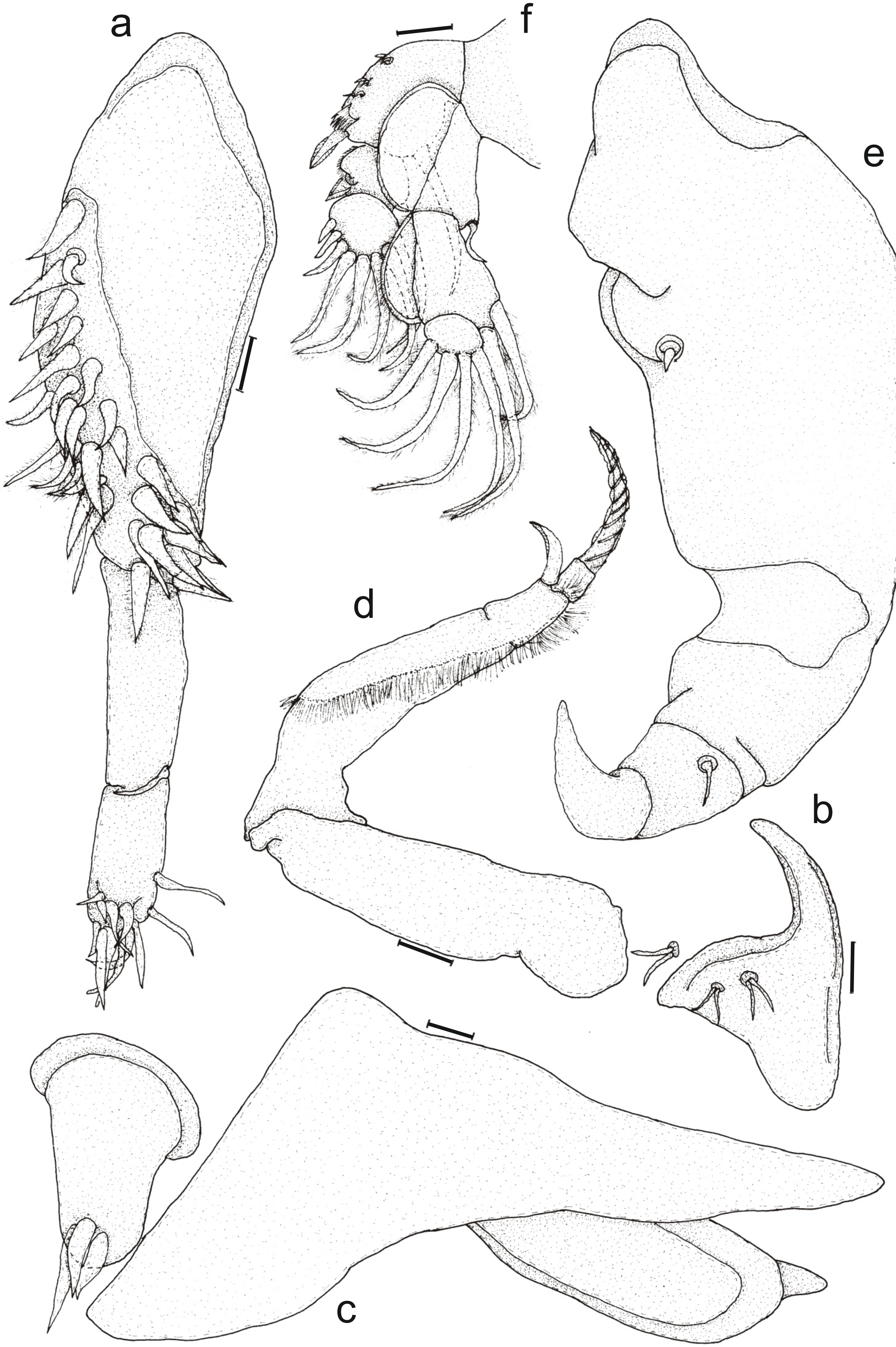

a
f
e
d
b
c

Fig. 28: Adult female Unidentified sp. 3

a. Antenna; b. Leg 1; c. Leg 2; d. Leg 4; e. Leg 5. Scale bars: a, 50 µm; b-d, 100 µm; e, 20 µm.


c
a
d
b
e

Fig. 29: Adult male Unidentified sp.

a. Habitus, ventral view; b. Habitus, dorsal view; c. Antennule; d. Mandible; e. Mandibles & Strigil. Scale bars: a, b, 0.5 mm; c, 50 μm; d, e, 20 μm.

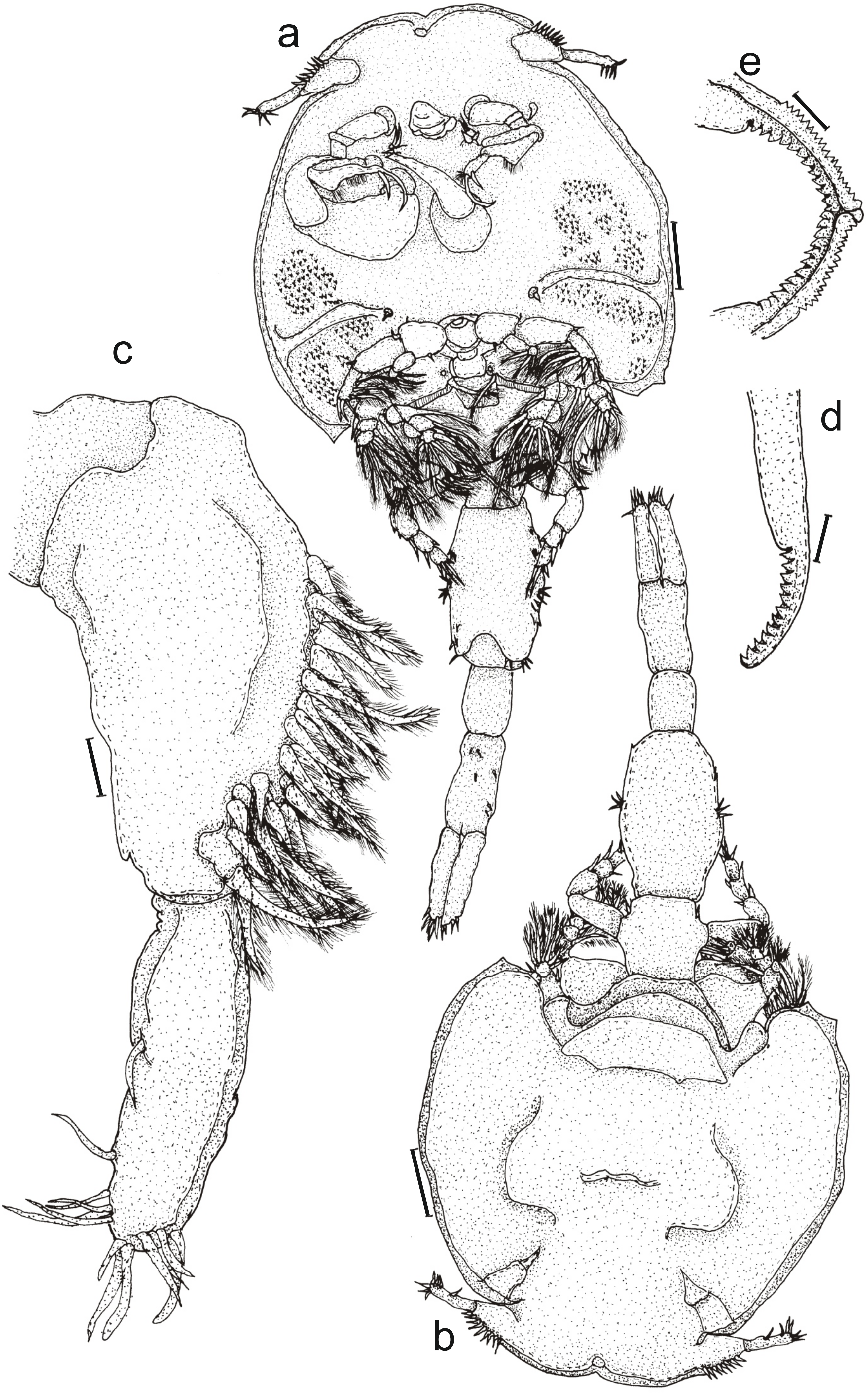

a
e
c
d
b

Fig. 30: Adult male Unidentified sp.

a. Caudal ramus; b. Antenna; c. Post-antennal process; d. Maxillule; e. Maxilla; f. Maxilliped; g. Leg 5. Scale bars: a-c, g, 50 μm; d, f, 100 μm.

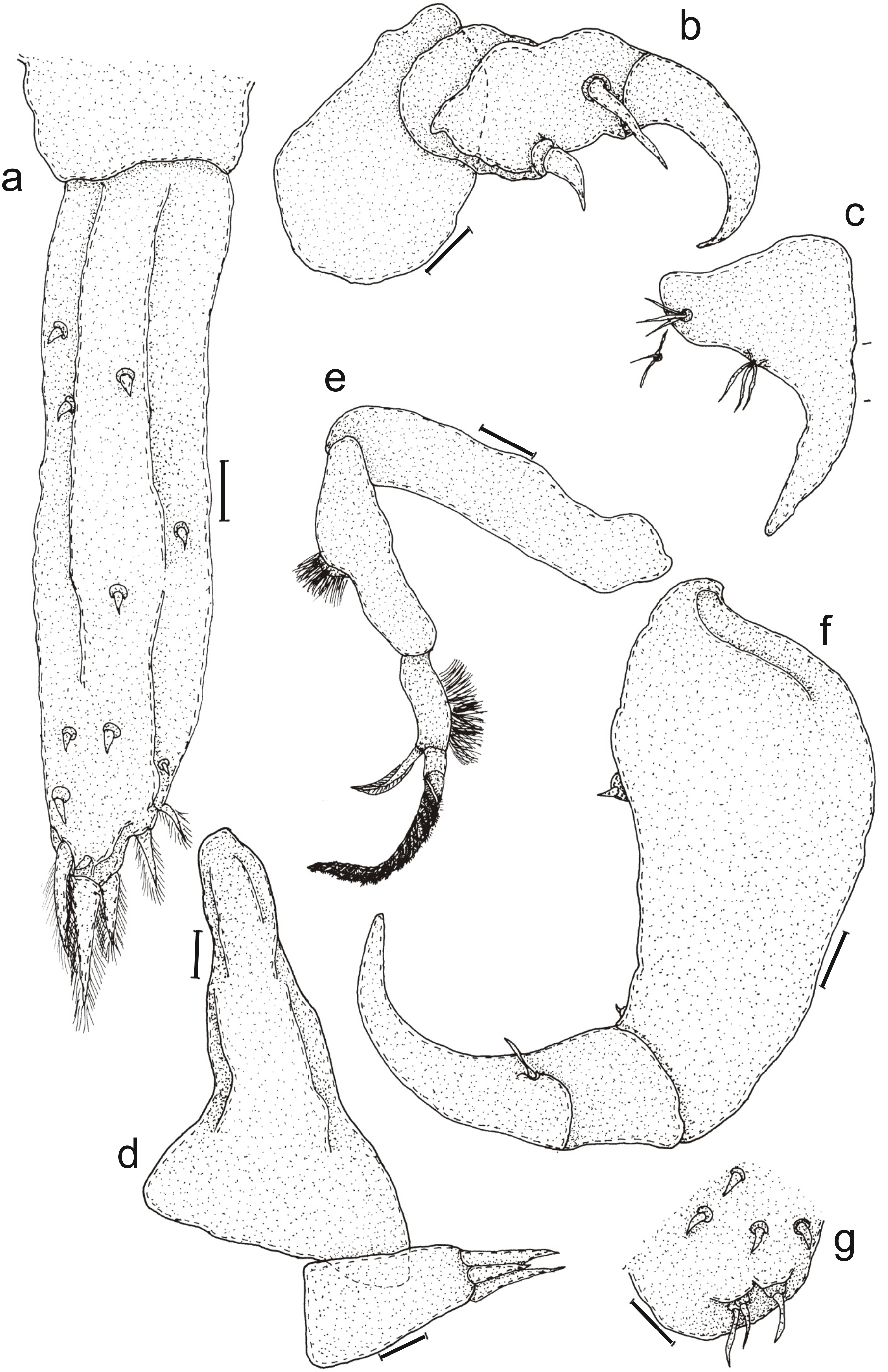
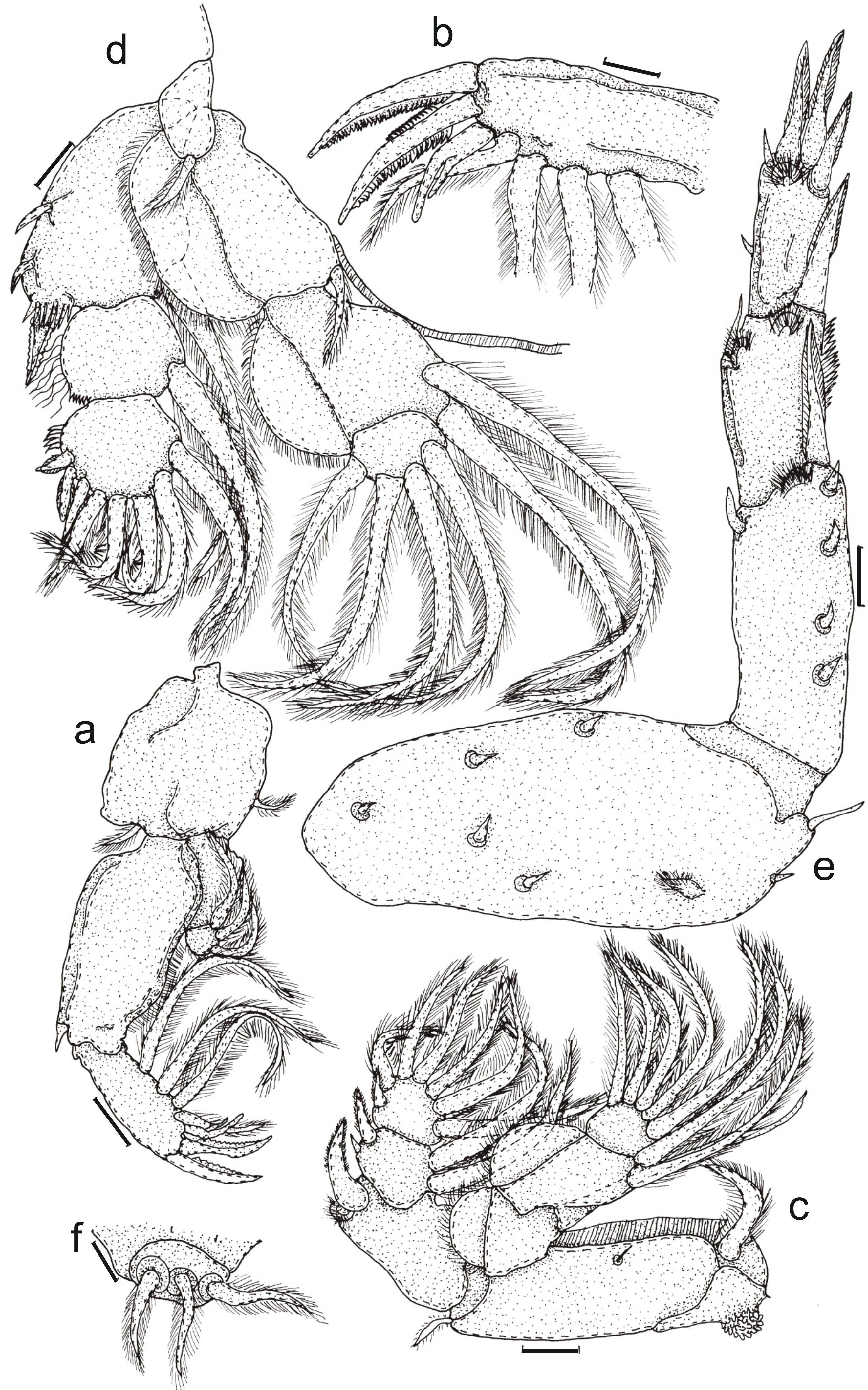

a
b
c
e
f
d
g

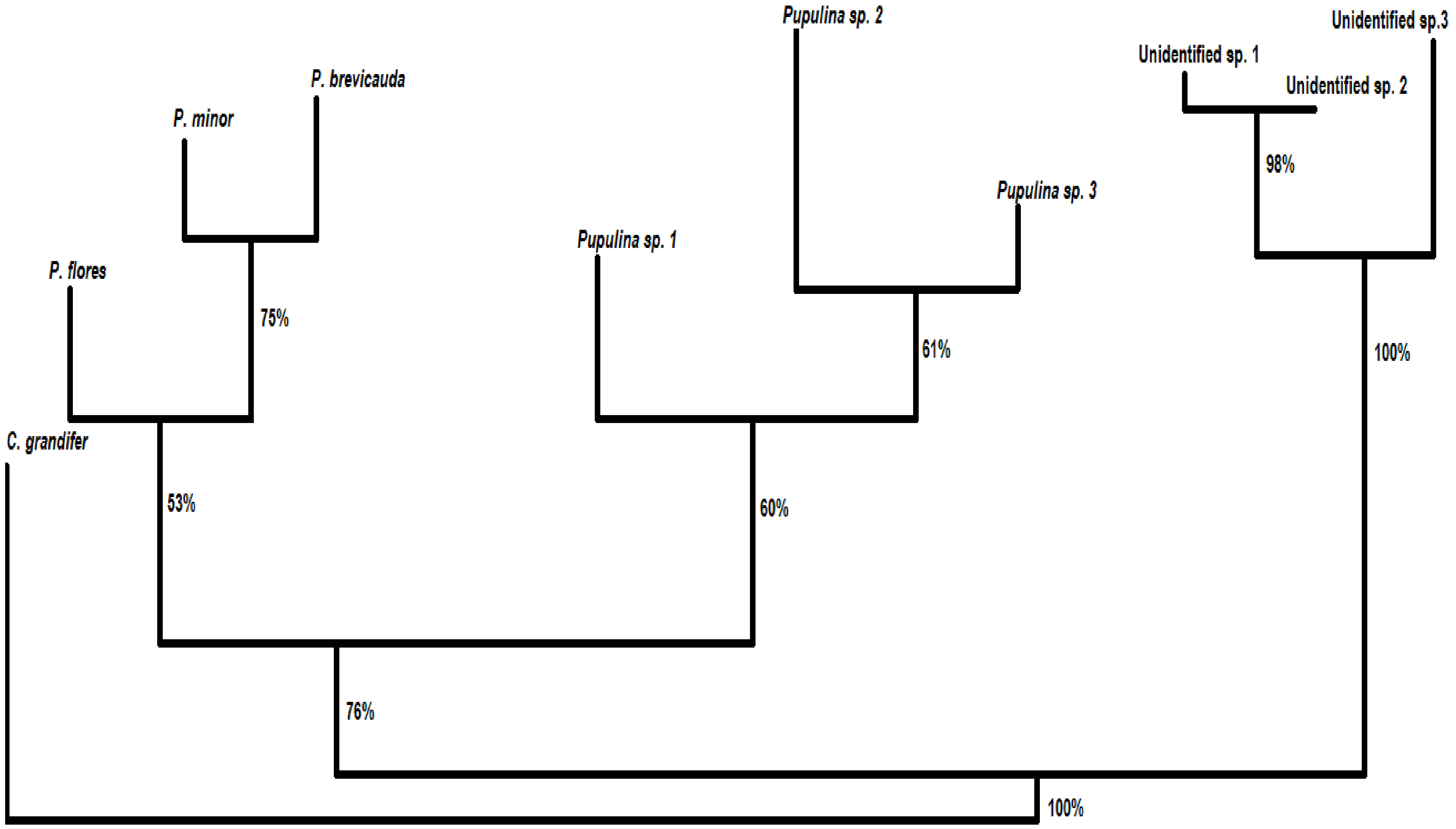
Fig. 31: Adult male Unidentified sp.

a. Leg 1; b. Tip of segment 2 of leg 1 exopod; c. Leg 2; d. Leg 3; e. Leg 4; f. Leg 5. Scale bars: a, c, 100 μm; b, d, e, 50 μm; f, 20 μm.



d
b
a
e
f
c

Fig. 32: Parsimony topology (TL = 85; CI = 0.7; RI = 0.7; HI = 0.3 and RCI = 0.5) estimating the phylogenetic relationships between *Pupulina flores* van Beneden, 1892; *Pupulina minor* Wilson, 1952; *Pupulina brevicauda* Wilson, 1952 *Pupulina* sp. 1; *Pupulina* sp. 2; *Pupulina* sp. 3; Unidentified sp. 1; Unidentified sp. 2; and Unidentified sp. 3 representing the in-group and Caligus *glandifer* Shiino, 1954 the out-group.


P. minor
P. brevicauda
P. flores
75%
C. grandifer
53%
Pupulina sp. 2
Pupulina sp. 3
Pupulina sp. 1
61%
60%
76%
Unidentified sp.3
Unidentified sp. 1
Unidentified sp. 2
98%
100%
100%

CHAPTER 4: Family Eudactylinidae C.B. Wilson, 1922

4.1 Introduction

Eudactylinidae was established by Wilson in 1922 as a sub-family of Dichelesthiidae (Kabata 1979; Boxshall & Montu 1997; Boxshall & Halsey 2004). It was later elevated to an independent family (Kabata 1979). This family currently consists of 65 species distributed over 12 genera (Boxshall 2010) with nine out of the 12 genera being monotypic (Benz *et al*. 2007; Boxshall 2010). The 12 genera include *Bariaka* Cressey, 1996 (one species); *Carnifossorius* Deets & Ho, 1988 (one species); *Eudactylina* van Beneden, 1853 (40 species); *Eudactylinella* C.B. Wilson, 1932 (one species); *Eudactylinoides* C.B. Wilson, 1932 (two species); *Eudactylinopsis* Pillai, 1968 (one species); *Heterocladius* Deets & Ho, 1988 (one species); *Jusheyus* Deets & Benz, 1987 (one species); *Nemesis* Risso, 1826 (15 species); *Protodactylina* Laubier, 1967 (one species); *Janinecaira* Benz, Smith, Bullard & Braswell, 2007 (one species) and *Dangoka* Izawa, 2011 (one species) (Boxshall 2010). Members of this family are predominantly parasitic on elasmobranch hosts (Kabata 1979; Deets & Ho 1988; Boxshall & Montu 1997; Benz *et al*. 2007; Izawa 2011). Two genera, *Heterocladius* and *Jusheyus* however, infect actinopterygian (Salmoniformes & Perciformes) hosts (Deets & Ho 1988; Boxshall & Montu 1997; Benz *et al*. 2007; Izawa 2011). Eudactylinid species are ectoparasitic, thus they infect the gill and nasal lamellae and filaments of their wide variety of hosts (Deets & Ho 1988; Benz 1994; Boxshall & Halsey 2004; Benz *et al*. 2007; Izawa 2011). One exception, *Carnifossorius* is mesoparasitic, with most of the anterior part of its elongated body deeply embedded into the wall of the branchial chamber of its host (Deets & Ho 1988).

Most species in this family have a high specificity for particular host species while others have broader host affiliations (Kabata 1979; Deets & Ho 1988; Benz 1994; Deets 1994; Benz *et al*. 2007; Izawa 2011). Only three eudactylinid species (*Eudactylina oliveri* Laubier, 1968; *Eudactylina diabolophila* Deets 1994 and *Eudactylina vaquetillae* Deets, 1994) have been reported from mobulid hosts from across all the worlds' oceans (Deets 1994). Members of this family are characterised by the presence of four free pedigerous segments between the cephalothorax and genital complex and the abundance of cuticular adornments in the form of scaly and spiny flaps present on the dorsal shield, appendages and swimming legs (Kabata 1979; Deets & Ho 1988; Benz 1994; Deets 1994).

4.2 Eudactylinids collected from examined hosts

4.2.1 Genus *Eudactylina* van Beneden, 1855

Introduction

Eudactylina is the largest and most species rich genus in the family Eudactylinidae (Kabata 1979; Romero & Kuroki 1991; Benz 1994; Deets 1994). This genus currently comprises about 40 accepted species (Boxshall 2010; Izawa 2011). The 40 accepted species include 10 species (*E. carchariaeglauci* Hesse, 1883; *E. breviabdomina* Pearse, 1952; *E. alata* Pillai, 1968; *E. mustelilaevis* Hesse, 1883; *E. parva* Castro-Romero & Baeza-Kuroki, 1991; *E. rhinobati* Raibaut & Essafi, 1979; *E. spinula* Pearse, 1950; *E. squatinaeangeli* Hesse, 1883; *E. valei* Nuñes-Ruivo, 1956; *E. vilelai* Nuñes-Ruivo, 1956), whose identities were considered doubtful due to inaccurate descriptions (Deets 1994). Three of the ten species (*E. carchariaeglauci*; *E. mustelilaevis* and *E. squatinaeangeli*) are listed as *species inquirenda* (Boxshall 2010). Additionally, during his revision, Deets (1994) described four new species (*E. dactylocerca* Deets, 1994; *E. diabolophila* Deets, 1994; *E. epaktolampter* Deets, 1994; *E. urophi* Deets, 1994) which are currently excluded from accepted species by Boxshall (2010) because they have not been published. Therefore the number of valid species is probably 44. Members of *Eudactylina* are distributed worldwide and mainly attach to the secondary gill and nasal lamellae of various elasmobranch hosts (Romero & Kuroki 1991; Deets 1994; Izawa 2011). They exhibit a high preference for batoid, squaloid, squatinoid and pristiophoroid elasmobranchs (Kabata 1979; Deets 1994; Boxshall & Halsey 2004) and have a high degree of host specificity (Deets 1994).

Eudactylina species reported from South African waters include *Eudactylina aspera* from *Carcharhinus limbatus* (Muller & Henle, 1839) and *Sphyrna lewini* (Griffith & Smith, 1834); *Eudactylina dollfusi* Brian, 1924 from *C. obscurus* (LeSueur, 1818) and *C. plumbeus* (Nardo, 1827); *Eudactylina hornbosteli* Deets, 1994 from *Aetobatus narinari* (Euphrasen, 1790); *Eudactylina oliveri* Laubier, 1968 from *Mobula kuhlii*; *Eudactylina pollex* Cressey, 1967 from *Galeocerdo cuvier* (LeSueur, 1822) and

Eudactylinodes niger (Wilson, 1905) from *Carcharias taurus* Rafinesque, 1810 (Dippenaar 2005; Dippenaar & Jordaan 2007). *Eudactylina* species are characterised by the morphology of the cuticular flaps, armature of the antenna, armature and segmentation of the swimming legs and character attributes of the caudal rami (Kabata 1979; Deets 1994). The 44 species can further be divided into two groups based on the morphology of the exopod of the second leg of the female species (Kabata 1979; Deets & Ho 1988; Deets 1994) (see Table 5). In the one group (comprising 40 species) the exopod of leg 2 is modified with the first segment greatly elongated and armed with stout, naked setae while in the other group (comprising four species) the exopod is unmodified and thus the exopodal segments more or less resemble the endopodal segments (Kabata 1979; Deets 1994). This grouping however, does not apply to the male specimens, since all described males have an unmodified exopod of leg 2 (Deets 1994).

4.2.1.1 *Eudactylina oliveri* Laubier, 1968

Material collected: From *M. kuhlii* 1130 ♀♀ and 9 ♂♂ --- 340 ♀♀ from five hosts off Umdloti (29.40S 31.08E) caught during January and April 2004, December 2010, January 2011 and February 2012; 59 ♀♀ from one host caught off Umhlanga (29.43S 31.05E) during December 2011; two ♀♀ from one host off St. Michaels (30.50S 30.24E) caught during May 2006; 182 ♀♀ and two ♂♂ from five hosts off Durban (29.51S 31.00E) caught during January and April 2004, November and December 2010 and December 2011; five ♀♀ and two ♂♂ from one host off Brighton Beach (29.56S 31.01E) caught during January 2011; 43 ♀♀ from one host off Warner Beach (30.05S 30.52E) caught during January 2010; seven ♀♀ and one ♂ from one host off Winkelspruit (30.06S 30.51E) caught during December 2010; three ♀♀ from two hosts off Umgababa (30.09S 30.50E) caught during January 2010 and November 2011; 259 ♀♀ from three hosts off Karridene (30.07S 30.37E) caught during January 2010 and November 2011; 88 ♀♀ and three ♂♂ from three hosts off Park Rynie (30.19S 30.44E) caught during January 2000 and January 2011; 43 ♀♀ from one host off Hiberdene (30.34S 30.34E) caught during April 2009; 99 ♀♀ from one host off Umzumbe (30.32S 30.51E) caught during January 2011. From *M. eregoodootenkee* 226 ♀♀ and 33 ♂♂ --- 266 ♀♀ and 33 ♂♂ from two hosts off Richards Bay (28.48S 32.06E) caught during September 2001.

All specimens were collected from the secondary gill lamellae of their examined hosts.

Morphological analysis:

Eudactylina oliveri was originally described from the secondary gill lamellae of *Mobula mobular*, caught off Narbonne Beach along the French Mediterranean by Laubier (Laubier 1968; Pillai 1985; Deets 1994). Subsequent host records include *M. kuhlii* caught off Cape Comorin, India (Pillai 1985) and the east coast of South Africa (Dippenaar & Jordaan 2007); *M*. *japanica*, *M*. *thurstoni* and *Mobula* sp. from the southern Sea of Cortez, Chanel islands in Southern California, Punta Arena de la Ventana and Nosé Be Madagascar respectively (Pillai 1985; Deets 1994). *Eudactylina oliveri* belongs to the group of eudactylinid females with a modified exopod of leg 2 and can be distinguished from all the other members of this group by the possession of spatulate spine-like setae on the sympods of legs 2, 3, 4 and reduced leg 5 (Laubier 1968; Pillai 1985; Deets 1994) (see Table 5).

Distribution statistics of component population

Eudactylina oliveri exhibited a prevalence of 87.1% and 100% (Fig. 1), a mean intensity of 42 and 130 individuals per host (Fig. 2) and a mean abundance of 37 and 130 (Fig. 3) individuals per host on *M*. *kuhlii* and *M*. *eregoodootenkee* respectively (Tables 1 & 2). Species component populations showed aggregated distribution patterns on both hosts (Tables 1 & 2).

4.2.1.2 *Eudactylina diabolophila* Deets, 1994.

Material collected: From *M*. *alfredi* eight ♀♀ and two ♂♂ --- five ♀♀ from one host off Karridene (30.07S 30.37E) caught during January 2011; two ♀♀ and one ♂ from one host off Park Rynie (30.19S 30.44E) caught during January 2011 and one ♀ and one ♂ from one host off San Lameer (30.57S 29.20E) caught during August 2010.

All specimens were collected from the secondary gill lamellae of the examined hosts.

Morphological analysis:

Eudactylina diabolophila was first described from the branchial lamellae of *M. birostris* caught off Sand and Line Islands in the Pacific Ocean (Deets 1994).

However, only female specimens were encountered and thus the male has never been reported and described. The female *E. diabolophila* belongs to the *Eudactylina* group with the unmodified exopod of leg 2 and can be distinguished from its congeners by the greatly extended distolateral regions of exopod segments 1 and 2 of legs 3 and 4, the unusually elongated caudal rami as well as the large, strongly curved claw of the antenna (Deets 1994) (Table 5).The current study marks the first record of *E. diabolophila* reported on *M. alfredi* from South African waters as well as the rest of the host's distribution across the oceans.

Adult Male (Figs. 33 - 35):

Overall length (excluding setae of caudal rami) approximately 3.75 mm. Cephalothorax (Fig. 33a, b, c) longer than wide. First four thoracic segments (Fig. 33a, c) wider than long, fourth free thoracic segment smaller than preceding segments, bearing leg 5. Genital segment (Fig. 33a, b, c) almost cuboid, longer than wide, bearing leg 6. Abdomen (Fig. 33a, b, c) slender, elongated, 4-segmented. Caudal rami (Fig. 33d) slender, armed with three robust, pinnate setae, one small naked seta laterally, rows of sub-triangular cuticular flaps on lateral margin and row of long setules on inner margin.

Antennule (Fig. 33e) indistinctly 7-segmented; segment 1 broad, armed with 2 small setae and row of cuticular flaps near base; segment 2 armed with nine short setae and one enlarged, robust spine with an accessory process at mid-length; segment 3 armed with six setae of varied sizes; segment 4 armed with three slender, elongate setae; segments 5 unarmed; segment 6 armed with three setae (one elongate aesthetasc); segment 7 small, armed with seven setae of varied length. Antenna (Fig. 34a) 3-segmented; segment 1 broad, unarmed; segment 2 armed with two slender setae with serrated membrane at base; segment 3 broad, elongated, armed with one seta on inner distal margin and an elongated claw armed with one small, auxiliary spine near base. Mouth tube (Fig. 34b) short and blunt, with circular flaps on dorsal surface. Maxillule (Fig. 34b) biramous; endite without cuticular flaps and armed with two apical papiliform setae; palp armed with row of small, triangular cuticular flaps along inner margin and two apical setae. Maxilla (Fig. 34c) branchiform, 2-segmented; syncoxa (lacertus) broad, large, armed with small, semicircular cuticular flaps on dorsal and lateral surfaces; basis (branchium) armed with semicircular and triangular cuticular flaps on lateral surface, a patch of slender, elongate setules distally and a thick claw with cuticular folds around base. Maxilliped (Fig. 34d) chelate, indistinctly 3-segmented; syncoxa armed with a patch of small semicircular, cuticular flaps and a well-produced denticulated, styliform process (myxa) armed with circular cuticular flaps; basis elongate and unarmed; subchela slender, armed with one large curved claw.

Armature of rami of legs 1-4 (Figs. 34e; 35a, b, c) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	II-0; IV-0	I-0; II-0
Leg 2	I-0; I-1; II-5	0-1; 0-1; 0-6
Leg 3	I-0; I-1; II-5	0-1; 0-1; 0-4
Leg 4	I-0; I-0; II-4	0-1; 0-1; 0-5

First four pairs of legs (Figs. 34e; 35a, b, c) biramous; Leg 1 (Fig. 34e) with 2-segmented rami and legs 2, 3 and 4 with 3-segmented rami.

Leg 1 (Fig. 34e) sympod armed with one outer seta distally; exopod segment 1 armed with semi-circular cuticular flaps along outer margin and two sub-apical spines; segment 2 armed with four terminal spines; endopod segments 1 and 2 each armed with semicircular cuticular flaps on dorsal margin; segment 1 with one inner, naked spine; segment 2 armed with two terminal, naked spines. Leg 2 (Fig. 35a) sympod armed with small triangular and semicircular cuticular flaps on dorsal surface; exopod segments 1 and 2 each armed with semicircular cuticular flaps and one distal spine on outer margin (spine of segment 2 flanged with serrated membrane); segment 2 armed with one long, inner naked seta and semicircular flaps along inner margin; segment 3 armed with cuticular flaps on outer margin, two terminal, unilaterally serrated spines and five (one pinnate, three naked) elongated setae; endopod segments 1, 2 and 3 each armed with semicircular and triangular cuticular flaps on dorsal surface; segments 1 and 2 each armed with one inner naked seta; segment 2 with rows of fine setules along outer margin; segment 3 armed with six naked setae. Leg 3 (Fig. 35b) sympod unarmed; exopod segments 1 and 2 each armed with one outer spine (spine of segment 2 unilaterally serrated) and semicircular cuticular flaps along dorsal margin; segment 2 with inner, naked seta; segment 3 armed with cuticular flaps on dorsal margin, two terminal unilaterally serrated spines and five naked setae; endopod segments 1 and 2 each armed with one inner seta (segment 1 seta pinnate); segment 3 armed with four naked setae. Leg 4 (Fig. 35c) sympod unarmed; exopod segments 1 and 2 each armed with cuticular flaps along outer margin and one outer spine (segment 2 spine unilaterally serrated); segment 3 armed with cuticular flaps along outer margin, two terminal unilaterally serrated spines and four naked setae; endopod segments 1 and 2 each armed with one inner, pinnate seta; segment 3 armed with five naked setae. Leg 5 (Fig. 35d) small, flattened, and armed with short, triangular cuticular flaps and 3 pinnate setae. Leg 6 (Fig. 35e) a small papilla armed with 2 pinnate setae.

Remarks:

The current study provides the first description of the male as well as the first record of this species from *M*. *alfredi* frequenting South African waters (Indian Ocean). The described male specimen is morphologically different from the female in most characteristics. This male is however, similar to the female by possessing the longer than wide cephalothorax; the fourth free thoracic segment that is much smaller than preceding segments; the caudal rami that are armed with sub-triangular cuticular flaps on the lateral margins and the first segments of the antennules which are armed with cuticular flaps along the posterolateral margins. The male of *E*. *diabolophila* can be distinguished from its known male congeners described thus far by the lack of cuticular flaps on the dorsal surface of the general habitus; the armature and segmentation of the antennules and the 3-segmented antennae while other males possess 4, 5 or 6-segmented antennae and only the palps of the maxillules are armed with cuticular flaps along the inner margins while in others both the lobes or none of them possess cuticular flaps. The most distinguishing features of the *E*. *diabolophila* male include the caudal rami which are each armed with rows of sub-triangular cuticular flaps along the inner margins, three robust pinnate setae and one small, outer, naked seta; the armature (formulae of setae and spines) of the first four pairs of legs and endopod segments 1, 2, and 3 of legs 3 and 4 are without cuticular flaps on the dorsal and ventral surfaces.

Distribution statistics of component population:

Eudactylina diabolophila exhibited a prevalence of 28.57% (Fig. 1), a mean intensity of 5 individuals per host (Fig. 2) and a mean abundance of 1 individual per host (Fig. 3) on *M*. *alfredi* (Table 3).

4.2.1.3 Remarks

According to Deets (1994), *Eudactylina* species exhibit a high degree of ecological and host specificity and both *E*. *oliveri* and *E*. *diabolophila* show an affinity for hosts of the family Mobulidae. *Eudactylina oliveri* is more widespread within the *Mobula* host family since it has been reported from *M*. *mobular*; *M*. *kuhlii*; *M*. *japanica*; *M*. *thurstoni*; *Mobula* sp. (Laubier 1968; Deets 1994) and *M*. *eregoodootenkee*, while *E*. *diabolophila* is more restricted to the host genus *Manta*, reported only from *M*. *birostris* (Deets 1994), and *M*. *alfredii* in the current study. Since *Eudactylina* species are host specific, males found with females probably belong to the same species. Thus the male specimens encountered with the females of *E*. *diabolophila* on *M*. *alfredi* in the current study were thus identified as *E*. *diabolophila* males. Males of *Eudactylina* do not conform to the separation into two groups based on the nature of the exopod of leg 2 since they all possess an unmodified exopod of leg 2. However, they have distinguishing characteristics that include the segmentation and armature of the antennules and the antennae; the armature (formulae of setae and spines) of the first four pairs of legs and the armature of the caudal rami (Laubier 1968; Pillai 1985; Romero & Kuroki 1991; Deets 1994; Izawa 2011).

Deets (1994), reported that members of this genus generally exhibit a low parasite load on their hosts and it is common to find less than five individuals of *Eudactylina* species on each type of host. The results of the current study however, are contradictory to this statement with the mean intensity of infection by *E*. *oliveri* being 42 and 103 individuals on *M*. *kuhlii* (Table 1; Fig. 2) and *M*. *eregoodootenkee* (Table 2; Fig. 2) respectively, while *E*. *diabolophila* exhibited a mean intensity of 5 individuals per host on *M*. *alfredi* (Table 3; Fig. 2). The maximum number of *E*. *oliveri* on *M*. *kuhlii* was 340 individuals (Table 1) and 226 individuals on *M*. *eregoodootenkee* (Table 2); while *E*. *diabolophila* exhibited a maximum number of eight individuals on *M*. *alfredi* (Table 3). It therefore appears as if a higher parasite intensity of infection is consistent with infection by *E*. *oliveri* on different types of hosts while lower parasite intensity is observed with infection by *E*. *diabolophila*. Furthermore, the secondary gill lamellae of elasmobranchs provide abundant habitat, in terms of space on hemibranchs, for small parasites such as species of *Eudactylina* (Dippenaar *et al*. 2008; Dippenaar *et al*. 2009). Thus the high mean intensity of *E*. *oliveri* on *M*. *kuhlii* and *M*. *eregoodootenkee* (Tables 1 & 2) could be a result of their ability to infect in large numbers because of their small size and the abundance of habitat.

Similar results were obtained for *Nemesis lamna* from *C*. *carcharias* with a mean intensity of 74 individuals per host (Dippenaar *et al*. 2008). In terms of size, *N*. *lamna* is much larger than *E*. *oliveri*, thus *N*. *lamna* takes up more space on the gill lamellae while *E*. *oliveri* species, because of their smaller size, have abundant space to attach in between the gill filaments of the examined hosts. Additionally, a previous study of *E*. *pusilla* on *Galeocerdo cuvier* Dippenaar *et al*. (2009), concluded that the large surface area of the secondary gill lamellae did not necessarily lead to higher infection. Therefore, the high infection rate by *E*. *oliveri* may not necessarily be due to the large surface area of the gill lamellae of their examined hosts or their ability to infect a host in large numbers because of their small size. Degree of infection could largely depend on preference of a particular host by symbionts or pure random chance (Bush *et al*. 2001) therefore a deeper investigation of host parasite relationships will contribute to the current knowledge of infection rate by symbiotic siphonostomatoids.

Eudactylina oliveri exhibited an aggregated distribution pattern on both *M*. *kuhlii* and *M*. *eregoodootenkee* (Tables 1 & 2). Similar results of aggregated distribution patterns were observed for *E*. *pusilla* on *G*. *cuvier* (Dippenaar *et al*. 2009) and *N*. *lamna* on *C*. *carcharias* (Dippenaar *et al*. 2008). In both reports, it was suggested that the only plausible explanation for the aggregated pattern was the presence of suitable resources and for mating purposes (Dippenaar *et al*. 2008; Dippenaar *et al*. 2009). Therefore *E*. *oliveri* may also exhibit an aggregated distribution pattern as a result of mutual feeding or the need for reproduction.

4.2.2 Genus *Nemesis* Risso, 1826

Introduction:

Nemesis is the second largest genus in the family Eudactylinidae with nine described species (Hewitt 1969; Kabata 1979). These species include *Nemesis lamna* Risso, 1826; *N. robusta* (van Beneden, 1851); *N. atlantica* C.B. Wilson., 1922; *N. pilosus* Pearse, 1951; *N. macrocephalus* Shiino, 1957; *N. sphyrnae* Rangnekar, 1984; *N. spinulosus* Cressey, 1970; *N. tiburo* Pearse, 1952 and *N. versicolor* C.B. Wilson, 1913 because *N. mediteranea* Heller, 1865; *N. vermi* Scott A, 1929 and *N. carchariaeglauci* (Hesse, 1883) were synonymized with *N. lamna* while *N. pallida* C.B. Wilson., 1932 and *N. aggregatus* Cressey, 1967 were synonymized with *N. robusta* (Hewitt 1969; Kabata 1979). However, Boxshall (2010) reported that there are 15 accepted *Nemesis* species since the synonomies of five species (*N. carchariaeglauci*; *N. aggregatus*; *N. vermi*; *N. pallida* and *N. mediteranea*) previously synonymized by Hewitt (1969) and Kabata (1979) were not considered. Members of this genus are characterized by four free thoracic segments separated from each other by transverse constrictions; a sub-spherical genital complex which is smaller than the preceding segment; a two or three segmented abdomen; a maxilliped that is sub-chelate and the first four pairs of legs biramous with 2-segmented rami (Kabata 1979; Pillai 1985; Boxshall & Halsey 2004).

The morphological criterion used to discriminate between the species of *Nemesis* is the widths of the cephalothorax, free thoracic segments and the genital segments relative to each other (Kabata 1979). This character however, only distinguishes and separates the existing species into two groups (Kabata 1979). In the one group (containing the single *Nemesis lamna*), the four free thoracic segments are more or less the same width and in the other group (containing the rest of the smaller species), the fourth free thoracic segment is narrower than the preceding three segments (Hewitt 1969; Cressey 1970; Kabata 1979). *Nemesis* species belonging to the group with smaller members are very similar and specific identification is largely based on minor differences (Kabata 1979). These minor characteristics include the shape and size of the cephalothoracic shield; the presence or absence of anterolateral expansions on the cephalothorax; the armature of the antennae and maxillae; the size of the maxillipeds and the shape, size and quantity of the spines and setae of the swimming legs (Wilson 1922; Pearse 1951; Shiino 1957; Hewitt 1969; Cressey 1970; Kabata 1979). Members of this genus are considered cosmopolitan, thus they are found on a wide variety of elasmobranch hosts with a wide distribution range (Wilson 1922; Pearse 1951; Shiino 1957; Hewitt 1969; Cressey 1970; Kabata 1979; Pillai 1985; Boxshall & Halsey 2004). *Nemesis* species reported from South African waters include *Nemesis lamna* from *C. carcharias* and *Isurus oxyrinchus* Rafinesque, 1810 and *Nemesis robusta* from *Alopias vulpinus* (Bonnaterre, 1788) and *Prionace glauca* (Linnaeus, 1758). No single species in the genus *Nemesis* has ever been reported from hosts of the family Mobulidae.

4.2.2.1 *Nemesis* sp.

Material collected: From *Manta alfredi* --- five ♀♀ from one host off Ramsgate (30.53S 30.20E) caught during August 2010. All specimens were found attached to the edges of the gill filaments of the examined host.

Adult female (Figs. 36 – 38):

Total length of habitus (excluding setae of caudal rami) about 3.42 mm. Cephalothoracic (Fig. 36a) shield oval, wider than long, posterior margin barely overlapping with first free thoracic segment. First three thoracic segments (Fig. 36a, b, c) free, with well-developed segmentation and of equal widths. Fourth free thoracic segment (Fig. 36a, b, c) about ½ as wide as the three preceding segments. Genital segment (Fig. 36a, b, c) small, wider than long, as wide as fourth free thoracic segment and ⅔ the width of the cephalothorax. Abdomen (Fig. 37a, b, c) 3-segmented; all three segments of equal length and width. Caudal rami (Fig. 37a) short and stout, longer than wide, armed with minute spinules on dorsal surface and six naked terminal setae of varying sizes.

Antennule (Fig. 36d, e) 12-segmented; segment 1 broad, armed with one naked seta; segment 2 with six naked setae; segment 3 with two naked setae on outer margin; segment 4 armed with four naked setae; segments 5-11 each armed with one naked seta and segment 12 slender, elongated and armed with eight naked setae and one aesthetasc. Antenna (Fig. 36f) 3-segmented; segment 1 broad, with patch of spinules on dorsal surface; segment 2 elongate and unarmed; segment 3 a stout, slightly curved claw armed with one short, slender seta near base and a short, blunt inner process. Mouth tube longer than wide; mandible not observed. Maxillule (Fig. 37b) biramous; endite, more pronounced armed with three naked setae; palp armed with two naked setae. Maxilla (Fig. 37c) branchiform, 2-segmented; syncoxa (lacertus) broad, unarmed; basis (branchium) armed with an outer patch of elongated setules, a short highly spinulated claw and an inner protrusion covered with spinules on outer margin. Maxilliped (Fig. 37d) sub-chelate, 2-segmented; syncoxa slender, elongated, unarmed; subchela an elongated, slightly curved claw armed with two short naked setae.

Armature of rami of legs 1-4 (Figs. 37e; 38a, b, c) as follows (Roman numerals indicate spines and Arabic numerals indicate setae):

	Exopod	Endopod
Leg 1	I-0; II-0	I-0
Leg 2	II-0; 0-6	I-1; 0-7
Leg 3	II-0; 0-4	I-1; 0-7
Leg 4	II-0; 0-4	I-1; 0-8

First four pairs of legs biramous, with 2-segmented rami (endopod of leg 1 uniramous). Sympods armed with band of spinules along distal margin of coxa and band of spinules and one inner, naked seta along distal margin of basis (not observed on sympod of leg 1).

Leg 1 (Fig. 37e) modified, sympod broad, unarmed; exopod segment 1 elongated, armed with a row of short spinules along outer margin, one terminal spine with circular cuticular flaps and a slightly curved, prominent, spinulated protuberance distally; segment 2 short, armed with two spines bearing circular cuticular flaps; endopod segment 1 broad, armed with a row of short spinules along inner margin and one terminal spine. Leg 2 (Fig. 38a) exopod segment 1 broad, armed with row of short spinules along outer margin, band of spinules along distal margin and two enlarged (one outer and one inner) spines; segment 2 armed with six short, naked setae; endopod segment 1 armed with row of short spinules along outer margin, band of short spinules along distal margin, one inner and one outer, short spines; segment 2 armed with seven apical setae. Leg 3 (Fig. 38b) exopod segment 1 armed with one inner and one outer spines; segment 2 armed with four apical setae; endopod segment 1 armed with row of short spinules along outer margin, a band of spinules along distal margin, one outer spine and one slender spine on inner margin; segment 2 armed with seven, slender apical setae. Leg 4 (Fig. 38c) exopod segment 1 broad, armed with band of short spinules along distal margin and two (one inner and one outer) robust spines; segment 2 armed with four short apical setae; endopod segment 1 armed with band of short spinules along distal margin, one inner and one outer, short spines; segment 2 armed with eight apical setae. Leg 5 (Fig. 38d) small, prominent papilla armed with three pinnate setae. Leg six not observed.

Remarks:

Nemesis sp. marks the first record of a *Nemesis* species found on a mobulid host. In the general configuration of the habitus, *Nemesis* sp. resembles *N. robusta* especially with the cephalothoracic shield lacking anterolateral expansions; the lengths of the first three, free segments which are half the widths of the same segments; the character and armature of the terminal claws of the antennae, which are slightly curved and are each armed with one short seta and one short blunt process on the inner margin; while the claws are more straightened and each armed with two short, blunt processes and one seta on the inner margin in other species. The terminal claw of the maxilla is armed with short spinules around the tip in *Nemesis* sp. and *N. robusta*, and naked in other species. The described species is however different from *N. robusta* by the absence of a row of short spinules on the outer margin of segment 2 of the antennae; the segmentation and armature of the antennules as well as the shape, size and armature of the caudal rami. The most distinct characteristics that define *Nemesis* sp. include the fourth free thoracic segment which is about $^3/_4$ as wide as preceding segments while the fourth free thoracic segment appears ½ as wide as the preceding segments in the other species; genital segment about ⅔ the width of the cephalothorax in *Nemesis* sp. and about the same width as that of the cephalothorax in its congeners. *Nemesis* sp. also possesses a 1-segmented endopod of leg 1 while the other species all possesses a 2-segmented endopod of leg 1; the armature and formulae (spines and setae) of all first four pairs of legs appears to be unique for each species.

Distribution statistics of component population:

Nemesis sp. exhibited a prevalence of 14.28% (Fig. 1), a mean intensity of 5 individuals per host (Fig. 2) and a mean abundance of 1 individual per host (Fig. 3) on *M. alfredi* (Table 3).

4.2.2.2 Remarks

Though more detailed morphological studies on the genus *Nemesis* have been conducted, it is still very difficult to distinguish between *Nemesis* species based on descriptions in the available literature (Kabata 1979; Pillai 1985). Only minor morphological differences separate species of *Nemesis* from each other (Wilson 1922; Shiino 1957; Cressey 1967; Hewitt 1969; Cressey 1970; Kabata 1979; Pillai 1985). *Nemesis* sp. encountered in the current study is clearly not *N. lamna*, as it is smallish and the fourth free thoracic segment is narrower than the preceding three segments. *Nemesis* sp. therefore belongs to the group of smaller species and it is mostly morphologically similar to *N. robusta*. *Nemesis robusta* is distinct from its congeners in the narrowness of the cephalothorax, which is about ½ the width of the second free thoracic segment (Hewitt 1969; Kabata 1979) while the cephalothorax is about $^{2}/_{3}$ the width of the second free thoracic segments in other species, *Nemesis* sp. included. *Nemesis* sp. morphologically resembles *N. robusta* in the lack of anterolateral expansions on the cephalothoracic shield; the length of the free thoracic segments which are half their widths; the slightly curved terminal claws of the antennae which are armed with one short seta and one short, blunt process on inner margin and the claws of the maxillae which are armed with short spinules around the tip (Hewitt 1969; Cressey 1970; Kabata 1979). *Nemesis* sp. can however be distinguished from *N. robusta* by the segmentation and armature of the antennules as well as the shape, size and armature of the caudal rami.

Shiino (1957) provided a table depicting differences between eight *Nemesis* species based on the length and width of the cephalothorax relative to the lengths and widths of the four free thoracic segments. Using the same method, *Nemesis* sp. was distinguished from other species by the length of the fourth free thoracic segment which is about $^{3}/_{4}$ as long as preceding segments and a genital segment which is about $^{2}/_{3}$ the width of the cephalothorax, while all other species within this genus possess genital segments that are the same width as the cephalothorax.

Furthermore, the formulae of the spines and the setae of the first four pairs of legs appear variable for each species in this genus (Cressey 1967; Hewitt 1969; Cressey 1970; Kabata 1979). However, identification of *Nemesis* species using minor characteristics such as the length and width of the cephalothorax relative to the lengths and widths of the four free thoracic segments as well as segmentation and formulae of the armature of the antennules and the first four pairs of legs is not sufficient to regard all the smaller species as different to *N. robusta*.

The previous state of knowledge caused researchers to concentrate on gross morphology in discriminating between the species of *Nemesis*, where each author observed different characteristics that distinguished between species within this genus. For example, while Shiino (1957) used the length and width of the cephalothoracic shield relative to the lengths and widths of all free thoracic segments to discriminate between *Nemesis* species, Pearse (1952) and Cressey (1967) used the spinules on the second segment of the antennae as well as the armature of the claws of the maxillae to discriminate between the different *Nemesis* species. Furthermore, Cressey (1970) used the armature of the caudal rami and the formulae of the armature (spines and setae) of the first four pairs of swimming legs to distinguish between species while Wilson (1922) used the segmentation and armature of the antennules as well as the formulae of the spines and setae of the first four pairs of legs to discriminate between the *Nemesis* species. Hewitt (1969) attempted to distinguish the other smaller species from *N. robusta* by using biometric appraisal where he came to the conclusion that two of the species (*N. pallida* and *N. aggregatus*) were synonymous to *N. robusta*.

Since the different characters used by the different authors somewhat contradict each other, a deeper analysis of their morphology, particularly on the details of the appendages is required for useful comparative analyses and revision of all the known species within this genus is vital. Applications of molecular techniques might also help discriminate between the species of *Nemesis* and determine whether or not they are different to *N. robusta*. However, molecular techniques may only aid in distinguishing between groups and may therefore not specify the actual differences between the species in this genus. Therefore, more detailed molecular techniques, morphological studies and a deeper investigation of all known *Nemesis* species from each type of host will aid in distinguishing between all the known and unknown species in this genus. Since *Nemesis* species might have some degree of host specificity (Pillai 1985), it is possible that *Nemesis* sp. encountered in the current study, as the first record of a *Nemesis* species from *M*. *alfredi* and mobulid hosts in general, represents a new species within this genus. The taxonomic distinctness of the current species should however, be treated as doubtful until the entire genus has been thoroughly investigated and scrutinised by revising previously collected specimens and validating them with further collections.

Though a number of studies have been conducted on species of the genus *Nemesis*, the host parasite relationships with regard to distribution patterns of each parasite component population on each type of host have received limited attention. Thus previous authors did not closely scrutinize host parasite relationships of *Nemesis* species on their wide variety of hosts. A prevalence of 14.28% and a mean intensity of 5 individuals per host were calculated for *Nemesis* sp. on *M*. *alfredi* (see Table 3; Figs. 1, 2). Compared to a prevalence of 80% and a mean intensity of 74 individuals per host of *N*. *lamna* on *C*. *carcharias* (Dippenaar *et al*. 2008), *Nemesis* sp. seems to exhibit a low parasite load on *M*. *alfredi*. However, no informed conclusions can be made due to lack of comparative literature on the distribution patterns of *Nemesis* species on their wide variety of hosts, in particular mobulid hosts. Therefore in addition to a deeper scrutiny on morphological features that distinguishes between *Nemesis* species, a closer look at the host parasite relationships of all *Nemesis* species on their various hosts will contribute towards a better understanding of the species dynamics of this genus.

Table 5: The two groups of *Eudactylina* van Beneden, 1853 species according to the morphology of the exopod of the second leg, reported hosts and distribution and the distinguishing features of each species (Laubier 1968; Kabata 1979; Deets 1994; Diebakate & Raibaut 2000; Izawa 2011).

Groups of *Eudactylina*	*Eudactylina* sp.	Reported host and distribution	Distinguishing characteristics
Leg 2 exopod modified	*E. acuta* van Beneden, 1853.	- *Squatina squatini* (Linnaeus, 1758) - Northwestern Atlantic and the Mediterranean.	- The nature and the branching, digiform claw-like setae of the exopods of legs 3 and 4.
	E. aphiloxenos Deets, 1994.	- *Squatina californica* Ayres, 1859 - Southern California bight.	- Relatively elongated fifth leg. - The relatively small and slender setae on the second segments of the modified exopods of leg 2.
	E. aspera Heller, 1865.	- *Chiloscyllium punctatum* Müller & Henle, 1838 - *Rhizoprionodon acutus* (Rüppel, 1837), - *Carcharhinus brevipinna* (Müller & Henle, 1839) -*Sphyrna lewini* (Griffith & Smith, 1834)	- Large spatulate processes on the second segments of the antennae. - The elongated proximal segments and the denticulated claw-like setae on the distal segment of the exopod of leg 2. - The branching setae of legs 5.

		- Indian Ocean near Nosé Be Madagascar, east coast of South Africa, Tunisian waters.	
	E. chilensis Ho & McKinney, 1981.	- *Aculeola nigra* De Buen, 1959 - Coquimbo, Chile.	- Presence of cuticular flaps on the second, third and fourth segments of the antennae with the reduced spiniform processes on the third segments. - The unique, tiny lateral setae on the caudal rami.
	E. corrugata Bere, 1930.	- *Raja erinacea* (Donovan, 1807) - St Andrews, New Brunswick and Woods Hole, Massachusetts.	- The large truncate setae on the terminal segment of the exopod of leg 2. - Two-segmented endopods of legs 1 and 4.
	E. dactylocerca Deets, 1994.	- *Rhinobatus productus* Ayres, 1817 - The inshore waters of the Southern California bight.	- Modified caudal rami. -The fine denticulations along the lateral margins of the setae on exopods of legs 3 and 4.
	E. dollfusi Brian, 1924.	- *Squalus* sp. - *Carcharias commersoni*	- Huge, modified claw-like fused endopod of leg 4.

		(Nardo, 1827) - *Carcharhinus milberti* Müller & Henle, 1839 - Woods Hole. - *Carcharhinus obscurus* (LeSueur, 1818) and *Carcharhinus plumbeus* (Nardo, 1827) - East Coast South Africa.	
	E. epaktolampter Deets, 1994.	- *Etmopterus pusillus* (Lowe, 1839) - Mississippi Delta and Atlantic Liberia	- The spinulations covering the concave surfaces of the maxillae. - The minute medial setae on the caudal rami apically. - The large orbicular lateral shield on the maxilliped claw.
	E. hornbosteli Deets, 1994.	- *Myliobatis* sp. - Nosé Be, Madagascar.	- The extremely elongated setae on the second, third and fourth segments of the antennules. - The large digiform sub-apical tines found on the setae of

			exopods of legs 3, 4 & 5.
	E. indivisa Castro & Baeza, 1991.	- *Myliobatis peruvianus* (Garman, 1939) - *Myliobatis chilensis* Phillipi, 1892 - Antofagasta, Chile.	- The unusual presence of cuticular flaps on the large spines on the third segments of the antennules. - The long, straight, denticulated spine-like setae on the second segment of the exopod of leg 2.
	E. insolens Scott & Scott, 1913.	- *Galeorhinus galeus* (Linnaeus, 1758), - Irish sea and North sea, off Norfolk, - *Mustelus mustelus* (Linnaeus, 1758) - *Mustelus mediterraneus* Quignard & Capape, 1972 - *Mustelus asterias* Cloquet, 1821 - Mediterranean sea near Tunisia.	- The unusual absence of cuticular flaps on the dorsal surfaces of the cephalothorax and free thoracic segments. - Coupled with the distally extended lobes of the second segments of the modified endopods of leg 2. - Denticulated claws of the maxillae.

	E. longispinosa Bere, 1936.	- *Sphyrna tiburo* (Linnaeus, 1758) - Lemon Bay Florida in the Gulf of Mexico and Tampa Bay Florida.	- The bizarrely modified terminal segments on the endopods of legs 3 and 4 forming long, blunt processes.
	E. myliobatidos Luque and Farfan, 1991.	- *Myliobatis chilensis* Phillipi, 1892 - Inshore waters near Chorrillos, Peru.	- The large rectangular cuticular flaps on the proximal segments of the modified exopod of leg 2.
	E. nykterimyzon Deets, 1994.	- *Myliobatis californica* Gill, 1865 - Inshore waters near El Segundo Californica and Punta Arena de la Ventana in the southern sea of Cortez.	- Three cuticular flaps on the third segments of the antennae.
	E. oliveri Laubier, 1968.	- *Mobula japonica* (Müller & Henle, 1841) - Punta Arena de la Ventana, Mexico and Anacap Island, - *Mobula thurstoni* (Lloyd, 1908) - *Mobula kuhlii* (Müller &	- The spatulate spine-like setae located on the basipods of legs 2, 3 and 4 and on the reduced leg 5.

		Henle, 1838) - East coast of South Africa; Punta Arena, Nosy Bè, Madagascar and near Narbonne Beach along the French Mediterranean and Cape Comorin, India.	
	E. papillosa Kabata, 1970.	- *Dasyatus kuhlii* (Müller & Henle, 1838) - Morton Bay, Queensland, Australia.	- The papiliform (nipple-like) setae located on the modified exopod of leg 2.
	E. peruensis Luque & Farfan, 1991.	- *Rhinobatus planiceps* Garman, 1880 - Inshore waters near Chorrillos, Peru.	- The uniquely modified caudal rami.
	E. pristophori Deets, 1994.	- *Pristiophorus cirratus* (Latham, 1794) - Greens beach at Tamar River mouth, northern Tasmania.	- The very short, stout and apically curving terminal setae on the caudal rami. - The stout papiliform setae on the second segments of the antennules.
	E. pusilla Cressey, 1967	- *Galeocerdo cuvier* (Peron &	- The large, blunt (nearly amorphous) denticulated setae

		LeSueur, 1822) - East Coast of South Africa; Sarasota, Florida and Madagascar.	on the caudal rami. - The unusually sharp terminal spines of the segments on the modified exopod of leg 2.
	E. similis T. Scott, 1902.	- *Torpedo californica* Ayres, 1958 - *Raja binoculata* Girard, 1836 - Palos Verdes, southern California.	- Many small denticles scattered over the convex surfaces of the large prehensile claws on segments 2 of the antennules.
	E. tuberifera Castro & Baeza, 1987	- *Squatina armata* (Phillipi, 1887) - Eastern South Pacific near Antofagasta, Chile.	- The serrated membranes on the lateral edges of the setae on exopod of leg 1. - The row of cuticular flaps along the lateral edges of the modified exopods of leg 2.
	E. turgipes Bere, 1936	- *Gymnura micruna* (Bloch & Schneider, 1871) - Lemon Bay, Florida and Tunisian waters - *Gymnura altevela* (Linnaeus, 1858)	- The peculiarly swollen modified exopods of leg 2. - The bimerite condition of the exopods and endopods of leg 1.

		- Lemon Bay, Florida.	
	E. urophi Deets, 1994.	- *Urolophus halleri* Cooper, 1837 - From LA harbor Seal beach, California.	- Possess only four setae on caudal rami.
	E. vaquetillae Deets, 1994.	- *Mobula tarapacana* (Phillipi, 1892) - Punta Arena de la Ventana in the southern sea of Cortez.	- The glubose mammiform setae located on the exopods of legs 2. - The very elongated legs 5.
	E. minuta Scott T, 1904.	- *Dasyatus pastinaca* T. Scott, 1904 - British waters. - *Dasyatus kuhlii* (Müller & Henle, 1838) - Queensland, Australia.	- The powerful spine on the anterior margins of the second segments of the antennae.
	E. leptochariae Diebakate & Raibaut, 2000.	- *Leptocharias smithii* (Müller & Henle, 1838) - Coastal area of Dakar (Senegal).	- The absence of a prominent lobe at an angle of the last segment of leg 2 exopods.

	E. lancifera Pillai, 1968.	- *Pristis* sp. - *Rhynchobatus* sp. - Trivandrum, India.	- Has an elongated fifth thoracic segment.
	E. parva Castro-Romero & Baeza-Kuroki, 1991.	- *Sympterygia brevicaudata* Cope, 1877 - Santiago, Chile.	- Third segments of the antennae are armed with two short processes and one seta.
	E. musteli Izawa, 2011.	- *Mustelus griseus* Pietschmann, 1908 - Seto, Wakayama, Japan.	- The first exopodal spine of leg 2 is sub terminal. - Legs 5 are longer than wide and unornamented.
	E. squatini Izawa, 2011.	- *Squatina japonica* Bleeker, 1858 - Seto, Wakayama, Japan.	- The widest body portion is the second thoracic segment.
	E. dasyati Izawa, 2011.	- *Dasyatis akajei* (Müller & Henle, 1841) - Seto, Wakayama, Japan.	- The cephalic shield and terga of segments 2-5 are covered with cuticular flaps. - Leg 1 endopod is 3-segmented.

	E. taeniuri Izawa, 2011.	- *Taeniura meyeni* Müller & Henle, 1841 - Seto, Wakayama, Japan.	- The slender elongated caudal rami. - Rami of swimming legs 1-4 are 3-segmented.
	E. gymnuri Izawa, 2011.	- *Gymnura japonica* (Temminck & Schlegel, 1850) - Tanabe Bay, Wakayama.	- The terminal segments of the antennules are tapering to form claw-like tips.
	E. rhinobati Raibaut and Essafi, 1979.	- *Rhinobatus rhinobatus* (Linnaeus, 1758) - *Rhinobatus cemiculus* Geoffrey, 1817 - Gulf of Gabes.	- Leg 2 exopods with two spines well developed on the third segments. - Segment 2 of the antennae with spiniform lobes.
	E. uncinata C.B. Wilson, 1908.	- *Galeorhinus galeus* (Linnaeus, 1758) - La Jolla, California.	- Its slender and tapering body form. -The large claws on the antennules.
	E. valei Nuñes-Ruivo, 1956.	- *Mustelus canis* (Mitchill, 1815) - Benguela. - *Squalus acanthias* (Linnaeus,	- Leg 2 exopods equipped with four curved spines distally.

		1758) - Luderidz, Namibia.	
	E. vilelai Nuñes-Ruivo, 1956.	- *Squalus uyato* Rafinesque, 1810 - Luanda, Angola. - *Squalus fernandinus* Molina, 1782 - Luderidz, Namibia.	- Proximal segments of legs 1 exopods developed and more elongated than the other two.
With leg 2 exopod unmodified	*E. acanthii* Scott, 1901.	- *Squalus acanthias* (Linnaeus, 1758) - Irish Sea, Sea of Japan and the Atlantic Sea board of North America.	- Has no lateral cephalothoracic processes.
	E. diabolophila Deets, 1994.	- *Manta birostris* (Donndorff, 1798) - Sand and Line islands in the Pacific Ocean.	- The greatly extended distolateral regions of segments 1 and 2 on the exopods of legs 3 and 4, - The unusually elongated caudal rami.
	E. squamosa Bere, 1936.	- *Rhinoptera bonasus* (Mitchill,	- The thick setae of segments 3

		1815) - West coast of Florida (Gulf of Mexico) - *Rhinoptera steindacheri* Evermann & Jenkins, 1891 - Punta Arena de la Ventana in the southern sea of Cortez.	of the antennae.
	E. pollex Cressey, 1967	- *Sphyrna mokarran* (Rüppel, 1837) - *Sphyrna lewini* (Griffith & Smith, 1834) - East Coast of South Africa, Sarasota, Florida; Madagascar and Caribbean sea.	- The fused uncinate claw-like nature of the endopods of leg 3 and especially leg 4 endopods. - The lateral expansion of the proximal segments of the endopods of legs 2, 3 and 4.

Fig. 33: Adult male *Eudactylina diabolophila*

a. Habitus, dorsal view; b. Habitus, ventral view; c. Habitus lateral view; d. Caudal ramus; e. Antennule. Scale bars: a-c, 0.5 mm; d, 50 µm; e, 20 µm.

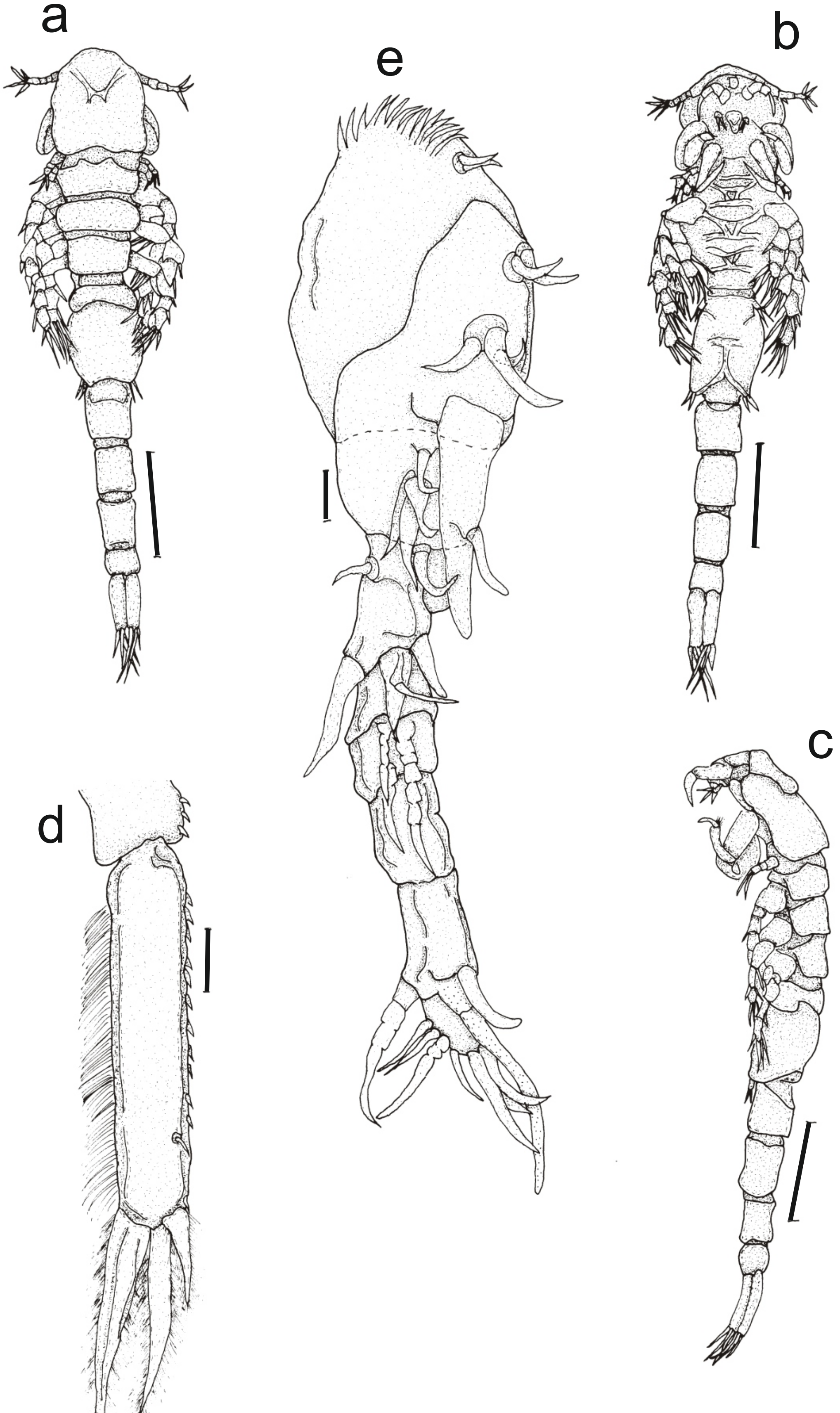

a
e
b
d
c

Fig. 34: Adult male *Eudactylina diabolophila*

a. Antenna; b. Mouth tube & Maxillule; c. Maxilla; d. Maxilliped; e. Leg 1. Scale bars: a-e, 20 µm.

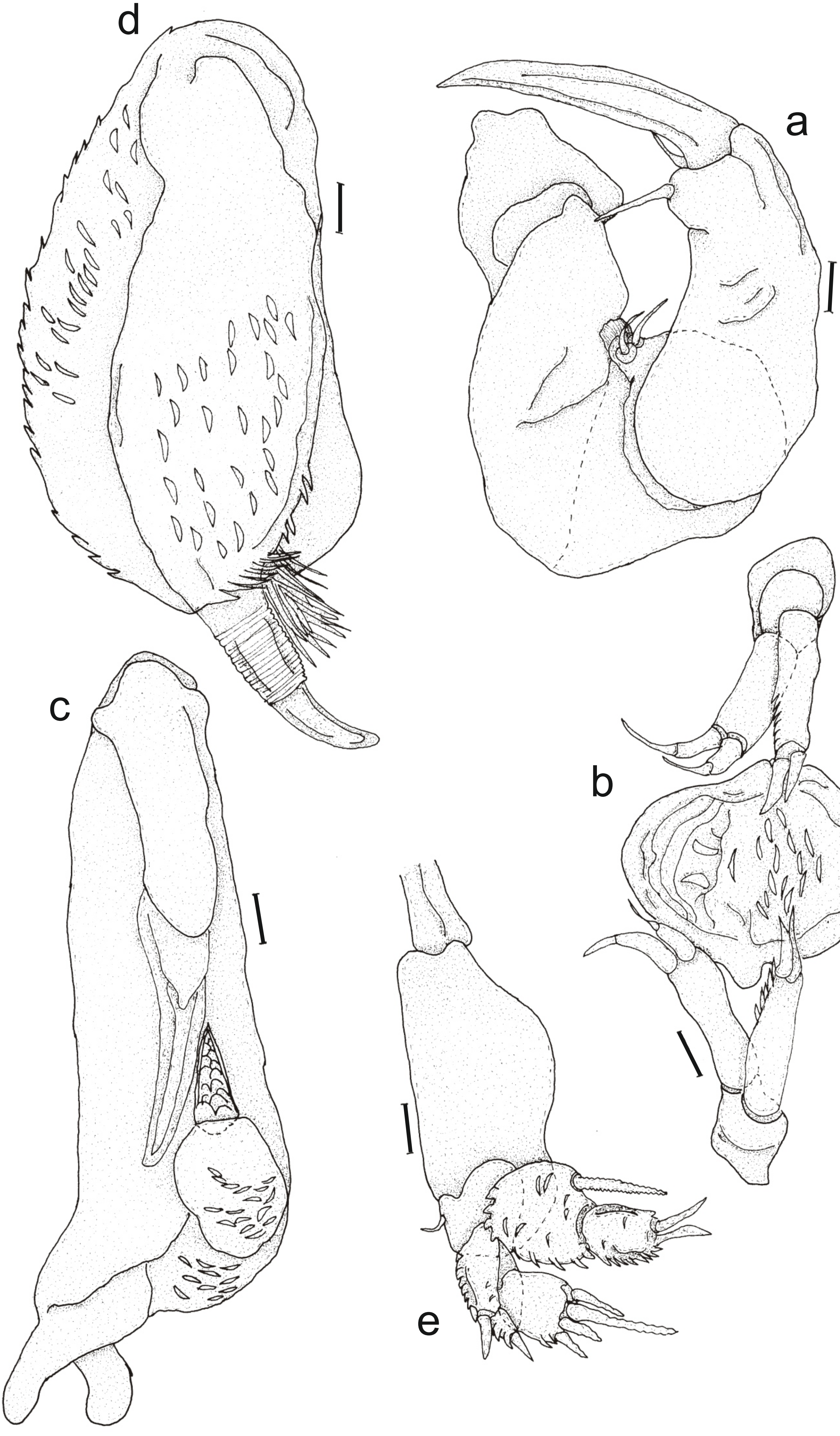

d
a
c
b
e

Fig. 35: Adult male *Eudactylina diabolophila*

a. Leg 2; b. Leg 3; c. Leg 4; d. Leg 5; e. Leg 6. Scale bars: a-d, 50 µm; e, 20 µm.

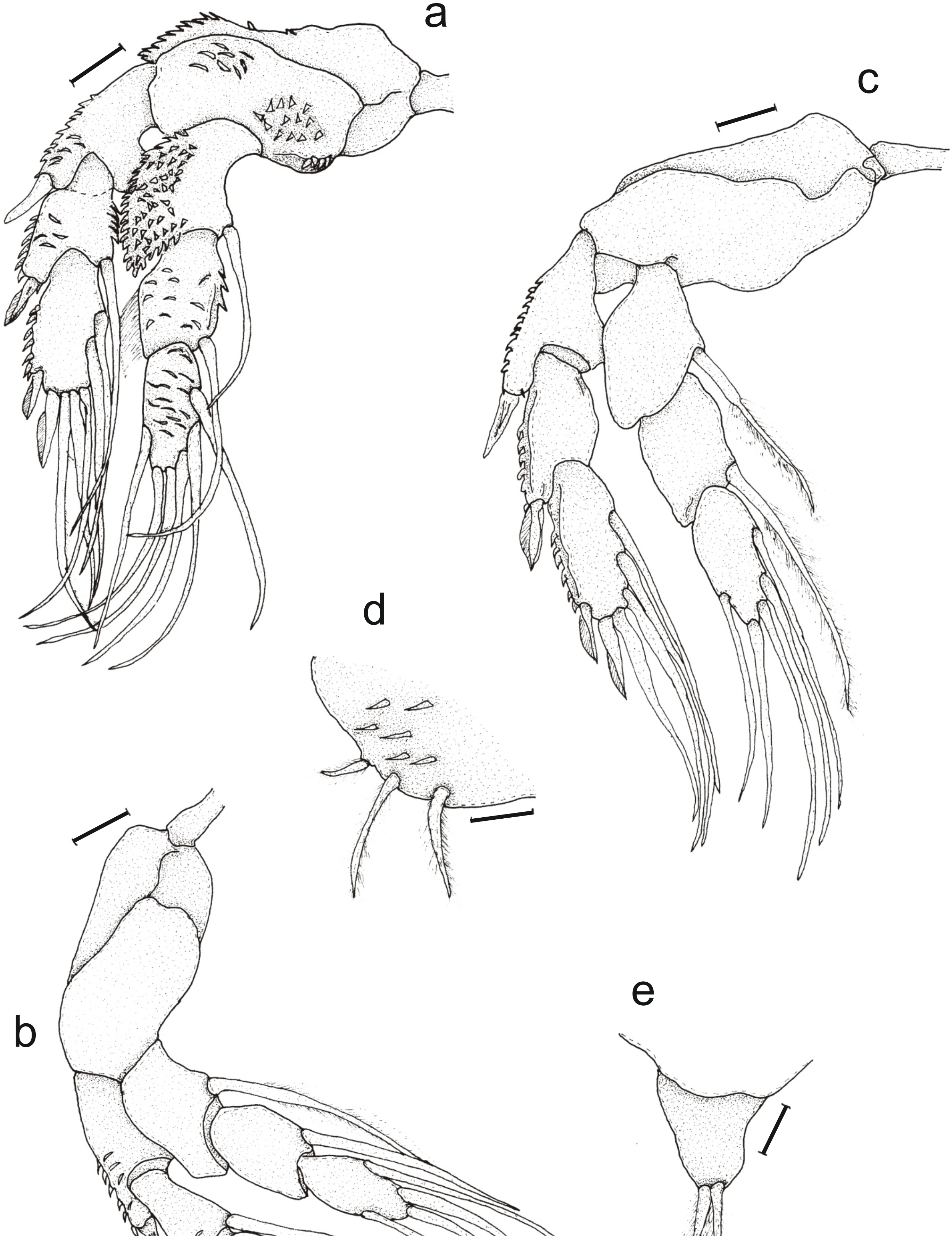

a
c
d
b
e

Fig. 36: Adult female *Nemesis* sp.

a. Habitus, dorsal view; b. Habitus, lateral view; c. Habitus ventral view; d. Antennule; e. Tip of antennule; f. Antenna. Scale bars: a-c, 0.5 mm; d, f, 50 µm; e, 20 µm.

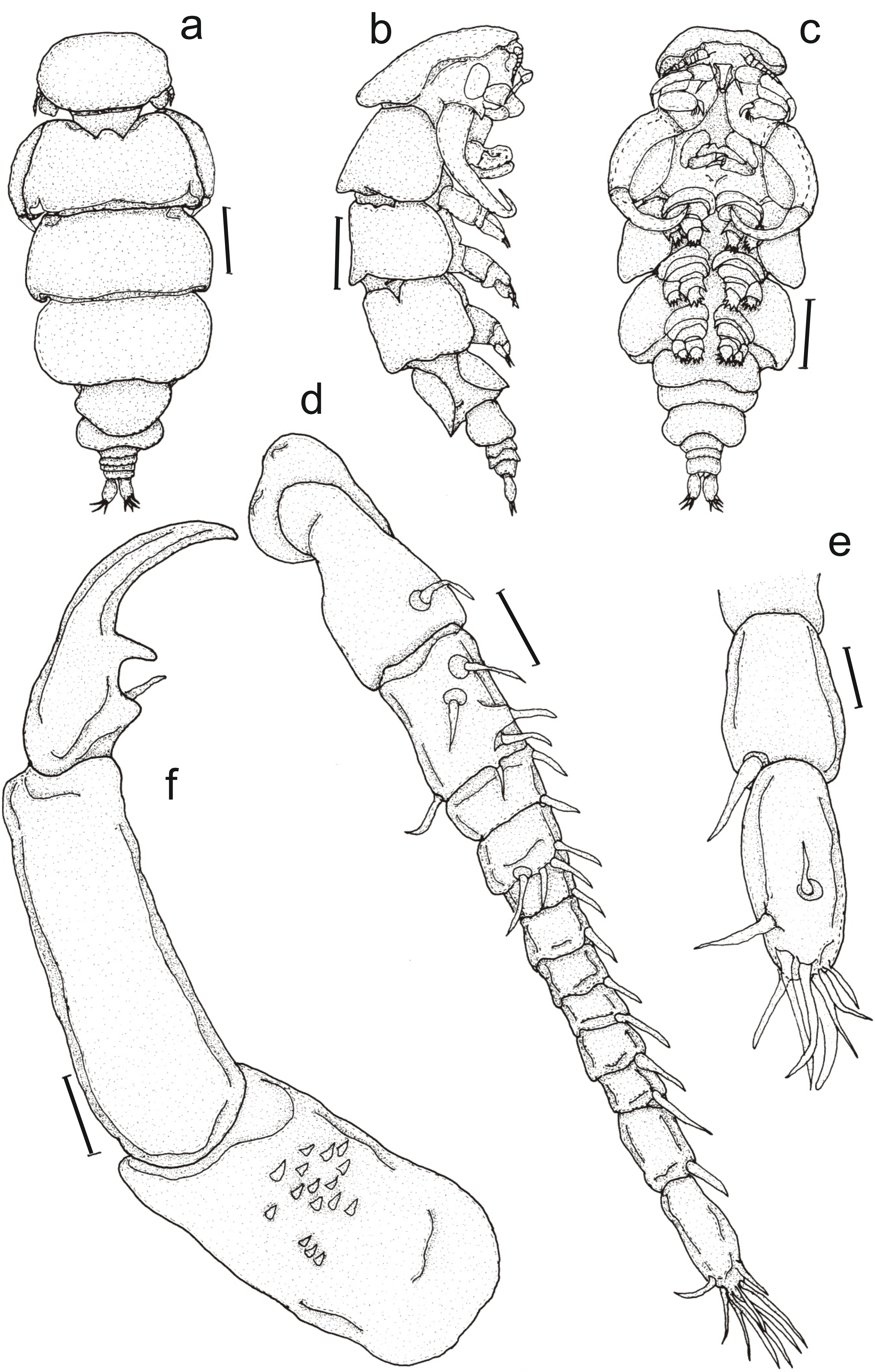

a
b
c
d
e
f

Fig. 37: Adult female *Nemesis* sp.

a. Caudal ramus; b. Maxillule; c. Maxilla; d. Maxilliped; e. Leg 1. Scale bars: a, 20 µm; b, c, 50 µm; d, 100 µm.

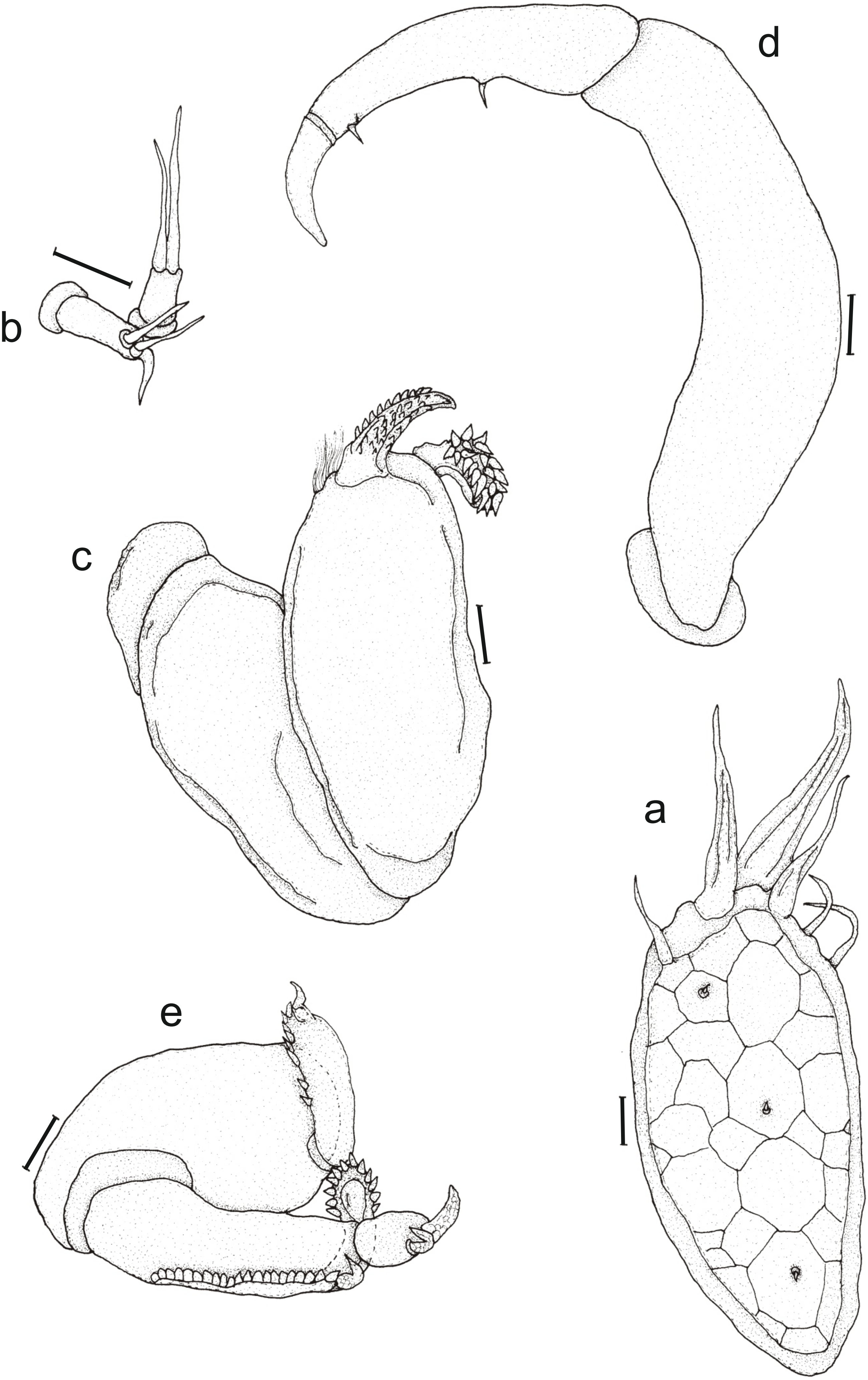
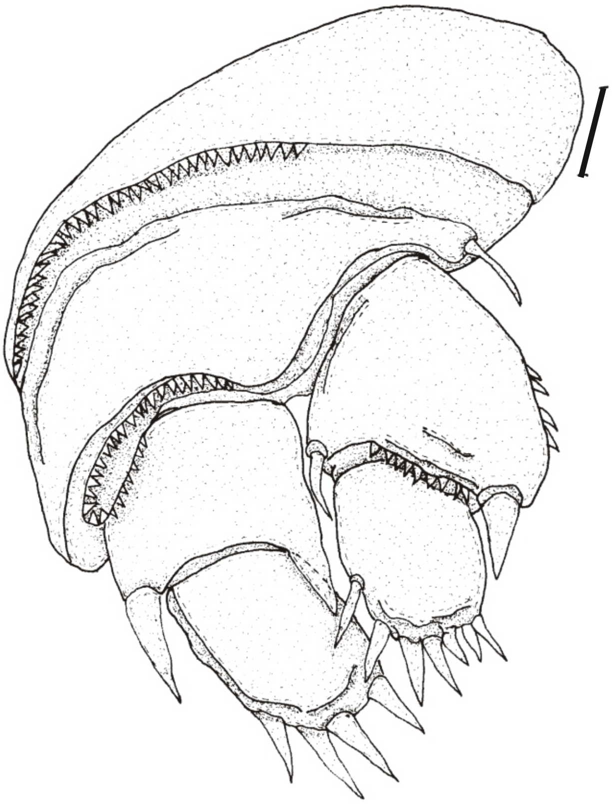

d
b
c
a
e

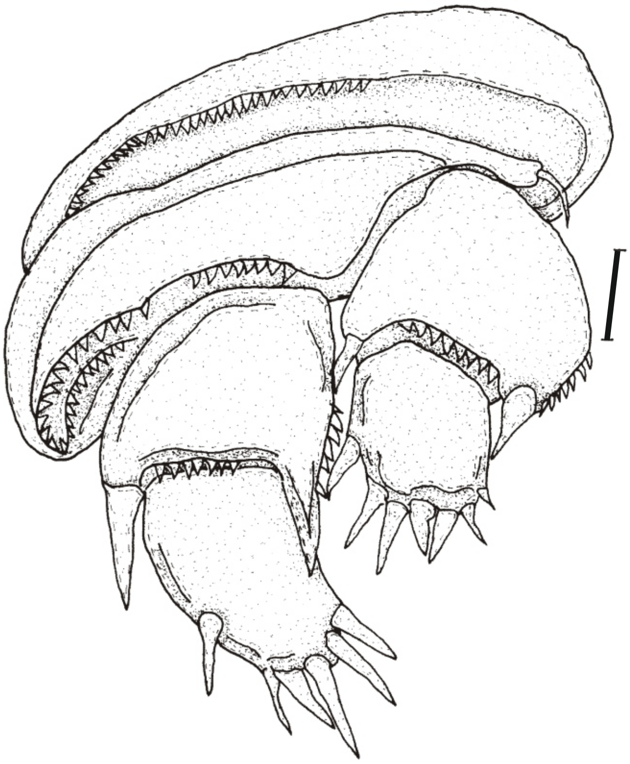
Fig. 38: Adult female *Nemesis* sp.

a. Leg 2; b. Leg 3; c. Leg 4; d. Leg 5. Scale bars: a-c, 50 µm; d, 20 µm.

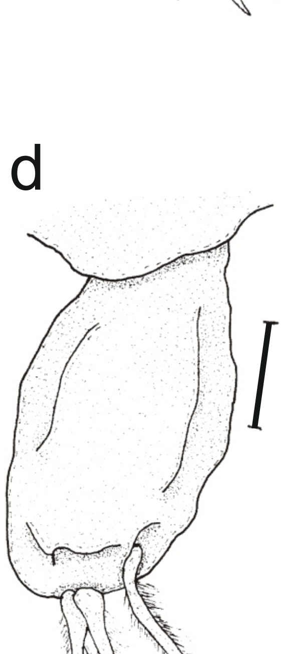
b



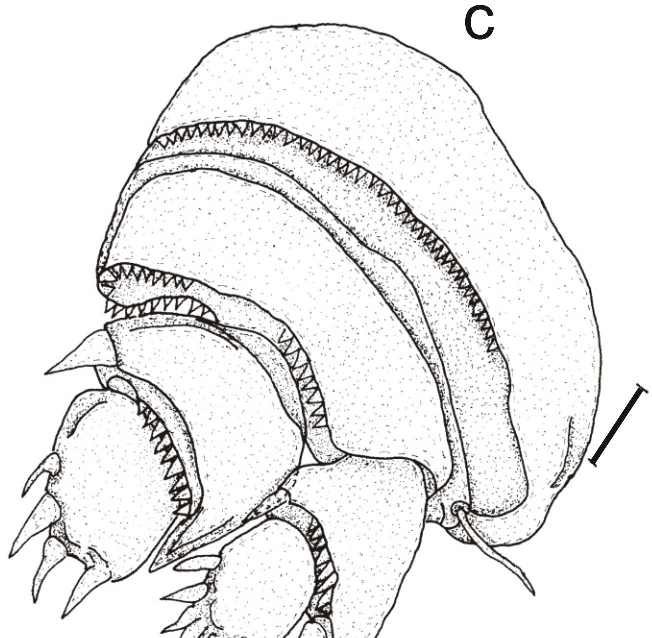
a



d



c



CHAPTER 5: Family Kroyeriidae Kabata, 1979

5.1 Introduction

The family Kroyeriidae was established by Kabata (1979) in order to accommodate the two genera *Kroyeria* van Beneden, 1853 and *Kroeyerina* Wilson, 1932, which were previously placed in the family Eudactylinidae Wilson, 1922 by Wilson in 1932 (Kabata 1979; Deets 1987; Boxshall & Halsey 2004). This family is mainly characterised by the possession of four pairs of biramous legs; the presence of three free leg bearing thoracic segments and a chelate antenna (Kabata 1979). Kroyeriidae is represented by 27 species distributed over three accepted genera (Deets 1987; Benz *et al*. 2001; Boxshall 2010). The genera include *Kroyeria*, with 15 accepted species and three *inquirenda* (total species equals 18) and *Kroeyerina* with eight accepted species (Walter & Boxshall 2008; Boxshall 2010). The third genus, *Prokroyeria* Deets, 1987 was established to accommodate *Kroeyerina meridionalis* Ramirez, 1795 which was elevated to *Prokroyeria meridionalis* (Deets 1987; Benz *et al*. 2001). Representatives of this family predominantly infect elasmobranch hosts of the families Triakidae (hound sharks); Carcharhinidae (requiem sharks) and Sphyriidae (hammerheads and bonnet heads) from localities in both the Indian and Atlantic Oceans, except the monotypic *Prokroyeria* reported from a holocephalan host (Deets 1987). Members of this family are ectoparasites and mostly infect the gills and olfactory lamellae, with one exception, *Kroyeria caseyi* (Benz & Deets, 1986), a mesoparasite found embedded in the interbranchial septa, below the free distal tips of the filaments, of their hosts (Benz & Deets 1988; Boxshall & Halsey 2004).

5.2 Kroyeriidae collected from examined mobulids

5.2.1 Genus *Kroeyerina* Wilson, 1952

Introduction

Kroeyerina, with only eight described species, is restricted to the nasal lamellae of various elasmobranch hosts (Deets 1987). The eight accepted species are *K. elongata* C.B. Wilson, 1932; *K. scottorum* Cressey, 1972; *K. nasuta* C.B. Wilson, 1932; *K. deborahae* Deets, 1987; *K. mobulae* Deets, 1987; *K. cortezensis* Deets, 1987; *K. benzorum* Deets, 1987 and *K. deetsorum* Benz, Smith Bullard, 2001 (Deets

1987; Benz *et al*. 2001). Of the eight accepted species of *Kroeyerina*, only *Kroeyerina scottorum*, found attached to the nasal lamellae of *S. lewini* has been reported from the east coast of South Africa (Dippenaar *et al*. 2001; Dippenaar 2005; Dippenaar & Jordaan 2007).

Although morphologically similar to both *Kroyeria* and *Prokroyeria*, *Kroeyerina* can easily be distinguished from *Kroyeria* by the absence of posterior sinuses and dorsal stylets on the dorsal shield of the cephalothorax (Deets 1987); and from *Prokroyeria* by the possession of two setae on the second segment of leg 1 endopod and the modified claw-like posterolateral seta on the caudal rami (Deets 1987). Representatives within this genus can mainly be distinguished from each other by the shape, size and appearance of the terminal setae of the caudal rami (Deets 1987; Benz *et al*. 2001).

5.2.1.1 *Kroeyerina mobulae* Deets, 1987

Material collected: From *M. kuhlii* one ♀ and one ♂ --- one ♂ from one host off Durban (29.51S 31.00E) caught during December 2005 and one ♀ from one host off Umgababa (30.09S 30.50E) caught during August 2005. Both specimens were collected from the nasal lamellae of their examined hosts.

Morphological analysis:

Kroeyerina mobulae was first reported from the nasal lamellae of *Mobula thurstoni* and *Mobula japanica* from the southern sea of Cortez (Deets 1987). A key to the species of *Kroeyerina* is provided and Table 6 provides host distribution records and distinguishing features of the eight *Kroeyerina* species using previous descriptions by Deets (1987); Benz *et al*. (2001) and Izawa (2008). *Kroeyerina mobulae* can easily be distinguished from its conspecifics by the robust subchela of the maxilliped and the reduced terminal setae of the caudal rami (see Table 6).

5.2.1.2 Key to the eight species of *Kroeyerina*

1. a. Rostral processes prominent upturned horns……………………………2

   b. Rostral processes sub-quadrate…………………………………………..6

2. a. Leg 1 endopod segment 2 with armature formula (0-1)…………………...3

   b. Leg 1 endopod segment 2 with armature formula (0-2); apical setae of caudal rami distally constricted……………………………………….*K. benzorum*

3. a. Leg 1 exopod segment 2, seta 2 unilaterally flanged with serrated marginal membranes………………………………………………………………..4

   b. Leg 1 exopod segment 2, seta 2 with serrated marginal membranes; caudal rami armed with three naked setae and 3 semi pinnate setae……………………………………………………………………..*K. elongata*

4. a. Leg 2 exopod segment 3, seta 2 with one side pinnate and the other side with serrated marginal membranes………………………………………………..5

   b. Leg 2 exopod segment 3, seta 2 with serrated marginal membrane on both sides and apical setae of the caudal rami are naked and elongate……………………………………………………………*K. cortezensis*

5. a. Caudal rami with two pinnate and 4 naked setae; rostrum sub-quadrate without lobes……………………………………………………..*K. deetsorum*

   b. Caudal rami with 2 semi pinnate setae and 4 naked setae……………………………………………………………………*K. scottorum*

6. a. Leg 3 exopod segment 3, seta 2 with serrated marginal membranes………………………………………………………………………..7

   b. Leg 3 exopod segment 3, seta 2 reduced and naked; caudal rami setae of medium length, thick and naked…………………………...……..*K. deborahae*

7. a. Leg 3 endopod segment 3, seta 2 reduced, naked and spiniform; caudal ramus with one reduced, naked distolateral spine and five pinnate setae terminally………...……………………………………………….....*K. nasuta*

   b. Leg 3 endopod segment 3, seta 2 reduced, with serrated marginal membrane; caudal rami with tremendously reduced naked setae……………………………………………………………………*K. mobulae*

5.2.1.3 Remarks

Kroeyerina scottorum is the only species of this genus reported from an elasmobranch host in South African waters (Dippenaar 2005; Dippenaar & Jordaan 2007). Since *K. mobulae* was previously reported from *M. japanica* and *M. thurstoni* from the sea of Cortez (Deets 1987), the current study provides the first record of this species from *M. kuhlii* off the east coast of South Africa (see Table 6). This report confirms the conclusion that *K. mobulae* is specific to representatives of the family Mobulidae (Deets 1987). The low numbers of *Kroeyerina* species reported on various elasmobranch hosts from South African shores (Dippenaar 2005; Dippenaar & Jordaan 2007), is in part due to the fact that most collections of symbiotic siphonostomatoid species have focused mainly on examination of the external skin surfaces, oral cavity and gills of each type of host (S.M. Dippenaar, pers. comm.). Thus, nasal lamellae of elasmobranch hosts from South African waters have received little attention and resulted in limited reports and knowledge of symbiotic siphonostomatoids that inhabit the olfactory lamellae of their hosts. Deets (1987) reported several females and one male of *K. mobulae* from the nasal lamellae of *M. japanica* and *M. thurstoni*, thus each host was infected by few individuals of *K. mobulae*. Similarly, only one female and one male were encountered in the current study (Table 1), therefore *K. mobulae* exhibits an apparent low parasite load on its mobulid hosts. However, comparisons and conclusions regarding intensity of infection by *K. mobulae* on its mobulid hosts cannot be made due to lack of comprehensive evidence, therefore a deeper scrutiny into host parasite relationships as well as examination of more possible hosts will likely add to the existing knowledge.

Table 6: Reported hosts, distribution and distinguishing characteristics of all eight accepted *Kroeyerina* C.B Wilson, 1932 species (Deets 1987; Benz *et al*. 2001; Dippenaar & Jordaan 2007; Izawa 2008).

Kroeyerina sp.	Reported host and distribution	Distinguishing characteristics
K. elongata Wilson C.B, 1932.	- *Prionace glauca* Linnaeus, 1758 - Martha's Vineyard, Western North Atlantic, Southern California Bight and Southern Sea of Cortez, - *Galeocerdo cuvier* (Peron & Lesueur, 1822) - Florida (Pacific Ocean).	- Genital complex is 75% total body length. - Caudal rami armed with three naked setae and three semi pinnate setae.
K. scottorum Cressey, 1972.	- *Sphyrna lewini* (Griffith & Smith, 1834) - East coast of South Africa and West coast Florida, - *Sphyrna zygaena* (Linnaeus, 1758) - Southern Sea of Cortez.	- Robust claw of the antennae and corpus of the maxilliped, - Setal armature of caudal rami (two semi-pinnate setae and four naked setae).
K. nasuta Wilson C.B, 1932.	- *Dasyatus centoura* (Mitchell, 1815) - Woods Hole, western north Atlantic.	- One reduced naked, distolateral spine of leg 1. - Five pinnate setae on the caudal rami.
K. deborahae Deets, 1987.	- *Rhinobatus productus* Ayres, 1854	- Six distal thick and naked, setae of medium length on caudal rami.

	- Palos Verdes, California.	
K. mobulae Deets, 1987.	- *Mobula thurstoni* Beebe & Tee-Van, 1938 - *Mobula japanica* (Muller & Henle, 1841) - Southern Sea of Cortez.	- The robust subchela of the maxilliped. - The tremendously reduced, naked setae on the caudal rami.
K. cortezensis Deets, 1987.	- *Carcharhinus falciformis* (Bribon, 1839) - Southern Sea of Cortez.	- The naked, elongate, distally constricted apical setae of the caudal rami.
K. benzorum Deets, 1987.	- *Alopias vulpinus* (Bonnaterre, 1788) - *Isurus oxyrinchus* Rafinesque, 1810 - Southern Sea of Cortez.	- The squat distally constricted apical setae of the caudal rami.
K. deetsorum Benz, Smith & Bullard, 2001.	- *Rhizoprionodon terranovae* (Richardson, 1836) - Gulf of Mexico and North western Atlantic.	- Extremely small size of adults. - Possesses a sub-quadrate rostrum without lobes or upturned horns.

CHAPTER 6: Family Cecropidae Dana, 1849

6.1 Introduction

The family Cecropidae consists of only a few members and forms part of the caligiforms which include members of the families Caligidae, Dissonidae, Pandaridae, Trebiidae and Cecropidae (Kabata 1979; Dojiri 1983; Benz & Deets 1988). Representatives of the Cecropidae can however, be distinguished from its caligiform allies by the frontal plates of the cephalothorax that are fused with the anterior margin of the thoracic shield (Kabata 1979; Benz & Deets 1988). This family is closely associated with the family Pandaridae as they share similar morphological characteristics (Kabata 1979; Benz & Deets 1988; Boxshall & Montu 1997). Kabata reviewed the relationship between Cecropidae and Pandaridae and concluded that the structure of the corpus maxillipedis of the female (which is slender in Cecropidae and squat in Pandaridae) is the only character that separates the two families (Kabata 1979). Cecropidae currently comprises of 11 accepted species, distributed over five genera. The five genera include *Cecrops* Leach, 1816 (with five species); *Orthagoriscicola* Poche, 1902 (with one species); *Philorthagoriscus* Horst, 1897 (with one species); *Luetkenia* Claus, 1864 (with three species) and *Entepherus* Bere, 1936 (with one species), (Kabata 1979; Benz & Deets 1988; Boxshall & Montu 1997; Boxshall 2010).

Representatives of this family are parasitic on epipelagic fish species (Kabata 1979; Boxshall & Montu 1997). A phylogenetic analysis of the family Cecropidae and their host associations placed the three genera, *Cecrops*, *Orthagoriscicola* and *Philorthagoriscus* in a monophyletic group parasitizing *Mola mola* (Benz & Deets 1988). The two remaining species, considered odd members of the family, were reported from *Luvarus imperialis* Rafinesque, 1810 (*Luetkenia* species) and mobulid hosts (*Entepherus laminipes*) from various localities (Kabata 1979; Benz & Deets 1988; Benz 1994; Boxshall & Montu 1997). Adult females of this family are massive and can reach a length of 30 cm while the males resemble the females closely but lack the plate-like expansions on the genital complex (Kabata 1979; Benz & Deets 1988; Benz 1994). Members of this family typically attach themselves to the branchial filters or gill lamellae of their hosts, though there have been reports of some found in the oral cavity of *Manta birostris* (Benz & Deets 1988; Benz 1994).

6.2 Cecropids collected from examined mobulids

6.2.1 Genus *Entepherus* Bere, 1936

Introduction:

Entepherus laminipes was first reported from *Mobula hypostoma* from the Gulf of Mexico in 1936 by Bere (Benz & Deets 1988; Benz 1994). She placed this genus in the family Pandaridae but it was later transferred to the family Cecropidae by Yamaguti in 1963 (Benz & Deets 1988; Benz 1994; Boxshall & Montu 1997). *Entepherus* is considered to be a cosmopolitan parasite, thus it is found infecting various hosts from different localities and these include reports from *M*. *birostris*, *M*. *thurstoni*, *M*. *japanica*, *M*. *tarapacana* from the southern sea of Cortez near Punta Arena de la Ventana (Benz & Deets 1988; Benz 1994) and *M*. *rochebrunei* captured near Nosy Bé Madagascar (Benz & Deets 1988; Boxshall & Montu 1997). The first and only record of an *Entepherus* species, *E*. *laminipes*, from South Africa was reported from *Manta birostris* caught off the Kwa Zulu-Natal coast (Dippenaar & Jordaan 2007). This monotypic genus is the only representative of the family Cecropidae known to infect elasmobranch hosts and based on previous records, *E*. *laminipes* appears to be restricted to hosts of the family Mobulidae (Benz & Deets 1988; Benz 1994; Boxshall & Montu 1997).

6.2.1.1 *Entepherus laminipes* Bere, 1936

Material collected: From *Mobula kuhlii* one ♀ from one host off Karridene (30.07S 30.37E) caught during January 2011. From *Manta alfredi* one ♀ and four ♂♂ --- four ♂♂ from two hosts off Karridene (30.07S 30.37E) caught during January 2011 and one ♀ from one host off Park Rynie (30.19S 30.44E) caught during January 2011. All specimens were found attached to the branchial filters of their hosts by their antennae and maxillipeds.

Morphological analysis:

Entepherus laminipes typically attaches to the gill rakers of mobulid hosts (Benz 1994) with at least one record where it attached to the tooth band of *Manta birostris* (Benz & Deets 1988). The most striking feature of *E*. *laminipes* is that the dorsal plate covering the genital complex is slightly indented on the posterior midline (Kabata 1979; Benz & Deets 1988; Boxshall & Halsey 2004).

<u>Distribution statistics of component population:</u>

E. *laminipes* exhibited a prevalence of 3.22% and 42.85% (Fig. 1); a mean intensity of 1 and 2 (Fig. 2) individuals per host and a mean abundance of 0 and 1 individual per host (Fig. 3) on *M*. *kuhlii* (Table 1) and *M*. *alfredi* respectively (Table 3).

6.2.1.2 Remarks

Results of the current study included, *E*. *laminipes* has only ever been reported from mobulid hosts (Benz & Deets 1988; Benz 1994; Boxshall & Montu 1997; Dippenaar & Jordaan 2007). This species therefore has high host specificity for mobulid hosts. As a cosmopolitan parasite of mobulid hosts (reported from *M*. *birostris*; *M*. *tarapacana*; *M*. *thurstoni*; *M*. *japanica* and *M*. *rochebrunei* from the Atlantic and Indian Oceans), *E*. *laminipes* typically attaches to the branchial filters of its hosts. Furthermore, *E*. *laminipes* was first reported from *M*. *birostris* caught in South African waters by Dippenaar and Jordaan (2007). However, since *Manta birostris* was thought to be a monotypic species occurring in the Indian Ocean, *E*. *laminipes* could have actually been reported from *M*. *alfredi*. No subsequent host records from South Africa existed for *E*. *laminipes* until the present study. The current study marks the first record of *E*. *laminipes* from *M*. *kuhlii* and *M*. *alfredi* worldwide.

Several (usually one or two) females and males were collected from *M*. *birostris*; *M*. *japanica*; *M*. *tarapacana*; *M*. *thurstoni* and *M*. *rochebrunei* (Kabata 1979; Benz & Deets 1988). Similarly, only one female was collected from *M*. *kuhlii* and one female and four males were collected from *M*. *alfredi* in the current study (Table 3). With a prevalence of 3.22% and 48.85% calculated for *E*. *laminipes* on *M*. *kuhlii* (Table 1; Fig. 1) and *M*. *alfredi* (Table 3; Fig. 1) respectively, this species exhibited a low parasite load on each examined host. Additionally, intensity of infection for *E*. *laminipes* was restricted to 1 and 2 individuals per respective host (Tables 1 & 3; Figs. 2, 3), as in most cases where a few individuals were encountered per previous examined hosts (Benz & Deets 1988).

CHAPTER 7: Family Dichelesthiidae Edwards, 1840

7.1 Introduction

Established in 1840 by Milne Edwards, the family Dichelesthiidae is represented by three species distributed over three monotypic genera (Kabata 1979; Benz 1994; Benz *et al*. 2002; Boxshall & Halsey 2004). The three monotypic genera include the extant *Anthosoma* Leach, 1816 and *Dichelesthium* Herman, 1804; and the extinct *Kabatarina* Cressey & Boxshall, 1989 (Pillai 1985; Benz *et al*. 2002; Boxshall & Halsey 2004). Representatives of this family are parasitic on various fish species (Hewitt 1968; Kabata 1979; Boxshall & Halsey 2004). *Anthosoma* typically infects elasmobranch hosts; *Dichelesthium* parasitizes hosts of the family Acipenseridae and the fossil form *Kabatarina* was recovered from two fossilised skulls of *Cladocylus gardeni* (Wilson 1922; Hewitt 1968; Kabata 1979; Boxshall & Halsey 2004). Members of this family are characterised by a cephalothorax that is completely fused with the first leg bearing segment; a well-developed dorsal shield; the unarmed, lobate caudal rami; the presence of a dorsolateral elytra on the second leg bearing segment; the retractile, sub chelate antennae and maxillae with a prehensile tip (Kabata 1979; Boxshall & Halsey 2004).

7.2 Dichelesthiids collected from examined mobulids

7.2.1 Genus *Anthosoma* leach, 1816

Anthosoma crassum is a cosmopolitan parasite, thus it has been reported from various elasmobranch hosts distributed over the Pacific, Indian and Atlantic Oceans *Anthosoma* predominantly parasitizes elasmobranch hosts belonging to the suborder Galeoidea, in particular members of Lamniformes (Hewitt 1968; Kabata 1979; Benz *et al*. 2002; Boxshall & Halsey 2004). However, this species has also been reported twice from *Mola mola*, which is considered an unusual host species for *Anthosoma* (Kabata 1979). This monotypic genus typically attaches to the regions about the upper and lower jaws and gill arches, by deeply embedding its retractile antennae into the tissue, of its examined host (Wilson 1922; Hewitt 1968; Kabata 1979; Benz *et al*. 2002). Deep and extensive lesions of the hosts' tissue always accompany infection by *Anthosoma* (Hewitt 1968; Benz *et al*. 2002). Furthermore, severe infections by *Anthosoma* have been associated with possible morbidity and mortality of hosts (Benz *et al*. 2002).

Anthosoma has a close affinity to the genus *Dichelesthium* and they both share the apomorphic 6-segmented antennules; the retractile antennae; the prehensile apex of the maxilla; the 2-segmented maxilliped; the folacious leg 3 and the loss of leg 4 (Kabata 1979); while *Kabatarina* retained pleisiomorphic characteristics such as the 21-segmented antennule; the non-retractile antennae; an opposable structure at the tip of the maxilla; the 3-segmented maxilliped and the presence of leg 4 (Boxshall & Halsey 2004). From South African waters, this cosmopolitan siphonostomatoid has been reported from *Carcharias* species; *Isurus* species; *Odontaspis* species; *Prionace* species; *Carcharodon* species and *Lamna nasus* (Bonnaterre, 1788), all distributed over the east and south coasts of South Africa (Dippenaar 2005; Dippenaar & Jordaan 2007). The widely distributed host record of *Anthosoma* was extended for the first time to mobulid hosts by Dippenaar and Jordaan (2007) where species were recorded from *M. birostris* caught off the east coast of South Africa.

7.2.1.1 *Anthosoma crassum* (Abildgaard, 1794)

Material collected: From *M. alfredi* eight ♀♀ and three ♂♂ --- one ♀ and one ♂ from one host off Karridene (30.07S 30.37E) caught during January 2011; four ♀♀ and one ♂ from one host off Park Rynie (30.19S 30.44E) caught during October 2004; one ♀ and one ♂ from one host off Ramsgate (30.53S 30.20E) caught during August 2010 and two ♀♀ from one host off San Lameer (30.57S 29.20E) caught during August 2010.

All specimens were found attached to the upper and lower regions of the jaws of their examined hosts.

Morphological analysis:

Anthosoma crassum is a relatively conspicuous parasite that typically attaches to the gill arches and jawlines of elasmobranch hosts (Benz *et al*. 2002). *Anthosoma crassum* can easily be distinguished from its congeners by possession of fused leg bearing segments; the second leg bearing segment which is armed with a dorsal elytra and folacious legs 1 and 2 that are without separate rami (Hewitt 1968; Kabata 1979). This species is easily observed because of the deep lesions and tissue proliferations that accompany the site of infection on its various hosts (Hewitt 1968; Kabata 1979; Benz *et al*. 2002).

Distribution statistics of component population:

Anthosoma crassum (Table 3) exhibited a prevalence of 14.28% (Fig. 1), a mean intensity of six individuals per host (Fig. 2) and a mean abundance of two individuals per host (Fig. 3).

7.2.1.2 Remarks

Results of the current study included, *A*. *crassum* appears to have a higher preference for elasmobranch hosts (Benz *et al*. 2002), even though Kabata (1979) reported this species once from the oral cavity and gill rakers of the ocean sunfish, *Mola mola*. *Anthosoma crassum* was reported for the first time on a mobulid host in South African waters from *M*. *birostris* (Dippenaar & Jordaan 2007). However, since this was most likely *M*. *alfredi*, the current study marks the second record from the east coast of South Africa. Proliferation of host tissue and severe lesions were observed on the attachments sites of *A*. *crassum* on *M*. *alfredi* as was previously reported on other host species (Hewitt 1968; Kabata 1979; Benz *et al*. 2002). The severity of the lesions tends to increase with a higher parasite load on each attachment site (Hewitt 1968). Similarly, on the examined *M*. *alfredi* hosts, single infections by *A*. *crassum* were associated with less severe lesions whereas multiple infections seemed to increase the severity of the lesions on each attachment site.

A prevalence of 14.28% and a mean intensity of 6 individuals per host were recorded for *A*. *crassum* on *M*. *alfredi* (Table 3; Fig. 1). Compared to previous encounters of this species on other elasmobranch hosts where specimens collected include 20 females and nine males encountered on *L*. *nasus*; 17 females and eight males on *I*. *oxyrinchus*; two females and one male on *C*. *carcharias* by Hewitt (1968); a few females and males from *I*. *oxyrinchus* by Benz *et al*. (2002) and from *M*. *alfredi* by Dippenaar & Jordaan (2007) it can be deduced that *A*. *crassum* tends to exhibit a low parasite load on each examined host. However, the degree and intensity of infection by this species varies with each encounter (Wilson 1922; Hewitt 1968) and Hewitt (1968) suggested that intensity of infection increases with host size. Observations made in the current study agree with those of Wilson (1922), who states that intensity of infection by *A*. *crassum* on each type of host varies with each encounter, regardless of size. *Anthosoma crassum* individuals show no preference for any particular elasmobranch host, but most have been known to infect hosts within the Lamniformes (Benz *et al*. 2002), thus they are generalists with a cosmopolitan occurrence.

CHAPTER 8: General discussion

Previous records of symbiotic siphonostomatoids reported from mobulids (*Mobula kuhlii* and *Manta alfredi*) off the east coast of South Africa include four species distributed over four families (Dippenaar & Jordaan 2007). The reported species include *Eudactylina oliveri* (Eudactylinidae); *Caligus elongatus* (Caligidae); *Entepherus laminipes* (Cecropidae) and *Anthosoma crassum* (Dichelesthiidae) (Dippenaar & Jordaan 2007). Despite examination of only three types of mobulid hosts (*M. kuhlii*, *M. ergoodootenkee* and *M. alfredi*) in the current study, thirteen different siphonostomatoid species distributed over five different families were collected and reported. The collected specimens were identified as members of the family Caligidae (*C. chrysophrysi*, *Pupulina* sp. 1, *Pupulina* sp. 2, *Pupulina* sp. 3, Unidentified sp. 1, Unidentified sp. 2 and Unidentified sp. 3); Eudactylinidae (*E. oliveri*, *E. diabolophila* and *Nemesis* sp.); Kroyeriidae (*K. mobulae*); Dichelesthiidae (*A. crassum*) and Cecropidae (*E. laminipes*). Out of the thirteen collected species nine (*C. chrysophrysi*, the three *Pupulina* species, the three Unidentified sp. species, *E. diabolophila* and *K. mobulae*) represent the first records from *M. kuhlii*, *M. ergoodootenkee* and *M. alfredi* from South African waters as well as the rest of the hosts distribution. Additionally, the three *Pupulina* species identified represent three new species while the three Unidentified sp. species are different from the genus *Pupulina* and may represent a new genus within the family Caligidae. However, validation of the possible new species will be confirmed through examination of more specimens from additional mobulid host species. Considering the number of new reports, an examination of all seven possible mobulid hosts that frequent South African waters will surely increase the number of reported siphonostomatoids along the east coast of South Africa.

Most symbiotic copepods tend to exhibit some degree of host specificity (Kabata 1979; Benz 1994; Bush *et al*. 2001; Deets 1994). The host generalists collected in this study includes *A. crassum* which is a cosmopolitan parasite of elasmobranchs (Hewitt 1969; Kabata 1979; Benz *et al*. 2002; Boxshall & Halsey 2004) while *C. chrysophrysi* is a possible generalist since it has been reported from both *R. sarba* (Pillai 1985) and mobulid hosts, though more findings of this species on other possible hosts will provide more reliable data. The remaining species collected in the current study each have specificity to a particular host. Thus, the three *Pupulina* species, *E*. *oliveri* and *K*. *mobulae* are all specific to *Mobula* hosts; *E*. *diabolophila* appears specific to *Manta* hosts while *E*. *laminipes* is restricted to hosts within the family Mobulidae.

There is certain expectancy about the types and numbers of symbiotic copepods infecting members of the family Mobulidae based on previous observations from around the world. Previous records revealed that an individual mobulid host species has never been simultaneously infected by more than three different species (including the external body surface, the nasal lamellae, the branchial lamellae and the buccal cavity). The highest number of different species ever recorded from a host species was two, and examples include *P*. *minor* and *P*. *brevicauda* both reported from *M*. *thurstoni* and *M*. *mobular* (Wilson 1952; Pillai & Padmanabhan 1963; Pillai 1964); *E*. *oliveri* and *C*. *elongatus* reported from *M*. *kuhlii* and *E*. *laminipes* as well as *A*. *crassum*, both reported from *M*. *alfredi* (Dippenaar & Jordaan 2007). This could be attributed to the fact that these hosts were not carefully studied for copepod infection previously and that copepods reported from them were probably found by chance. The current study, however, exceeds the expected number of different species encountered on a single of host species. The host species that was infected with the highest number of different copepod species in the current study was *M*. *alfredi* with eight species (*C*. *chrysophrysi*; three Unidentified sp. species; *E*. *diabolophila*; *Nemesis* sp.; *E*. *laminipes* and *A*. *crassum*), followed by *M*. *kuhlii* with six different species (*C*. *chrysophrysi*; *Pupulina* sp. 1; *Pupulina* sp. 2; *E*. *oliveri*; *K*. *mobulae* and *E*. *laminipes*) and lastly *M*. *eregoodootenkee* with four different species (the three *Pupulina* species and *E*. *oliveri*) (see Tables 1, 2 & 3). However, it seems more likely that a single species of host can be infected by more different species of copepods than reported in the current study, especially external body surface dwellers. Due to the fact that some of the collected hosts were caught, washed and frozen for some time before they were examined, it may have caused some specimens to be lost in the process. This could also be the primary reason behind the low infection intensities for most of the collected species on each particular host. Despite the smaller size of the hosts, the highest intensity of infection was recorded for *E*. *oliveri* on *M*. *eregoodootenkee* with 130 individuals per host (Table 2; Fig. 2) and on *M*. *kuhlii* with 42 individuals per host (Table 1; Fig. 2). The third highest was recorded for *Pupulina* sp. 1 on *M*. *kuhlii* with 41 individuals per host (Table 1; Fig. 2) while the rest of the collected specimens exhibited an infection intensity that ranged between 1 and 6 individuals per host (see Tables 1, 2, & 3; Fig. 2). It may be possible that more *E*. *oliveri* were collected since they inhabit the secondary gill filaments that may be protected against the process of washing the hosts, but similarly *K*. *mobulae* should have been protected in the nasal filaments and *Pupulina* species on the gill filaments as well. There is always variation regarding the intensity of infection by symbiotic siphonostomatoids on each type of examined host, thus the degree and intensity of infection varies with each encounter, regardless of host size (Wilson 1922). It could also be argued that *E*. *oliveri* is able to infect a host in large numbers because of its small size, but *E*. *diabolophila* and *K*. *mobulae* are equally small and they both exhibited low infection rates even provided with similar attachment surface area as *E*. *oliveri*. Therefore, degree of infection of any symbiotic siphonostomatoid could just be attributed to the preference of a particular host by a particular copepod or just pure random chance of infection by the infective stages. *Eudactylina oliveri* and *Pupulina* sp. 1 both exhibited an aggregated dispersion pattern on *M*. *kuhlii* and *M*. *eregoodootenkee* (see Tables 1 & 2), which is typical for most parasitic species in a natural environment (Bush *et al*. 2001). A deeper scrutiny into the host parasite relationships of all symbiotic siphonostomatoids, especially those that occur on mobulid hosts, is needed in order to understand the dispersion of each component population on each type of host.

CHAPTER 9: Conclusions

More than 90% of all examined mobulid hosts were infected with different symbiotic siphonostomatoids. A total of 13 different species of the order Siphonostomatoida distributed over five families were identified from just three species of mobulid hosts. The current study provides the first record of *C*. *chrysophrysi* (as such), *Pupulina* sp. 1, *Pupulina* sp. 2, *K*. *mobulae* and *E*. *laminipes* on *M*. *kuhlii*; *E*. *oliveri*, *Pupulina* sp. 1, *Pupulina* sp. 2 and *Pupulina* sp. 3 on *M*. *eregoodootenkee*; and *E*. *diabolophila*, *Nemesis* sp., *C*. *chrysophrysi*, *E laminipes*, *A*. *crassum* and the three Unidentified species on *M*. *alfredi*. The three *Pupulina* species collected and identified in the current study represent three new species within the genus while the three Unidentified sp. species may represent a new genus within the family Caligidae. However, examination of more specimens is needed to establish the correct identity of these specimens. Most of the specimens identified in the current study are specific to hosts within the family Mobulidae, with the exception of *A*. *crassum* and *C*. *chrysophrysi* which appear to be host generalists.

Despite South Africa’s richness and diversity of aquatic species, there is still limited knowledge regarding symbiotic copepods infecting elasmobranch hosts. Additionally, South Africa has significant diversity of elasmobranchs and they are all possible hosts of symbiotic siphonostomatoid copepods. The current study makes a small, but significant contribution to a larger study of the metazoan parasites of elasmobranch hosts along the coast of South Africa. The current study provides evidence that a careful and thorough examination of all possible mobulid hosts and host species will result in more reports of symbiotic Copepoda from South African waters. This will further contribute to the knowledge of marine invertebrate diversity, in particular the symbiotic copepods that occur along the South African east coast as well as across the world’s oceans.

CHAPTER 10: REFERENCES

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Appendix I: Character numbers and character states of six *Pupulina* species and three Unidentified species

Character 1: Cephalothorax – as wide as long/ longer than wide/ wider than long (0/1/2)

Character 2: Cephalothorax (ventrolateral borders) – without long slender hairs/ with long slender hairs (0/1)

Character 3: Cephalothorax (posterior margin) – without spinules dorsally/ with spinules dorsally (0/1)

Character 4: Fourth free thoracic segment – wider than long/ as wide as long/ longer than wide (0/1/2)

Character 5: Fourth free thoracic segment (dorsal surface) – not spiny/ spiny (0/1)

Character 6: Fourth free thoracic segment (posterior edge) – without row of minute spinules/ with row of minute spinules (0/1)

Character 7: Genital segment – wider than cephalothorax/ half as wide as cephalothorax/ more than half as wide as cephalothorax/ less than half as wide as cephalothorax (0/1/2/3)

Character 8: Genital segment (anterior margin) – rounded/ corners slightly extended upward/ square-like (0/1/2)

Character 9: Genital segment (posterolateral corners) – extending to mid length of abdomen/ almost reaching segment 1 of abdomen/ reaching well beyond caudal rami/ lateral expansions absent/ slightly extended (0/1/2/3/4)

Character 10: Genital segment (dorsolateral edges) – without row of spinules/ with row of spinules (0/1)

Character 11: Genital segment (posterodorsal surface) – without patch of spinules/ with patch of spinules (0/1)

Character 12: Abdomen – slender and short/ slender and elongate (0/1)

Character 13: Abdomen – 2-segmented/ 3-segmented/ 1-segmented (0/1/2)

Character 14: Abdomen (dorsal surface) – naked/ spinulated (0/1)

Character 15: Abdomen (ventral surface) – naked/spinulated (0/1)

Character 16: Abdomen – shorter than genital complex/ longer than genital complex/ as long as genital segment (0/1/2)

Character 17: Caudal rami – shorter than abdomen/ longer than abdomen/ as long as abdomen (0/1/2)

Character 18: Caudal rami (surface) – without scattered spinules/ with scattered spinules (0/1)

Character 19: Caudal rami – armed with 6 setae/ armed with 5 setae (0/1)

Character 20: Antennules – 2-segmented/ 3-segmented (0/1)

Character 21: Antenna – 3-segmented/ 4-segmented/ apparently 2-segmented (0/1/2)

Character 22: Antenna (segment 1) – with blunt posteromedial process/without blunt posteromedial process (0/1)

Character 23: Post antennal process – small rounded protrusion with more robust tine/ small rounded protrusion with slender tine (0/1)

Character 24: Mouth tube – short and blunt siphon/ longer than wide siphon (0/1)

Character 25: Mandible – with 12 teeth/ with 11 teeth (0/1)

Character 26: Maxillule (palp) – flattened process with 3 naked setae/ elongated process with 3 naked setae (0/1)

Character 27: Post-maxilluliary process – absent/ sub-triangular/ orbicular with pointed tip (0/1/2)

Character 28: Sternal furca – present/ absent (0/1)

Character 29: Lunules – present/ absent (0/1)

Character 30: Maxilla (segment 2) – with serrated membranous flabellum on posterior margin/ with patch of fine setules along posterior and anterior margin/ with row of setules extending to base of calamus (0/1/2)

Character 31: Maxilla (Calamus) – with parallel serrated membranes/ with coiled serrations (0/1)

Character 32: Leg 3 (endopod segment 1) – without semi-circular velum/ with extremely large semi-circular velum/ with medium size semi-circular velum (0/1/2)

Character 33: Leg 4 (sympod) – without long stiff hairs along dorsal surface/ with long stiff hairs along dorsal surface (0/1)

Character 34: Caudal rami – with row of fine setules along inner margin/ with long stiff hairs along inner margins/ no ornamentations on inner margin/ with rows of spinules lining inner margin (0/1/2/3)

Character 35: Post-maxillipedial process – absent/ present (0/1)

Character 36: Leg 4 (sympod) – without short spinules dorsally/ with short spinules dorsally (0/1)

Character 37: Leg 4 (sympod) – without membranes dorsally/ with 2 spinulated membranes dorsally/ with a pectinate membrane basal to distolateral spinule (0/1/2)

Character 38: Leg 1 (sympod) – without cobblestone-like patches anteriorly/ with cobblestone-like patches anteriorly (0/1)

Character 39: Leg 1 (exopod segment 1) – with rows of setules on medial margin/ with picket fence-like extensions on medial margin/ with rows of short spinules on medial margin (0/1/2)

Character 40: Leg 2 (sympod) – without cobblestone-like or spinulated patches anteriorly/ with cobblestone-like patches anteriorly /with spinulated patches anteriorly (0/1/2)

Appendix II: Data matrix derived from morphological characteristics of six *Pupulina* species and three unidentified species.

Taxon/ character	*Caligus grandifer*	*Pupulina flores*	*Pupulina minor*	*Pupulina brevicauda*	*Pupulina* sp. 1	*Pupulina* sp. 2	*Pupulina* sp. 3	Unidentified sp. 1	Unidentified sp. 2	Unidentified sp. 3
1	0	1	1	3	0	3	0	2	2	2
2	0	0	1	1	0	1	1	0	0	0
3	0	0	1	1	0	1	0	0	0	0
4	0	0	0	1	0	0	0	0	1	2
5	0	0	1	0	0	1	0	0	0	0
6	0	0	1	0	0	1	1	2	2	3
7	0	1	1	2	1	1	2	0	0	0
8	0	0	0	0	1	2	0	2	2	1
9	0	1	1	2	3	4	4	4	3	4
10	0	0	1	1	0	1	0	1	1	0
11	0	0	1	1	0	1	0	1	1	0
12	0	1	0	0	1	1	1	1	1	1
13	0	1	0	0	0	0	1	1	1	1
14	0	0	0	0	1	1	1	0	0	0
15	0	0	0	0	1	1	1	0	0	0
16	0	1	0	1	2	0	1	2	1	1
17	0	0	1	0	0	2	0	0	0	0
18	0	0	1	1	1	1	1	1	1	1
19	0	1	1	0	0	0	0	0	0	1
20	0	0	0	0	0	0	0	1	1	1
21	0	1	1	1	0	0	0	2	2	2
22	0	0	0	0	0	0	0	1	1	1
23	0	1	1	1	1	1	1	0	0	0
24	0	1	0	0	1	1	1	1	1	1
25	0	0	0	0	1	1	1	0	0	0
26	0	0	0	0	0	0	0	1	1	1

Taxon/ character	*Caligus grandifer*	*Pupulina flores*	*Pupulina minor*	*Pupulina brevicauda*	*Pupulina* sp. 1	*Pupulina* sp. 2	*Pupulina* sp. 3	Unidentified sp. 1	Unidentified sp. 2	Unidentified sp. 3
27	0	1	1	1	1	1	1	0	0	2
28	0	1	1	1	1	1	1	1	1	1
29	0	1	1	1	1	1	1	1	1	1
30	0	0	0	0	1	1	1	2	2	2
31	0	0	0	0	0	0	0	1	1	1
32	0	1	1	1	1	1	1	2	2	2
33	0	1	0	0	0	1	1	0	0	0
34	0	2	2	2	3	1	2	2	2	2
35	0	1	1	1	0	1	1	0	0	0
36	0	1	1	1	1	1	1	1	1	1
37	0	0	0	0	0	3	0	0	0	2
38	0	0	0	0	0	0	0	1	1	2
39	0	0	0	0	0	0	0	1	2	0
40	0	0	0	0	0	0	0	1	0	0