A TAXONOMIC STUDY OF SELECTED REPRESENTATIVES OF SIPHONOSTOMATOIDA (COPEPODA) FROM OSTEICHTHYES IN COASTAL WATERS OFF SOUTHERN AFRICA

by

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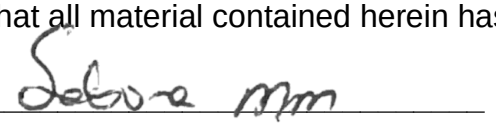
DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Masters 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 execution, and that all material contained herein has been duly acknowledged.



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ABSTRACT

Currently Copepoda consists of 14 600 species of which 2 275 species are members of the Siphonostomatoida. Siphonostomatoida consists of 40 families, with 17 families symbiotic on fish. Sphyriidae has 44 accepted species in eight reported genera, of which four genera infect teleosts and the remaining four infect elasmobranchs. Adult females undergo transformation through loss of locomotory appendages to suit their mesoparasitic lifestyle and develop outgrowths on the cephalothorax or neck for attachment to the host. To date, only 176 marine siphonostomatoid species have been reported from South African waters, with only nine sphyriid species.

Sphyriids previously collected from marine bony fish off the east, south and west coasts of southern Africa and preserved in 70% ethanol were studied. Specimens were examined with stereo- and compound microscopes and identified using published literature. Selected specimens were stained in lactic acid with added lignin pink, appendages were dissected and illustrated with the aid of a drawing tube. Selected specimens were also studied through scanning electron microscopy.

The examined specimens were identified as species of *Sphyrion* and *Lophoura*. Re-descriptions were done for all valid *Sphyrion* species females (*S. laevigatum*, *S. lumpi* and *S. quadricornis*) and new descriptions for the males of *S*. *laevigatum* and *S*. *quadricornis*. Post-metamorphosis females of *Sphyrion* species can be differentiated by the shape of cephalothorax, length of the neck in relation to the length of the trunk and the length of posterior processes in relation to the trunk length, while males are mostly very similar. New information is provided regarding the appendages of *S. laevigatum* and *S. quadricornis*. The appendages of the three species bear close resemblance to one another. Additionally, an identification key for the post-metamorphosis females of *Sphyrion* species is provided.

Re-descriptions were done for five female *Lophoura* species (*L. caparti*, *L. cornuta*, *L. cf edwardsi*, *L. tetraloba* and *Lophoura* sp.) and a new description of the male of *L. tetraloba.* Differences between young and post-metamorphosis females of *L. cf edwardsi* and *L. tetraloba* were observed in the width of the holdfast organ processes and the length of porous peduncle and stalks of the posterior processes which appear to grow with age. The difference between the young and adult male of *L. tetraloba* lies in the lengths of the cephalothorax in relation to the trunk length and segmentation visible on the trunk of the young male but not adult male. The post-metamorphosis females of *Lophoura* species can be differentiated by the shape and number of processes on the holdfast organ, in combination with the cephalothorax length in relation to the neck length, neck length in relation to the trunk length, shape of the trunk, and the length and structure of the posterior processes. An identification key was drawn up for all species of the *Lophoura* post-metamorphosis females.

An attempt was made to provide the COI barcodes for all the species of *Sphyrion* and five species of *Lophoura*. These would have confirmed the species identification of morphologically variable species e.g. *S. laevigatum* and *S. lumpi* and also provide an estimation of the interspecific divergence amongst the different species. Additionally, it would have assisted in distinguishing between *L. tetraloba* and *L*. *cf edwardsi* and provided an estimation of the amount of sequence divergence between the two genera. Unfortunately sequencing of apparently successfully amplified products was unsuccessful probably due to low DNA quality which possibly degraded due to collection methods used for the fish hosts and parasites and prolonged preservation of specimens.

This study provides new host records i.e. *Coelorinchus simorhynchus, Coelorinchus trunovi* and *Saurida undosquamis* for *Sphyrion quadricornis* off South Africa which is also a new geographical record. *Allocyttus verrucosus, Coelorinchus simorhynchus, Coelorinchus trunovi*, *Mesovagus antipodum* and *Ventrifossa nasuta* are also new host records for *S. lumpi*. Additionally, *Epigonus denticulatus* and *Bassanago albescens* are new host records for *Lophoura caparti* and *L. cornuta* respectively off

South Africa, which is a new geographical record for both species. Furthermore, *Coelorinchus fasciatus* and *Lucigadus ori* are new host records for *Lophoura tetraloba* and *L*. *cf edwardsi* off South Africa, which is also a new geographic record for both species. Thus, the results of the study improve the current knowledge of the marine siphonostomatoid biodiversity off South Africa as well as their distribution and infected hosts.

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RESEARCH OUTPUTS

Publications

Dippenaar SM, Sebone MM. 2022. Morphology of three *Sphyrion* (Copepoda: Siphonostomatoida: Sphyriidae) species infecting teleost fishes off South Africa with the first description of males of two species. *Diversity* 14(11): 929. https://doi.org/10.3390/d14110929

Sebone MM, Dippenaar SM. (in preparation). Reports and re-descriptions of selected *Lophoura* species (Copepoda: Siphonostomatoida: Sphyriidae) off South Africa.

Oral presentations

Sebone MM, Dippenaar SM. Sphyriidae females infecting marine teleosts off the South African coast. Virtual International Conference on Copepoda (e-ICOC) 25 – 30 July 2022.

Sebone MM, Dippenaar SM. The study of *Sphyrion* females infecting marine Osteichthyes off the South African coasts. 4th ICPOW & 50th Annual PARSA Conference, Kruger National Park, South Africa. 11 – 15 September 2022.

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CHAPTER 1: General Introduction

1.1. Copepoda Milne Edwards, 1840

This class consists of typically small aquatic crustaceans within the phylum Arthropoda. Copepoda exhibits high morphological diversity with abundant species (Boxshall and Halsey 2004). Currently, it consists of 10 orders (Platycopioida Fosshagen, 1985; Calanoida G. O. Sars, 1903; Misophrioida Gurney, 1933; Canuelloida Khodami, Vaun MacArthur, Blanco-Bercial & Martinez Arbizu, 2017; Gelyelloida Huys, 1988; Harpacticoida G. O. Sars, 1903; Mormonilloida Boxshall, 1979; Cyclopoida Burmeister, 1834; Siphonostomatoida Burmeister, 1835 and Monstrilloida Sars, 1901) with 14 600 species reported worldwide (Walter and Boxshall 2022). The members are morphologically diverse, ranging from free-living to symbiotic copepods inhabiting a wide variety of habitats, ranging from freshwater to marine and hypersaline waters (Boxshall and Halsey 2004; Eyun 2017).

Copepods are uniquely known by their broad, paddle-like swimming legs. They are typically small creatures with body lengths varying from about 0.2 mm to 2.8 mm. However, some parasitic copepods are large, with a body length of about 250 mm, such as members of the Pennellidae Burmeister, 1835 (Boxshall and Halsey 2004). A copepod's body is divided into the cephalosome, metasome and urosome. The cephalosome and urosome have 10 appendages each, used mostly for feeding and movement but also for attachment in symbiotic species (Huys and Boxshall 1991).

Copepods are of major importance to living systems. The aquatic food chain requires copepods which feed on phytoplankton and provide food for fish and other planktivorous organisms. Freshwater copepods feed on, amongst others, mosquito larvae, thus biologically controlling malaria. They also serve as intermediate hosts to other parasites, such as guinea worm and tapeworm (Boxshall and Halsey 2004). Some copepods can impact fisheries by parasitizing economically important fish. Free-living copepods can be biological indicators of climate change and symbiotic copepods

can be used as biological tags while also providing information (e.g. geographical, diet, phylogenetic, etc.) about their hosts (Goater et al. 2014).

1.2. Copepods as fish symbionts

Of the ten Copepoda orders, only two have species that are fish symbionts, namely Siphonostomatoida and Cyclopoida (Huys and Boxshall 1991; Khodami et al. 2017). These symbiotic copepods are mostly ectoparasitic, since they inhabit the host's external body surface such as eye orbit, oral cavity, gill cavity and nasal slits (Huys and Boxshall 1991). They use appendages such as the antennae, maxillae and/or maxillipeds for attachment, with exception of the family Lernaeopodidae Milne Edwards, 1840, which embed maxillary arms (bulla) into the host tissue for firm attachment. Mesoparasites include members of the families Sphyriidae Wilson C.B., 1919 and Pennellidae Burmeister, 1835 since they embed the anterior part of their bodies into the host tissues, aided by cephalic holdfasts for attachment (Kabata 1979; Boxshall and Halsey 2004). Endoparasitic copepods occur within the family Philichthyidae Vogt, 1877 with members inhabiting the skull bones (e.g. *Colobomatus mylionus* Fukui, 1965) and sensory canals of the lateral line system (e.g. *Colobomatus pupa* Izawa, 1974) (Madinabeitia et al. 2012, Madinabeitia and Iwasaki 2013).

1.3. Siphonostomatoida Burmeister 1835

The order Siphonostomatoida is a large order within the superorder Podoplea Giesbrecht, 1882 (Huys and Boxshall 1991), which consists of 40 families with 2 275 species (Walter and Boxshall 2022). Approximately 75% of the siphonostomatoid members are fish symbionts (Dippenaar 2004, 2016). Siphonostomatoids are mainly marine, although there are few symbionts of freshwater fish. This order is known for unresolved interfamilial relationships due to scanty knowledge of the representatives (Dippenaar 2009).

In addition to the 163 Siphonostomatoida from the checklist by Nunkoo (2019), 13 additional species (*Lernaeopoda mustelli* Thomson G.M., 1890 and *Lernaeopoda bidiscalis* Kane, 1892 (see Dippenaar 2020b); *Neoalbionella izawai* Dippenaar, 2020 (see Dippenaar 2020a); *Naobranchia cygniformis* Hesse, 1863 (see Dippenaar and

Sebone 2021); *Pseudocharopinus malleus* (Rudolphi in Nordmann, 1832) (see Dippenaar 2019); *Caligus lineatus* Hayes, Christison, Vaughan, Smit & Boxshall, 2021; *Caligus tumulus* Hayes, Christison, Vaughan, Smit & Boxshall, 2021; *Caligus longipedis* Bassett-Smith, 1898; *Lepeophtheirus spinifer* Kirtisinghe, 1937; *Lepeophtheirus acutus* Heegaard, 1943; *Caligus tenuis* (van Beneden, 1852); *Caligus rufimaculatus* Wilson, 1905 (see Hayes et al. 2021); and *Alella igillimpethu* Erasmus, Hadfield, Wepener & Smit, 2022 (see Erasmus et al. 2022)) have been reported from marine fish of South Africa. Thus, only 176 marine symbiotic siphonostomatoids were reported from South Africa in comparison to the 2 275 (Walter and Boxshall 2022) marine siphonostomatoids reported worldwide. This is most likely due to limited studies examining the hosts for possible symbiotic copepods since there is a shortage of taxonomists studying marine siphonostomatoids in South Africa (Smit and Hadfield 2015; Dippenaar 2016). Without sufficient studies, there is a possibility that some species, including siphonostomatoids, may become extinct (with their hosts) prior to description or report which may lead to lack of knowledge about the biodiversity of siphonostomatoids in South Africa but also worldwide (Woodcock 2002; Narendran 2008; Dippenaar 2016).

Members of order Siphonostomatoida possess a tubular mouth resembling a siphon, which is formed by the fused labium and labrum (Kabata 1979). There is sexual dimorphism in members of this order, with the extreme being reached in families such as Lernaeopodidae and Sphyriidae where males are dwarf and attach to large-bodied females (Kabata 1979; Huys and Boxshall 1991).

The lack of sufficient knowledge to identify and classify some members of the Siphonostomatoida is clear considering for example the family Lernaeopodidae Milne Edwards, 1840 with about 22 renamed genera and 522 renamed species, while Pennellidae has about 17 renamed genera and 116 renamed species and Sphyriidae Wilson C.B., 1919 has about seven renamed genera and 30 renamed species. Additionally, about 22 marine lernaeopodids and about 20 marine pennellids have incomplete descriptions for further taxonomic classification (Walter and Boxshall 2022).

1.4. Family Sphyriidae Wilson C.B., 1919

Species found in this family are mesoparasites of deep sea and open ocean fish (Huys and Boxshall 1991). The body of the adult females are transformed and the locomotory appendages lost or reduced after attaching to the host. Thus, the body can be divided into the head (cephalothorax), neck and genito-abdominal trunk (Kabata 1979). In cases where locomotion is required on the host's body surface, the female uses the maxillae and maxillipeds to aid the movement (Boxshall and Halsey 2004). These appendages are used for prehension but once the female becomes attached to a suitable spot on the host, it develops holdfast protuberances on the cephalothorax margins or neck which is embedded into the host tissue, and it becomes a mesoparasite. The cephalothorax protuberances aid with firm attachment and eventually the maxillae and/or maxillipeds may disappear (Kabata 1979). The males continue to use the second maxillae and maxillipeds both for locomotion and prehension to the adult female. The sphyriid dwarf males bear a close resemblance to the lernaeopodid males (Kabata 1979; Huys and Boxshall 1991).

Sphyriidae currently consists of eight genera (i.e. *Sphyrion* Cuvier, 1830; *Lophoura* Kölliker in Gegenbaur, Kölliker & Müller, 1853; *Tripaphylus* Richiardi in Anonymous, 1878; *Opimia* Wilson C.B., 1908; *Periplexis* Wilson C.B., 1919; *Paeonocanthus* Kabata, 1965; *Norkus* Dojiri & Deets, 1988; *Driocephalus* Raibaut, 1999) including 44 species (Walter and Boxshall 2022). The genera are distinguished based on the morphology of the cephalothorax, posterior processes (simple and cylindrical vs subdivided and complex) and a neck with or without holdfast outgrowths (Kabata 1979).

Sphyriids are parasites of both elasmobranchs and teleosts (Boxshall and Halsey 2004), with only four genera (*Paeonocanthus*, *Periplexis*, *Lophoura* and *Sphyrion*) found on teleosts (Gómez et al. 2010). To date, only three genera and nine species (*Lophoura elongata* Kensley & Grindley 1973 from *Histiobranchus bathybius* (Günther, 1877); *Sphyrion laevigatum* (Quoy & Gaimard, 1824) from *Atractoscion aequidens* (Cuvier, 1830), *Coelorinchus fasciatus* (Günther, 1878) and *Genypterus capensis* (Smith, 1847) (Barnard 1955; Payne 1986); *Sphyrion lumpi* (Krøyer, 1845) from *Antimora rostrata* (Günther, 1878), *Psychrolutes inermis* (Vaillant, 1888) and *Psychrolutes macrocephalus* (Gilchrist, 1904) (Barnard 1955; Kensley and Grindley

1973); *Tripaphylus elongatus* (Wilson C.B., 1932) from *Carcharhinus obscurus* (LeSueur, 1818) (Dippenaar and Jordaan 2007; Dippenaar 2018); *Tripaphylus benzi* Dippenaar, 2018 from *Mustelus palumbes* Smith, 1957 (Dippenaar 2018); *Tripaphylus hoi* Dippenaar, 2018 from *Mustelus palumbes* Smith, 1957; *Tripaphylus beatricae* Dippenaar, 2018 from *Mustelus mustelus* (Linnaeus, 1758) (Dippenaar 2018); *Tripaphylus lewisi* Dippenaar, 2018 from *Hemipristis elongata* (Klunzinger, 1871) (Dippenaar 2018); and *Tripaphylus vaissierei* (Delamare Deboutteville & Nuñes-Ruivo, 1954) from *Sphyrna lewini* (Griffith & Smith, 1834) (Dippenaar and Jordaan 2007; Dippenaar 2018)) were reported from South African waters.

1.5. Osteichthyes

Osteichthyes contributes 96% of fish species worldwide. The representatives are highly diverse, and their adaptation extends to extreme habitat conditions. Osteichthyes consists mostly of Actinopterygii (ray-finned fish) and Sarcopterygii (lobe-finned fish). The Actinopterygii has a bony skeleton, fins with spines, a cartilaginous skull (partly calcified), and a pair of gill openings enveloped in the operculum. Sarcopterygii members possess fleshy lobes which join fins to the body (Smith and Heemstra 1988). Fishes of the Osteichthyes differ from those of the Chondrichthyes by possessing bony skeletons rather than cartilaginous skeletons and also the presence of a swim bladder to improve buoyancy (Perry 2007). Currently, there are 1 810 Osteichthyes species reported from South African marine waters (Froese and Pauly 2022). Although there is a high diversity of bony fish, there is currently no copepodologist actively researching the symbiotic siphonostomatoids infecting Osteichthyes in South Africa. Most available studies of South African siphonostomatoids were reported from representatives of Chondrichthyes (Dippenaar 2016).

1.6. Coastal waters and their lifeforms

The South African coast ranks the second longest in Africa with about 3 650 km (Rautenbach et al. 2019). It is composed of three ecoregions (KwaZulu-Natal, Agulhas bank and Namaqua) with the cold Benguela current of the Atlantic Ocean along the west coast (Fig. 1.1) and the warm Agulhas current of the Indian ocean along the east coast (Fig. 1.1). The east coast has lower biodiversity compared to the west and the

south coasts, due to less nutrients in warm water whereas the west and south coast are enriched with abundant biodiversity (Griffiths et al. 2010). The South African marine waters provide habitats to more than 12 000 species, with about 2 013 known fish species (Froese and Pauly 2022).

1.7. Biological taxonomy

Taxonomy is the science of studying and classifying biotic organisms into respective taxa (Boxshall 2020). There are two methods widely used to identify invertebrates, i.e. classical and DNA-based taxonomy. Classical taxonomy includes a close observation of external traits and morphometric measurements. It has been used from ancient times before technological advancements (Wheeler 2008). DNA-based taxonomy includes the amplification of DNA fragments through polymerase chain reaction (PCR) to determine species-specific genetic variation, interrelationships within different taxa and systematics between taxa (Pfenninger et al. 2006). The integration of both classical and DNA-based taxonomy yields the best results for taxonomic purposes (Wheeler 2008) of species and overcomes the disadvantages arising when each method is used individually.

Biological taxonomy contributes to background knowledge about an organism, including the habitat preference, host association, geographical distribution, morphology and biology (Boxshall 2020). Taxonomy helps in discovering the number of species existing in the environment and gives an idea of local fauna and flora, thus helping in distinguishing endemic species (Frankham et al. 2002) and making biodiversity estimations.

1.8. Purpose of the study

1.8.1. Aim

The aim of this study was to study and report on selected representatives of Sphyriidae collected from marine bony fish in coastal waters off southern Africa and to attempt to clarify any existing confusion regarding their taxonomy and systematics.

1.8.2. Objectives

The objectives of this study were:

i. To identify all the collected specimens of the family Sphyriidae collected from marine bony fish to species level by studying the morphology through dissecting appendages, illustrating and completely describing each species.

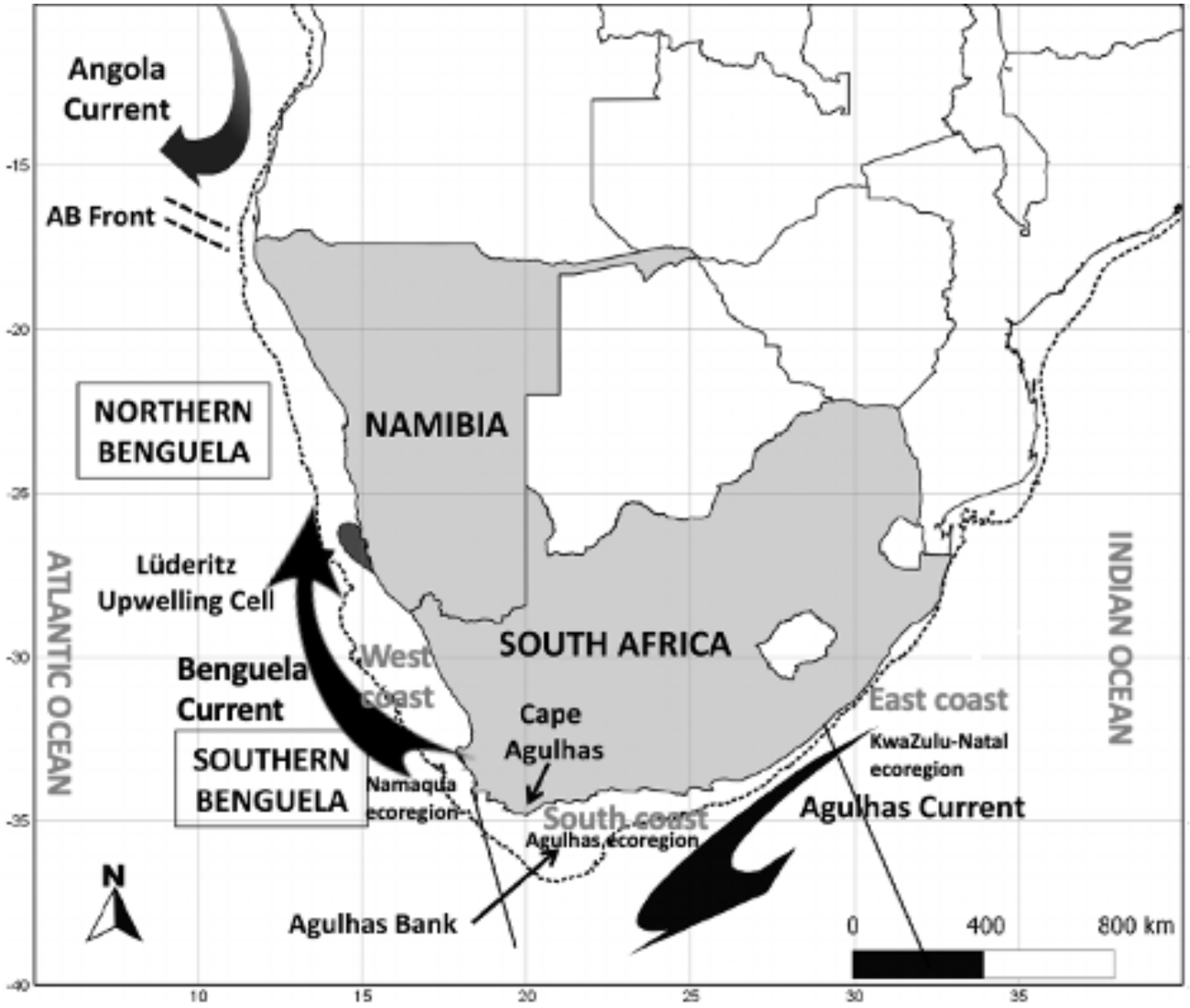
ii. To compare the identified species with those in the literature and to attempt to clarify the existing uncertainty regarding the number of valid species where necessary.

iii. To support classical taxonomy by DNA-based taxonomy of the identified species.

1.9. Significance of the study

This study will provide additional information to the limited knowledge regarding the biodiversity of marine siphonostomatoids occurring off South Africa. It may also add to our knowledge of the hosts being infected by these collected sphyriid species as well as information regarding the host specificity of the selected species. An important contribution will be made to current knowledge by the attempt to resolve the current valid species discrepancies and providing re-descriptions and illustrations of collected species. The knowledge of our world's symbiotic copepods can help the present and upcoming generations to better understand the world of copepods and their interactions with fish.

Figure 1.1: A South African map showing the positions of the coasts, oceans, currents and ecoregions (adapted from Roux et al. 2013 and modified)


Angola
Current
AB Front
NORTHERN
BENGUELA
NAMIBIA
Lüderitz
Upwelling Cell
ATLANTIC OCEAN
Benguela
Current
SOUTHERN
BENGUELA
West
coast
SOUTH AFRICA
Cape
Agulhas
East coast
KwaZulu-Natal
ecoregion
INDIAN OCEAN
Namaqua
ecoregion
South coast
Agulhas ecoregion
Agulhas Current
Agulhas Bank
N
0
400
800 km
-15
-20
-25
-30
-35
-40
10
15
20
25
30
35

CHAPTER 2: Methodology

2.1. Sampling

Specimens previously collected from marine bony fish off the east, south and west coasts of southern Africa, and preserved in 70% ethanol, were examined. These specimens were collected between 1991 and 2016 from fish caught as by-catch during demersal cruises, including hake assessment demersal cruises off the south and west coasts of South Africa on board the Department of Agriculture, Forestry and Fisheries (DAFF) research vessel *R/V* (*Africana*) as well as during demersal survey trawls off the South African east coast (Indian Ocean) (ACEP African Coelacanth Ecosystem Programme). The fish hosts were identified by researchers on board the vessels. The fish hosts examined from the west coast include *Allocyttus verrucosus* (Gilchrist, 1906); *Coelorinchus simorhynchus* Iwamoto & Anderson, 1994; *Nezumia umbracincta* Iwamoto & Anderson, 1994; *Lucigadus ori* (Smith, 1968); *Coelorinchus fasciatus* (Gunther, 1878); *Epigonus denticulatus* Dieuzeide, 1950; and *Genypterus capensis* (Smith, 1847); whereas the fish hosts from the east coast include *Coelorinchus trunovi* Iwamoto & Anderson, 1994; *Epigonus telescopus* (Risso, 1810); and *Ventrifossa nasuta* (Smith, 1935); and the fish hosts from south coast include *Mesovagus antipodum* (Hubbs & Iwamoto, 1977) and *Bassanago albescens* (Barnard, 1923). The host names were confirmed through Froese & Pauly (2022) and Fricke et al. (2022).

2.2. Data collection

2.2.1. Morphological data

The identification of collected specimens was performed using morphological characteristics. The collected specimens were stained in lactic acid with a small amount of dissolved lignin pink. The external morphology of collected specimens was studied using the wooden slide technique (Humes and Gooding 1964), through stereo- and compound microscopes and drawings done with the aid of drawing tubes. Studied specimens were dissected, and all appendages were drawn. Measurements were done using a 2 mm stage micrometer. Morphological terminology used is mostly according to Kabata (1979) and Huys and Boxshall (1991). Additionally, a series of

photographs of sections of selected specimens were taken using an imaging software Olympus Stream Essentials 2.2 and merged into one picture (since the size of specimens was too big to be captured in a single photo). Photographs of appendages of somes specimens were taken using an imaging software Olympus LCmicro 2.2. Morphological features were used to classify the species into the respective genera using relevant literature (Wilson 1919, 1935; Nigrelli and Firth 1939; Barnard 1955; Nuñes Ruivo 1962; Hewitt 1964; Szidat 1971; Kensley and Grindley 1973; Kabata 1979; Gaevskaya and Kovaleva 1984; Ho 1985; Hogans and Dadswell 1985; Payne 1986; Ho and Kim 1989; Boxshall 1989; Moran and Piasecki 1994; Boxshall 2000; Castro-Romero and Gonzalez 2009; Gómez et al. 2010; Kazachenko 2017).

Scanning Electron Microscopy

Cephalic areas of the specimens were dissected off the cephalothoraces and dehydrated through ethanol series (70% – 80% – 90% – 100% – 100%) with each over 30 minutes intervals whereafter the samples were transferred into hexamethyldisilazane for at least 30 minutes and then transferred into a petri dish to dry completely. Samples were mounted onto stubs, sputter-coated with carbon and gold palladium. Scanning electron microscopy observations were carried out through a FEI Quanta 250 FEG SEM.

2.2.2. Molecular data

Specimens were rehydrated from 70% ethanol to distilled water by gradually decreasing the ethanol concentration. Genomic DNA was isolated from trunk tissues of specimens of *Sphyrion laevigatum*, *Sphyrion lumpi*, *Sphyrion quadricornis*, *Lophoura edwardsi*, *Lophoura caparti* and *Lophoura tetraloba* (see Table 2.1) using proteinase K digestion and the Qiagen genomic purification kit, following the instruction of the manufacturer. Extracted DNA concentrations, and UV absorption at 260 nm (A260), UV absorption at 280 nm (A280), ratio 260/280 and ratio 260/230 were measured using a Nanodrop. The recommended DNA concentration for successful PCR is 50 ng/µl and thus attempts to increase the concentration of extractions with lower concentrations included multiple extractions made from an individual which were combined and incubated at 56°C (dry bath) for 1 hour to evaporate. Thereafter the concentration of DNA was measured again and if still less than 50 ng/µl the

concentration step was repeated until the desired concentration was reached. Samples consisting of DNA concentrations ranging from 76.8 ng/µl to 221.7 ng/µl were used to attempt to amplify part of the COI (cytochrome oxidase I) gene. Each polymerase chain reaction (PCR) was carried out in 20 µl volumes, containing 10 µM of both forward (cop-COI-1498F, LCO and LCO_t1) and reverse (HCO and HCO_t1) primers (see Table 2.2), 10 µl OneTaq® 2X Master Mix, 50 ng/µl of template DNA and distilled water to add up the volume. The PCR reactions were performed with the following conditions: initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 45°C – 48°C for 2 min (see Table 2.3), and extension at 70°C for 3 min. The final extension was carried out at 72°C for 10 min and cooling at 4°C for 30 min. The amplicons were separated on a 1.5% agarose gel stained with ethidium bromide, running at 150 V. PCR products were sent for sequencing to Inqaba Biotech laboratory.

Table 2.1: Specimens used for DNA extractions with their species names and host names.

Species name	Host name	No of specimens used	No of extractions per specimen
S. quadricornis	*Coelorinchus simorhynchus*	4	5
S. lumpi	*Coelorhinchus trunovi*	3	3 – 4
S. laevigatum	*Genypterus capensis*	4	7
S. quadricornis	*Coelorhinchus trunovi*	1	5
S. lumpi	*Allocytus verrucosus*	1	7
S. lumpi	*Coelorinchus simorhynchus*	1	3
S. lumpi	*Mesovagus antipodium*	1	7
S. laevigatum	*Genypterus capensis*	1	7
L. edwardsi	*Coelorhinchus fasciatus*	3	3
L. tetraloba	*Coelorhinchus fasciatus*	3	4

L. tetraloba	*Lucigadus ori*	1	4
L. caparti	*Epigonus denticulatus*	2	6
L. caparti	*Epigonus denticulatus*	1	6

Table 2.2: Primers used in PCR amplification of a part of mitochondrial gene (COI), with their sequences and product size anticipated.

Name of primer	Sequence	Annealing temperature	Anticipated gene size	Reference
LCO 2198	5'-GGTCAACAAATCATAAAGATATTGG-3'	45 °C	670 bp	Folmer et al. 1994
HCO 1490	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	45 °C, 48 °C	670 bp	Folmer et al. 1994
LCO 2198_t1	5'-TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG-3'	45 °C	670 bp	Messing 1983
HCO 1490_t1	5'-CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA-3'	45 °C	670 bp	Messing 1983
cop-COI-1498F	AAYCATAAAGAYATYGGDAC	48 °C	670bp	Bucklin et al. 2010

2.3. Data analysis

2.3.1. Cladistics analysis

Cladistic analysis was performed for all *Lophoura* species based on morphological characteristics, to determine evolutionary relationships among them. *Tripaphylus elongatus* (Wilson, 1932), one of members of family Sphyriidae, was used as the outgroup. Outgroup taxon helps to determine polarity of character states using the

outgroup comparison method. Thirteen morphological characters (Table 2.3) were identified (according to Benz et al. 2006) to use in compiling a character matrix for the 19 *Lophoura* species (see Tables 2.4). Parsimony analysis was performed with PAUP version 4.0b10 (Swofford 1985), using a heuristic search with tree-bisection reconnection (TBR) branch swapping technique to find the most parsimonious tree(s). The homoplasy index, retention index, consistency index and rescaled consistency index were used to measure the goodness of fit of the characters on the tree. In cases where there is more than one most parsimonious tree, the strict consensus (only display nodes that concur in all most parsimonious trees) and 50% majority rule consensus (display nodes that occur in 50% of most parsimonious trees) trees were determined.

Table 2.3: Characters with assigned character states used to compile a character matrix for *Lophoura* species (according to present study and Benz et al. (2006)).

Characters	States
1. Cephalothorax	circular……..…….……..……...…….0 longitudinally elongated, cylindrical..…… ……..……..……..1
2. Cephalothorax length vs neck length	cephalothorax length shorter than neck length.……..…….0 cephalothorax length longer than neck length.……..…..…….1
3. Cephalothorax vs trunk length	cephalothorax length shorter than trunk.…..……..……...…..0 cephalothorax length longer than trunk…….……..……..……1
4. Cephalothorax width	cephalothorax without longitudinal part.…….……0 longitudinal part of the cephalothorax with the same width throughout…….…..1 longitudinal part of the cephalothorax with different width..……...…….2
5. Neck length in relation to trunk length	longer than the trunk…….……...0 as long as trunk…….……..…..1 shorter than the trunk……...…..2

6. Holdfast organ	holdfast organ absent……..……...…….0 holdfast organ simple.……..…..1 holdfast organ complex, processes can be counted, with uniform shape…...2 holdfast organ complex, processes irregular/ without uniform shape.…..…… 3
7. Holdfast organ main processes	holdfast organ without main processes/absent…..…….……..…...0 holdfast organ with few (1 to 3) main processes…..……...1 holdfast organ with many (4 – 5) main processes..……..……...…....2
8. Holdfast organ processes	absent……..……..……0 without tentacle-like processes or outgrowths.…..……..…..1 with tentacle-like processes or outgrowths...……...……...……...2
9. Trunk shape	pyriform…….……...……..….0 quadrangular to circular……..…..…...1
10. Rows of depressions on trunk	Absent..……..……..…..0 present…….……..……..…..1
11. Posterior processes position	extending posteriorly to trunk..……..…….....……..…0 extending anteriorly on trunk…...…..……..…..…...1
12. Posterior processes stalks	absent…….....…….....…0 straight…….……..…..…1 slightly curved, not intertwined.……..……...……2 curved and intertwined…….....…..……..…3
13. Posterior processes stalks	absent……..……..…….....0 single stalks, do not branch……..……...……..1 each stalk branches into secondary stalks……..……..……...2

Table 2.4: Character matrix compiled for *Lophoura* species with *Tripaphylus elongatus* (Wilson, 1932) as the outgroup taxon (compiled from Wilson 1919, 1935; Nuñes Ruivo 1962; Hewitt 1964; Szidat 1971; Kensley and Grindley 1973; Stadler 1978; Kabata 1979; Ho 1985; Hogans and Dadswell 1985; Ho and Kim 1989; Boxshall 1989, 2000; Castro-Romero and Gonzalez 2009; Gómez et al. 2010; Dippenaar 2018).

Character	1	2	3	4	5	6	7	8	9	10	11	12	13

L. bipartita	1	1	1	2	0	2	1	2	1	1	1	3	1
L. bouvieri	1	0	0	1	0	1	1	1	1	1	0	1	1
L. brevicollum	1	0	0	2	2	2	2	1	1	1	0	1	2
L. caparti	1	0	1	1	0	1	1	1	0	0	0	1	2
L. cardusa	1	0	1	1	2	3	0	2	0	1	0	1	1
L. cornuta	1	0	0	1	2	2	2	2	0	1	0	1	1
L. edwardsi	1	0	0	1	0	2	2	1	1	1	0	1	1
L. elongata	1	1	1	1	0	2	2	1	0	1	0	1	1
L. gracilis	1	1	1	2	1	2	2	1	0	1	0	1	2
L. laticervix	1	0	0	1	2	2	1	1	0	1	0	1	1
L. magna	1	0	0	1	2	3	1	2	0	1	0	1	1
L. pentaloba	1	0	0	1	2	2	2	1	0	1	1	3	1
L. simplex	1	0	0	1	0	0	0	0	0	1	0	1	1
L. szidati	1	0	1	2	0	1	1	1	0	1	0	2	1
L. tetraloba	1	0	0	1	0	2	2	1	1	1	0	1	1
L. tetraphylla	1	0	0	1	2	2	2	1	1	1	0	2	1
L. tripartita	1	0	1	1	0	2	1	1	0	1	0	1	2
L. unilobulata	1	0	0	1	0	1	1	1	0	1	0	1	2
L. ventricula	1	0	0	1	0	2	2	2	0	1	1	3	1
T. elongatus	0	0	0	0	0	0	0	0	0	0	0	0	0

CHAPTER 3: Genus *Sphyrion* Cuvier, 1830

3.1. Introduction

Sphyrion is one of the genera belonging to family Sphyriidae, which Krøyer (1863) previously placed under the Pennellidae (see Kabata 1979). Currently there are only three accepted species, namely *Sphyrion laevigatum* (Quoy & Gaimard, 1824), *Sphyrion lumpi* (Krøyer, 1845) and *Sphyrion quadricornis* Gaevskaya & Kovaleva, 1984 (Walter and Boxshall 2022). Similar to other sphyriid females, *Sphyrion* adult females possess highly modified bodies.

Post-metamorphosis females possess transversely elongated (hammer-shaped) cephalothoraces of shapes and sizes varying from one species to the other, attached to a cylindrical neck. The posterior part of the neck expands into the dorsoventrally flattened trunk with suborbicular or pyriform shape, bearing a small abdomen posteriorly. The abdomen accommodates branched and grape-like posterior processes, and long egg sacs, with multiseriate eggs. Males attach to the female body (on the posterior processes) resemble lernaeopodid males with the body indistinctly divided into the cephalothorax and compact trunk (Wilson 1919; Kabata 1979).

Sphyrion species are cosmopolitan and infect a wide range of host species (Ho 1992; Moran & Piasecki 1994; Kazachenko 2017). To date, *S. lumpi* has been reported from 30 fish host species (i.e. *Antimora rostrata* (Günther, 1878); *Anarhichas denticulatus* Krøyer, 1845; *Anarhichas lupus* Linnaeus, 1758; *Boreogadus saida* (Lepechin, 1774); *Cyclopterus lumpus* Linnaeus, 1758; *Coelorinchus fasciatus* (Günther, 1878); *Coelorinchus marinii* Hubbs, 1934; *Coryphaenoides rupestris* Gunnerus, 1765; *Coryphaenoides armatus* (Hector, 1875); *Gaidropsarus ensis* (Reinhardt, 1837); *Gadus morhua* Linnaeus, 1758; *Glyptocephalus cynoglossus* (Linnaeus, 1758); *Laemonema laureysi* Poll, 1953; *Macrourus berglax* Lacepède, 1801; *Merluccius bilinearis* (Mitchill, 1814); *Molva dypterygia* (Pennant, 1784); *Psychrolutes inermis* (Vaillant, 1888); *Reinhardtius hippoglossoides* (Walbaum, 1792); *Sebastes norvegicus* (Ascanius, 1772); *Sebastes mentella* Travin, 1951; *Sebastes viviparus* Krøyer, 1845; *Macrourus holotrachys* Günther, 1878; *Merluccius merluccius* (Linnaeus, 1758); *Psychrolutes macrocephalus* (Gilchrist, 1904); *Sebastes fasciatus*

Storer, 1854; *Sebastes flammeus* (Jordan & Starks, 1904); *Solea solea* (Linnaeus, 1758); *Pagellus bogaraveo* (Brünnich, 1768); *Urophycis tenuis* (Mitchill, 1814)) (Ho 1992, Alves et. al 2013, Walter and Boxshall 2022) belonging to 15 families, whereas *S*. *laevigatum* has been reported from six fish host species (i.e. *Coelorinchus fasciatus* (Günther, 1878); *Genypterus blacodes* (Forster, 1801); *Genypterus capensis* (Smith, 1847); *Merluccius australis* (Hutton, 1872); *Merluccius hubbsi* Marini, 1933; *Merluccius polli* Cadenat, 1950) belonging to three families (Ho 1992, Walter and Boxshall 2022) and *S*. *quadricornis* has been reported from four fish host species (i.e. *Coelorinchus braueri* Barnard, 1925; *Coelorinchus innotabilis* McCulloch, 1907; *Coelorinchus fasciatus* (Günther, 1878); *Macrourus berglax* Lacepède, 1801) belonging to Macrouridae (Ho 1992, Walter and Boxshall 2022).

3.2. Materials and Methods

Refer to Chapter 2.

3.3. Results

3.3.1. Descriptions of *Sphyrion* species

3.3.1.1. a. *Sphyrion laevigatum* (Quoy & Gaimard, 1824)

Host: *Genypterus capensis* (Smith, 1847)

Locality: South coast of South Africa (Atlantic Ocean)

Material examined: 4♀♀ and 1♂ from *G*. *capensis* off the south coast (Atlantic Ocean)

Material collected: 30♀♀ and 3♂♂ all from *G*. *capensis* off the south and west coast (Atlantic Ocean)

Description of post-metamorphosis female (Figs. 3.1 – 3.3)

Body length from tip of cephalothorax to the tip of the abdomen 27.6 mm (*n* = 15; 22.3 – 34.3 mm), cephalothorax length 7.9 mm (*n* = 15; 6.3 – 9.8 mm), width 20.4 mm (*n* = 15; 15.4 – 25.9 mm); neck length 9.1 mm (*n* = 15; 7 – 14 mm), width 2 mm (*n* = 15; 1.4 – 2.8 mm); trunk length 10.7 mm (*n* = 15; 8.4 – 12.6 mm), width 14.9 mm (*n* = 15; 11.3 – 19.6 mm); posterior process length 13.4 mm (*n* = 15; 8.4 – 17.5 mm), width

14.5 mm (*n* = 15; 11.5 – 22.4 mm); egg-sac length 36.6 mm (*n* = 3; 35 – 39.2 mm), width 1 mm (*n* = 10; 0.8 – 1.8 mm).

Cephalothorax (Figs. 3.1A – C, 3.2A – B) transversely elongated, bearing 8 smooth protuberances, 1 pair of enlarged protuberances laterally, 2 pairs of protuberances off different sizes anteriorly and 1 pair of protuberances posteriorly; dorsal surface smooth; ventral surface (Figs. 3.1A – C, 3.2A) with two pairs of enlarged processes medially, i.e., antennary and maxillary processes respectively (Figs. 3.1D, 3.2C – D). Neck (Figs. 3.1A, 3.2A – B) elongated, cylindrical. Trunk (Figs. 3.1A, 3.2A – B) dorsoventrally flattened, sub-circular, wider than long with rudimentary abdomen. Posterior processes (Figs. 3.1A, 3.2A – B) divided numerously into profusely branched structures. Egg sacs (Figs. 3.1A, 3.2A – B) with multi-seriated eggs.

Cephalic region (Figs. 3.1 C – D, 3.2C – D) situated venteromedially on cephalothorax, bearing two pairs of enlarged processes (antennary and maxillary processes). Antennary processes (Figs. 3.1D, 3.2C) bear antennules and antennae, posteriorly to antennules. The mouth tube (Figs. 3.2C, 3.3B) situated between antennae. Maxillules (Figs. 3.1D, 3.2C) posterior to mouth tube. Maxillary processes (Figs. 3.1D, 3.2D) at the posterior end of cephalic region, with maxillipeds (Figs. 3.1D, 3.2D) posteromedially to maxillary processes.

Antennule (Fig. 3.2C, E) digitiform, with a pointed seta extending upwards. Antenna (Figs. 3.1E, 3.2F) bulbous, with four apical tubercles; two small tubercles, both bifid. Mouth tube (Figs. 3.2C, 3.3B) labrum (Fig. 3.3B) with blunt tubercle and labium (See Fig. 3.3B) with two tubercles (Fig. 3.3C). Maxillule (Figs. 3.1F, 3.3A) bulbous, endite with two pointed setae, palp with two setae. Maxilla represented by maxillary processes (Figs. 3.1D, 3.2D, 3.3D) with maxillary gland pores (Figs. 3.2D, 3.3E). Maxilliped (Figs. 3.1G, 3.3F) subchelate, robust corpus with prominent pointed spine at base and similar curved spine distomedially.

Male (Figs. 3.4A – H)

Body length from tip of cephalothorax appendages to the tip of abdomen 2 mm (*n* = 3; 1.9 – 2.2 mm), cephalothorax length 0.9 mm (*n* = 3; 0.8 – 1 mm), width 1 mm (*n* = 3; 0.8 – 1.1 mm); trunk length 1.1 mm (*n* = 3; 1 – 1.3 mm), width 1.1 mm (*n* = 3; 0.8 – 1.2

mm). Cephalothorax (Fig. 3.4A) less than half of total length, separated from the trunk by transverse constriction. Trunk (Figs. 3.4A, B) oval, bearing small caudal rami on posterior margin (Fig. 3.4B).

Antennule (Fig. 3.4C) 3-segmented, first segment with whip, second segment bearing solus, last segment with six apical setae (4 long and 2 short). Antenna (Fig. 3.4D) 3-segmented; sympod denticulated distoventrally; exopod shorter than endopod, 1-segmented, bulbous, denticulated apically with two long seta-like papillae and small medial tubercle; endopod 2-segmented, basal segment covered with denticles ventrally, distal segment with strong hook 1, long, thin spine 2, papilla 3, tubercle 4 with denticles (nr. 5) (see Kabata 1979). Maxillule (Fig. 3.4E) biramous; endite with 2 long, truncated and one small apical setae; palp with two apical setae of different lengths. Mandible (Fig. 3.4F) with 10 teeth slightly diminishing in size towards base. Maxilla (Fig. 3.4G) robust, two small tubercles medially, one short spine-like tubercle at the base of subchela; subchela broad at base with sharp, hook-like claw. Maxilliped (Fig. 3.4H) elongated; corpus with raised myxal area, covered with denticles, accommodating tip of claw; subchela sharply curved with tapering claw, long seta close to base, short seta basally.

3.3.1.1. b. *Sphyrion laevigatum* (Quoy & Gaimard, 1824)
Host: *Genypterus capensis* (Smith, 1847)

Locality: West coast of South Africa (Atlantic Ocean)

Material examined: 1♀ from *G*. *capensis* off the west coast

Material collected: 2♀♀ from *G*. *capensis* off the west coast (Atlantic Ocean)

Description of post-metamorphosis female (Figs. 3.5 – 3.6)

Body length from tip of cephalothorax to the tip of the abdomen 20.1 mm (*n* = 2; 18.5 – 22.4 mm), cephalothorax length 6.3 mm (*n* = 2; 5.3 – 7.2 mm), width 24.6 mm (*n* = 2; 23.4 – 25.7 mm); neck length 5.9 mm (*n* = 2; 5.7 – 6.1 mm), width 2.4 mm (*n* = 2; 2.2 – 2.6 mm); trunk length 8.3 mm (*n* = 2; 7.5 – 9.2 mm), width 12.5 mm (*n* = 2; 11.3

– 13.6 mm); posterior process length 7.2 mm (*n* = 2; 6.8 – 7.5 mm), width 11.8 mm (*n* = 2; 10.2 – 13.4 mm); egg-sac length 11.2 mm (*n* = 1), width 1.6 mm (*n* = 2; 1.4 – 1.8 mm).

Cephalothorax (Figs. 3.5A – C, 3.6A – B) transversely elongated, bearing a pair of smooth protuberances laterally, 2 pairs of protuberances of different sizes anteriorly and 1 pair of protuberances posteriorly; dorsal surface (Figs. 3.5B, 3.6B) smooth; ventral surface (Figs. 3.5A, C, 3.6A) with two pairs of enlarged processes medially, i.e., antennary and maxillary processes respectively (Fig. 3.6C). Neck (Figs. 3.5A – B, 3.6A – B) short and cylindrical. Trunk (Figs. 3.5A – B, 3.6A – B) dorsoventrally flattened, sub-circular, wider than long with rudimentary abdomen. Posterior processes (Figs. 3.5A – B, 3.6A – B) divided numerously into profusely branched structures. Egg sacs (Figs. 3.5A – B, 3.6A – B) short, with multi-seriated eggs.

Antennule (Fig. 3.6D) sub-oval base with bifid apical tips. Antenna (Fig. 3.6E) posterior to antennule (see Fig. 3.6C), sub-spherical with two apical tubercles bearing bifid tips. Maxillule (Fig. 3.6F) posterior to antenna, elongated, with pointed apical seta, palp sub-apically. Maxilla represented by maxillary processes (Fig. 3.6C). Maxilliped (Fig. 3.6G) posterior to maxillary processes (see Fig. 3.6C), subchelate; corpus broad with prominent pointed process at base and similar curved process medially close to subchela; subchela indistinctly subdivided in shaft and claw, with stout seta at base of claw; claw elongated, sharply curved with pointed tip.

Remarks:

Slight variations observed in the morphology of *S*. *laevigatum* individuals from the same host species (Figs. 3.1 – 3.3 versus Figs. 3.5 – 3.6) include mostly variations in relative sizes (Table 3.1).

Table 3.1: Comparisons between the morphology of *S*. *laevigatum* female individuals.

Traits	*S*. *laevigatum* (a) (Figs. 3.1 – 3.3)	*S*. *laevigatum* (b) (Figs. 3.5 – 3.6)

Cephalothorax	with pronounced ridge isolating cephalic area, slightly wider than trunk	without pronounced ridge, 2 times wider than the trunk
Neck	slightly shorter than trunk (9.1 mm vs 10.7 mm)	shorter than trunk (5.9 mm vs 8.3 mm)
Posterior processes	longer than the trunk, branched into irregular mass	shorter than the trunk, branched into 2 clustered masses
Egg sacs	longer than the total body length	shorter than the total body length

3.3.1.2. a. *Sphyrion lumpi* (Krøyer, 1845)

Host: *Coelorinchus trunovi* Iwamoto & Anderson, 1994

Locality: East coast of South Africa (Indian Ocean)

Material examined: 2♀♀ from *C*. *trunovi* off the east coast (Indian Ocean)

Material collected: 11♀♀ from *C*. *trunovi*, 1♀ from *Ventrifossa nasuta* (Smith, 1935) off the east coast (Indian Ocean) and 6♀♀ from *C*. *simorhynchus* Iwamoto & Anderson, 1994 off the west coast (Atlantic Ocean)

Description of post-metamorphosis female (Figs. 3.7 – 3.10)

Body length from tip of cephalothorax to the tip of the abdomen 21.8 mm (*n* = 3; 16.8 – 26.7 mm), cephalothorax length 5.5 mm (*n* = 3; 3.6 – 7.3 mm), width 11.3 mm (*n* = 3; 8.4 – 12.9 mm); neck length 10.2 mm (*n* = 3; 7.8 – 12.6 mm), width 1.8 mm (*n* = 3; 1.5 – 2 mm); trunk length 8.1 mm (*n* = 3; 5.6 – 10.1 mm), width 10.1 mm (*n* = 3; 6.4 – 12.6 mm); posterior process length 6.3 mm (*n* = 3; 4.2 – 9.7 mm), width 9.7 mm (*n* = 3; 7 – 10.2 mm); egg-sac length 19.6 mm (*n* = 1), width 1.6 mm.

Cephalothorax (Figs. 3.7A – D, 3.9A – D) transversely elongated, bearing smooth protuberances laterally (2 – 5 protuberances); dorsal surface smooth (Figs. 3.7C, 3.9A, C); ventral surface (Fig. 3.7B, D, 3.9B, D) with two pairs of enlarged processes medially, i.e., antennary and maxillary processes respectively (Figs. 3.7E, 3.9E). Neck (Figs. 3.7A – C, 3.9A – D) elongated and cylindrical. Trunk (Figs. 3.7A – C, 3.9A – D) slightly dorsoventrally flattened, sub-circular, slightly wider than long with rudimentary abdomen. Posterior processes (Figs. 3.7A – C, 3.9A – D) divided numerously into profusely branched structures. Egg sacs (Figs. 3.7A, C) long, eggs multi-seriate.

Cephalic region (Figs. 3.7E, 3.9E – F) situated ventromedially on cephalothorax, bearing two pairs of enlarged processes (antennary and maxillary processes). Antennary processes (Figs. 3.7E, 3.9E) bear antennules and antennae posteriorly to antennules. Mouth tube (Fig. 3.9F) situated between antennae, maxillules posterior to mouth tube. Maxillary processes (Figs. 3.7E, 3.9E) at posterior margin of cephalic region, with maxillipeds posteromedially to maxillary processes (Figs. 3.7E, 3.9E).

Antennule (Figs. 3.7F, 3.10A) bulbous with bifid apical tips. Antenna (Figs. 3.8A, 3.10B) bulbous, with two small tubercles apically. Labrum with a small tubercle (Fig. 3.10C) and labium with two small, pointed tubercles (Figs. 3.8B, 3.9F, 3.10D). Maxillule (Figs. 3.8C, 3.10E) bulbous, endite bearing two setae with small bifid palp sub-apically. Maxilla represented by maxillary processes (see Figs. 3.7E, 3.9E) with maxillary gland pores. Maxilliped (Figs. 3.8D, 3.10F) subchelate, robust corpus with prominent pointed spine at base and similar curved spine distomedially.

3.3.1.2. b. *Sphyrion lumpi* (Krøyer, 1845)

Host: *Allocyttus verrucosus* (Gilchrist, 1906)

Locality: West coast of South Africa (Atlantic Ocean)

Material examined: 2♀♀ from *A. verrucosus* off the west coast (Atlantic Ocean)

Material collected: 3♀♀ from *Allocyttus verrucosus* off the west coast (Atlantic Ocean) and 2♀♀ from *Mesovagus antipodum* (Hubbs & Iwamoto, 1977) off the south coast (Atlantic Ocean)

Description of post-metamorphosis female (Figs. 3.11 – 3.12)

Body length from tip of cephalothorax to the tip of the abdomen 66.8 mm (*n* = 2; 53.6 – 79.9 mm), cephalothorax length 8.9 mm (*n* = 2; 7.6 – 10.2 mm), width 16.5 mm (*n* = 2; 15 – 17.9 mm); neck length 38.9 mm (*n* = 2; 23.8 – 53.9 mm), width 2.2 mm (*n* = 2; 2.1 – 2.2 mm); trunk length 19.1 mm (*n* = 2; 18.5 – 19.6 mm), width 19.7 mm (*n* = 2; 15.5 – 23.9mm); posterior process length 16.7 mm (*n* = 2; 15.1 – 18.2 mm), width 23.8 mm (*n* = 2; 22.4 – 25.1 mm); egg-sac width 2.5 mm (*n* = 1).

Cephalothorax (Figs. 3.11A – D, 3.12A, C) transversely elongated, bearing smooth, slightly bifid protuberances laterally; dorsal surface smooth (Figs. 3.11C, 3.12C); ventral surface (Figs. 3.11D, 3.12A) with two pairs of enlarged processes medially, i.e., antennary and maxillary processes respectively (Figs. 3.12B). Neck (Figs. 3.11A – B) elongated and cylindrical. Trunk (Figs. 3.11A – B) dorsoventrally flattened, sub-circular slightly wider than long with rudimentary abdomen. Posterior processes (Figs. 3.11A – B) divided numerously into profusely branched structures. Egg sacs (Figs. 3.11A – B) long, with multi-seriate eggs.

Antennule (Fig. 3.12D) bulbous with bifid apical tips. Antenna (Figs. 3.12E, F) posterior to antennule (see Fig. 3.12B), subspherical and robust, with two small apical bifid tubercles. Maxillule (Fig. 3.12G) endite tipped with two pointed apical setae; small palp sub-apically with pointed seta. Maxilla represented by maxillary processes (see Fig. 3.12B). Maxilliped (Fig. 3.12H) posterior to maxillary processes (see Fig. 3.12B), subchelate; corpus robust with prominent pointed spine at base and similar curved spine below subchela; subchela not distinctly subdivided in shaft and claw, sharply curved with elongated pointed tip.

Remarks:

Slight variations in morphology between *S. lumpi* female individuals from different host species were observed (Figs. 3.7 – 3.10 versus Figs. 3.11 – 3.12), which mainly include differences in the morphometry and the structure of the cephalothorax protuberances. The variation in relative sizes in *S. lumpi* specimens can possibly be attributed to the depth range of the hosts in marine waters. According to Gómez et al. (2010), the difference in vertical distribution of the hosts can lead to allopatric

speciation of the ectoparasites. The increase in the size of *S*. *lumpi* in higher depths, might be an adaptation to hypoxic conditions (for large surface area) and strong turbulence similar as discussed of *Lophoura* species (Gómez et al. 2010). *Sphyrion lumpi* (Figs. 3.7 – 3.10) with smaller sizes were found infecting *C*. *trunovi* (occurring at depths of 421 – 552 m) and *C*. *simorhynchus* (occurring at depths of 139 – 986 m) which inhabit comparatively shallower waters whereas, *S*. *lumpi* (Figs. 3.11 – 3.12) with larger sizes were collected from *A*. *verrucosus* (occurring at depths of 0 – 1800 m) and *M*. *antipodium* (occurring at depths of 846 - 1300 m) both inhabiting deeper waters (Froese and Pauly 2022).

3.3.1.3. *Sphyrion quadricornis* Gaevskaya & Kovaleva, 1984

Host: *Coelorinchus simorhynchus* Iwamoto & Anderson, 1994 (Gadiformes: Macrouridae)

Locality: West coast of South Africa (Atlantic Ocean)

Material examined: 3♀♀ from and 2♂♂ from *C*. *simorhynchus* off the west coast

Material collected: 25♀♀ and 4♂♂ all from *C*. *simorhynchus* off the west coast (Atlantic Ocean), 17♀♀ from *C*. *trunovi* off the east coast (Indian Ocean) and 1♀ from *Saurida undosquamis* (Richardson, 1848) off the east coast (Indian Ocean)

Description of post-metamorphosis female (Figs. 3.13 – 3.15)

Body length from tip of cephalothorax to the tip of the abdomen 28.5 mm (*n* = 6; 25.8 – 35.8 mm), cephalothorax length 4.9 mm (*n* = 6; 3.6 – 5.6 mm), width 11 mm (*n* = 6; 8.4 – 12.6 mm); neck length 16.6 mm (*n* = 6; 1 – 22,4 mm), width 1.1 mm (*n* = 6; 0.84 – 1.26 mm); trunk length 7 mm (*n* = 6; 6.2 – 8.4 mm), width 8.1 mm (*n* = 6; 5.6 – 9.8 mm); posterior processes length 7.9 mm (n = 6; 6.58 – 9.8 mm); posterior processes width 8.8 mm (*n* = 6; 7.4 – 10.1 mm); egg-sac length 19.4 mm (*n* = 3; 14 – 23.1 mm), width 1.3 mm (*n* = 6; 1.1 – 1.5 mm).

Cephalothorax (Figs. 3.13A – C, 3.14A – B) transversely elongated, bearing bifid protuberances laterally (Figs. 3.13A – C, 3.14A – B); dorsal surface smooth (Figs. 3.13C, 3.14A); ventral surface (Figs. 3.13A – B, 3.14B) with two pairs of enlarged processes medially, i.e., antennary and maxillary processes respectively (Figs. 3.13D,

3.14C). Neck (Figs. 3.13A – B, 3.14A – B) elongated and cylindrical. Trunk (Figs. 3.13A – B, 3.14A – B) slightly dorsoventrally flattened, sub-circular, slightly wider than long with rudimentary abdomen. Posterior processes (Figs. 3.13A – B, 3.14A – B) divided numerously into profusely branched structures. Egg sacs (Figs. 3.13A – B, 3.14A – B) long, multi-seriate.

Cephalic region (Figs. 3.13D, 3.14C) situated ventromedially on cephalothorax, bearing two pairs of enlarged processes (antennary and maxillary processes). Antennary processes (Figs. 3.13D, 3.14C) bears antennules, antennae posteriorly to antennules. Mouth tube (See Figs. 3.14D, 3.15D) situated between antennae. Maxillules posterior to the mouth tube (see Fig. 3.14D). Maxillary processes (Figs. 3.13D, 3.14C) at posterior margin of cephalic region. Maxillipeds posteromedially to maxillary processes (Figs. 3.13D, 3.14C).

Antennule (Figs. 3.13E, 3.14E) bulbous with bifid apical tubercle. Antenna (Figs. 3.13F, 3.14F) bulbous, with two small tubercles. Labrum (see Fig. 3.15C) with a tubercle (Fig. 3.15C – D) bearing a bifid tip; labium (Fig. 3.15C) with small, pointed tubercles (Figs. 3.13G, 3.15C, E). Maxillule (Figs. 3.13H, 3.15A) bulbous, endite bearing two setae with small bifid palp sub-apically. Maxilla represented by maxillary processes (see Figs. 3.13D, 3.14C) with maxillary gland pores (Figs. 3.14C, 3.15B). Maxilliped (Figs. 3.13I, 3.15F) subchelate; robust corpus with prominent pointed spine at base and similar curved spine distomedially.

Male (Figs. 3.16A – I)

Body length from tip of cephalothorax to the tip of abdomen 2.3 mm (*n* = 3; 1.8 – 2.5 mm), cephalothorax length 1.1 mm (*n* = 3; 0.9 – 1.2 mm), width 1.2 mm (*n* = 3; 0.9 – 1.5 mm); trunk length 1.2 mm (*n* = 3; 1.1 – 1.3 mm), width 1.2 mm (*n* = 3; 0.8 – 1.2 mm). Cephalothorax (Fig. 3.16A) less than half of total length, separated from trunk by transverse constriction. Trunk (Fig. 3.16A) egg-shaped, bearing small caudal rami on posterior margin (Fig. 3.16B) near maxillipeds. Antennule (Fig. 3.16C) 3-segmented, first segment with the whip, second segment bearing solus, last segment with six apical setae (4 long and 2 short). Antenna (Fig. 3.16D) 3-segmented; exopod shorter than endopod, 1-segmented, bulbous, with denticles on margin, with two long

papillae; endopod 2-segmented; basal segment with a patch denticles ventrally; distal segment with strong hook 1, thin long spine 2, papilla 3, tubercle 4, with two spinules (nr. 5?) (see Kabata 1979), medial margin with denticles. Maxillule (Fig. 3.16E) biramous; endite with 2 long, truncated and one small apical setae, palp with 2 apical setae of different lengths. Mandible (Fig. 3.16F) with 12 teeth. Maxilla (Fig. 3.16G) robust, situated on medial process, medial area with three tubercles, basal one with 2 small setae; subchela broad at base with hook-like claw. Maxilliped (Figs. 3.16H, I) slender and elongated; corpus with raised myxal area, covered with denticles, accommodating tip of claw; subchela sharply curved with tapering claw, long seta close to base of claw, short seta medially at base of subchela.

3.3.2. Key to the adult females of *Sphyrion* species (according to current study):

1a. Neck shorter than length of trunk, multiple cephalothorax protuberances ……..... *S. laevigatum*

1b. Neck longer than trunk..……..…….2

2a. Cephalothorax symmetrical, with pair of bifid protuberances laterally.…… ……..…… *S. quadricornis*

2b. Cephalothorax variable (symmetrical or asymmetrical), unforked …...…...*S. lumpi*

3.4. Discussion

The studied specimens belong to genus *Sphyrion* due to possession of hammer-shaped cephalothoraces with protuberances and processes that mostly obscure the majority of the appendages. The trunks are mostly dorsoventrally flattened, sub-circular to pyriform, with reduced abdomens, branched posterior processes and multi-seriated egg sacs. Males are rarely encountered. All males found were attached to a female. However, *Sphyrion* males are very distinct from females, by being dwarf males possessing small bodies and distinct appendages similar to those of the lernaeopodid males (Kabata 1979).

Sphyrion lumpi has been reported many times, including Wilson (1919), Kabata (1979), Ho and Kim (1989) and Moran and Piasecki (1994). Moran and Piasecki (1994) provided complete descriptions for both the adult male and post-metamorphosis female. However, little information is available regarding the morphology of the appendages of *S*. *laevigatum* and *S*. *quadricornis*. The appendages of the studied post-metamorphosis *S. lumpi* females resemble those in Moran and Piasecki (1994) with small differences. The cuticular knob reported on the maxillary processes (reported as maxilla in Moran and Piasecki (1994); see Figs. 3.16, 4.22), was not observed in the studied specimens, although the maxillary process (Fig. 3.9E) and maxillary gland pore (Fig. 3.9E) were observed. Since Sphyriidae females transform with age, this may be due to a difference in age of the specimens with the studied *S*. *lumpi* specimens that may be older and thus already lost the cuticular knob observed in Moran and Piasecki (1994). Like the cuticular knob in Moran and Piasecki (1994) (see Fig. 4.22), the maxilla of *Tripaphylus* species (another sphyriids) in Benz and Boxshall (2017) (see Figs. 2B, 3A) and Dippenaar (2018) (Figs. 2E, 11B, 13B) is also represented by a knob situated on the maxillary spheres/processes.

Regardless of few other variations between *Sphyrion* post-metamorphosis females, the number of protuberances on cephalothorax plays a major role in differentiating these species. *Sphyrion laevigatum* possesses a cephalothorax with up to eight protuberances, *S*. *quadricornis* with four protuberances on the cephalothorax and *S*. *lumpi* with two (sometimes up to five, see Figs. 3.9C – D) protuberances (Table 3.2). Although this character seems to be determined by age, since it may slightly vary among individuals of the same species, for instance, some post-metamorphosis females of *S*. *laevigatum* possess six protuberances (see Figs. 3.6 A – C), and some *S*. *lumpi* post-metamorphosis females possess up to five protuberances (see Figs. 3.9C – D).

In this study, redescriptions and illustrations of all *Sphyrion* females are provided including the morphology of all appendages even though these are difficult to see clearly due to the reduced sizes and presence of host mucus. *Sphyrion lumpi* (Figs. 3.7F, 3.9F, 3.10A) and *S*. *quadricornis* (Figs. 3.13E, 3.14D, E) possess a bulbous

antennule with the apical tubercle bearing bifid tips while *S*. *laevigatum* possesses a digitiform antennule (Figs. 3.2C, E) with apical seta extending upwards. The bulbous process of the antennule seems reduced significantly in *S*. *laevigatum* (see Figs. 3.2A, 3.6D). All females have maxillules with a bifid palp, situated basally in *S*. *laevigatum* (Fig. 3.3A) and sub-apically in *S*. *lumpi* (Figs. 3.8C, 3.10E) and *S*. *quadricornis* (Figs. 3.13H, 3.15A). The maxillary spheres of all the *Sphyrion* species have maxillary gland pores, without the cuticular knob observed in Moran and Piasecki (1994), which might have been lost during development.

This study provides the detailed descriptions and illustrations of the morphology and appendages of the males of *S*. *laevigatum* (Figs. 3.4A – H) and *S*. *quadricornis* (Figs. 3.16A – I) (supplemented by Dippenaar and Sebone 2022). The studied male appendages bear a close resemblance to those of lernaeopodid males (Kabata 1979). The appendages of *S*. *laevigatum*, *S*. *quadricornis* and *S*. *lumpi* (See Moran and Piasecki 1994) are mostly similar. The antennule in *S*. *lumpi* (Figs. 2.5 – 2.6 in Moran and Piasecki 1994) is 4-segmented while 3-segmented in both *S*. *laevigatum* (Fig. 3.4C) and *S*. *quadricornis* (Fig. 3.16C). The antennae differ mostly regarding the armature of process 4 on the endopod, which in *S*. *laevigatum* (Fig. 3.4D) is armed with smooth denticles, in *S*. *lumpi* (Figs. 2.7 – 2.8 in Moran and Piasecki 1994) with pointed denticles and in *S*. *quadricornis* (Fig. 3.16D) with one bigger and one smaller denticles. The mandible of all three species varies only in the number of teeth (i.e. *S*. *laevigatum* (Fig. 3.4F) with 10 teeth, *S*. *quadricornis* (Fig. 3.16F) with 12 teeth and *S*. *lumpi* (Fig. 2.9 in Moran and Piasecki 1994) with 6 teeth). The maxillule of both *S*. *laevigatum* (Fig. 3.4E) and *S*. *quadricornis* (Fig. 3.16E) palp possess two setae, whereas only one seta was observed in *S*. *lumpi* (Fig. 2.10 in Moran and Piasecki 1994). The maxillae of all the species are similar, differing slightly in terms of the armature of tubercles on the corpus medial margin (see Dippenaar and Sebone 2022).

Sphyrion lumpi has been reported from 31 host species in 15 families worldwide, including three host species from the west coast (Atlantic Ocean) off South Africa (Barnard 1955; Kensley and Grindley 1973). *Sphyrion lumpi* from the Indian Ocean off South Africa constitutes a new geographical record, as well as new host records for *C*. *trunovi*, *V*. *nasuta*, *A. verrucosus* (new family record, i.e. Oreosomatidae), *C*. *simorhynchus* and *M*. *antipodum*. This report of *S*. *quadricornis* constitutes a new

geographical record from the Indian and Atlantic Oceans off South Africa as well as new host records for *C*. *simorhynchus*, *C*. *trunovi and S*. *undosquamis* (new family record, i.e. Synodontidae).

According to Gaevskaya and Kovaleva (1984) and Ho and Kim (1989), *Sphyrion* species have a high preference for deep-water hosts. From the 30 reported host species of *S*. *lumpi*, 28 species (four bathypelagic, eight benthopelagic, six bathydemersal and 10 demersal) inhabit deep waters. All four of the reported host species of *S*. *quadricornis* (three bathydemersal and one benthopelagic) and the six host species of *S*. *laevigatum* (two benthopelagic and four bathydemersal) inhabit deep waters. Therefore, confirming *Sphyrion* species preference for deep-water hosts, although they also inhabit pelagic (*Sebastes norvegicus* (Ascanius, 1772) and *Mola mola* (Linnaeus, 1758) both hosts for *S*. *lumpi*) host species. The preference for deep-water hosts may be the reason why *Sphyrion* species are not often reported and therefore there may still be other species that has not been encountered and described yet.

In future more deep-water fish species should be examined to find *Sphyrion* species in order to really determine the number of valid species and thus the diversity of this genus. Additionally, molecular data should be used from freshly collected and identified specimens to determine whether the observed morphological variations are intraspecific variation or maybe interspecific variations.

Table 3.2: Comparison of general habitus of different *Sphyrion* species (according to the current study).

Character	*Sphyrion laevigatum*	*Sphyrion lumpi*	*Sphyrion quadricornis*
Host species	*Genypterus capensis*	*Coelorinchus trunovi*, *Ventrifossa nasuta*, *Allocyttus verrucosus*, *Coelorinchus simorhynchus* and *Mesovagus antipodum*	*Coelorinchus simorhynchus*, *Coelorinchus trunovi and Saurida undosquamis*

Geographical record	Atlantic Ocean (off South Africa)	Atlantic Ocean (off South Africa) and Indian Ocean (off South Africa)	Atlantic Ocean (off South Africa) and Indian Ocean (off South Africa)
Cephalothorax	6 – 8 lateral protuberances on cephalothorax margin	known to lack bifurcations, with only 2 protuberances 2 – 5 protuberances observed	2 bifurcated lateral protuberances
Cephalothorax vs trunk width	cephalothorax 1.4 – 2 times wider than trunk	cephalothorax 1 – 0.8 times wider than trunk	cephalothorax about 1.4 times as wide as trunk
Neck length vs trunk length	shorter or equal to trunk length neck 0.9 – 0.7 times as long as trunk	longer than trunk neck 1.3 – 2.1 times longer than trunk	longer than trunk neck 2.4 times longer than trunk
Trunk shape (trunk length vs trunk width)	trunk wider than long trunk 0.7 – 0.7 times as long as wide	trunk wider than long trunk 0.8 – 1 times as long as wide	trunk wider than long trunk 0.9 times as long as wide
Posterior processes	branched, grape-like posterior processes, 1.3 – 0.9 times as long as trunk	branched, grape-like posterior processes, 0.8 – 0.9 times as long as trunk	branched, grape-like posterior processes, 1.1 times as long as trunk
Abdomen	rudimentary	rudimentary	rudimentary
Eggs	elongated egg sacs with multi-seriate eggs	elongated egg sacs with multi-seriate eggs	elongated egg sacs with multi-seriate eggs

Figure 3.1: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) post-metamorphosis female. A. general habitus, cephalothorax ventral view, trunk dorsal view; B. cephalothorax, posteroventral view; C. cephalothorax, anteroventral view; D. cephalothorax, cephalic region with appendages; E. antenna; F. maxillule; G. maxilliped. Scale bars: A – D = 1 mm and E – G = 10 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mx1 – maxillule, mp – maxillary processes, mxp – maxilliped)

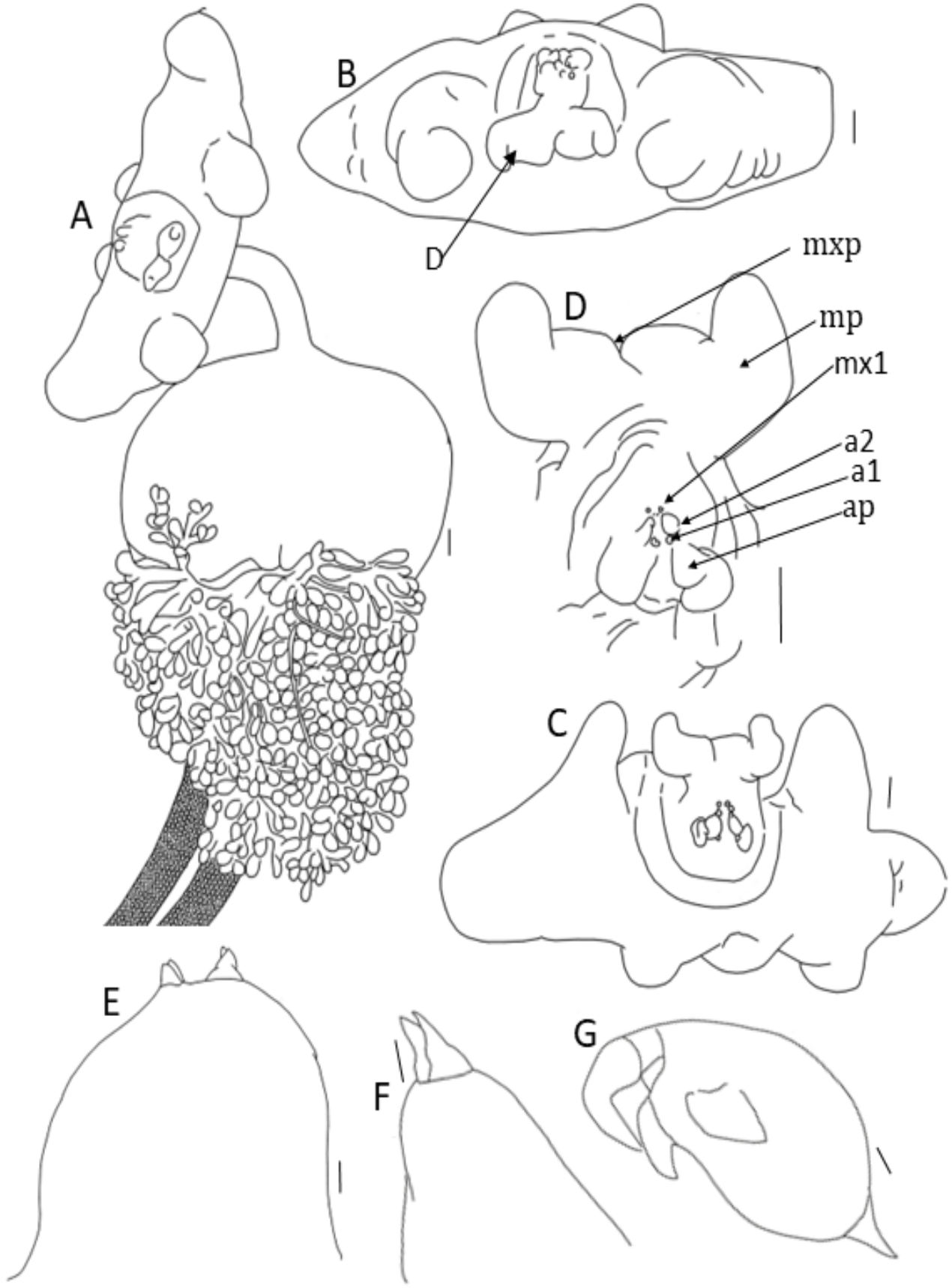

A
B
D
mxp
mp
mx1
a2
a1
ap
C
E
F
G

Figure 3.2: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) post-metamorphosis female. A. general habitus, cephalothorax ventral view, trunk dorsal view; B. general habitus, cephalothorax posterodorsal view, trunk ventral view. Scanning electron micrographs: C. cephalic region, ventral; D. cephalic region, posteroventral view; E. antennule; F. antenna. Scale bars: A, B, D = 1 mm; C = 500 µm; B; E = 10 µm; F = 100 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mt – mouth tube, mx1 – maxillule, mp – maxillary processes, mgp – maxillary gland pore, mxp – maxilliped).

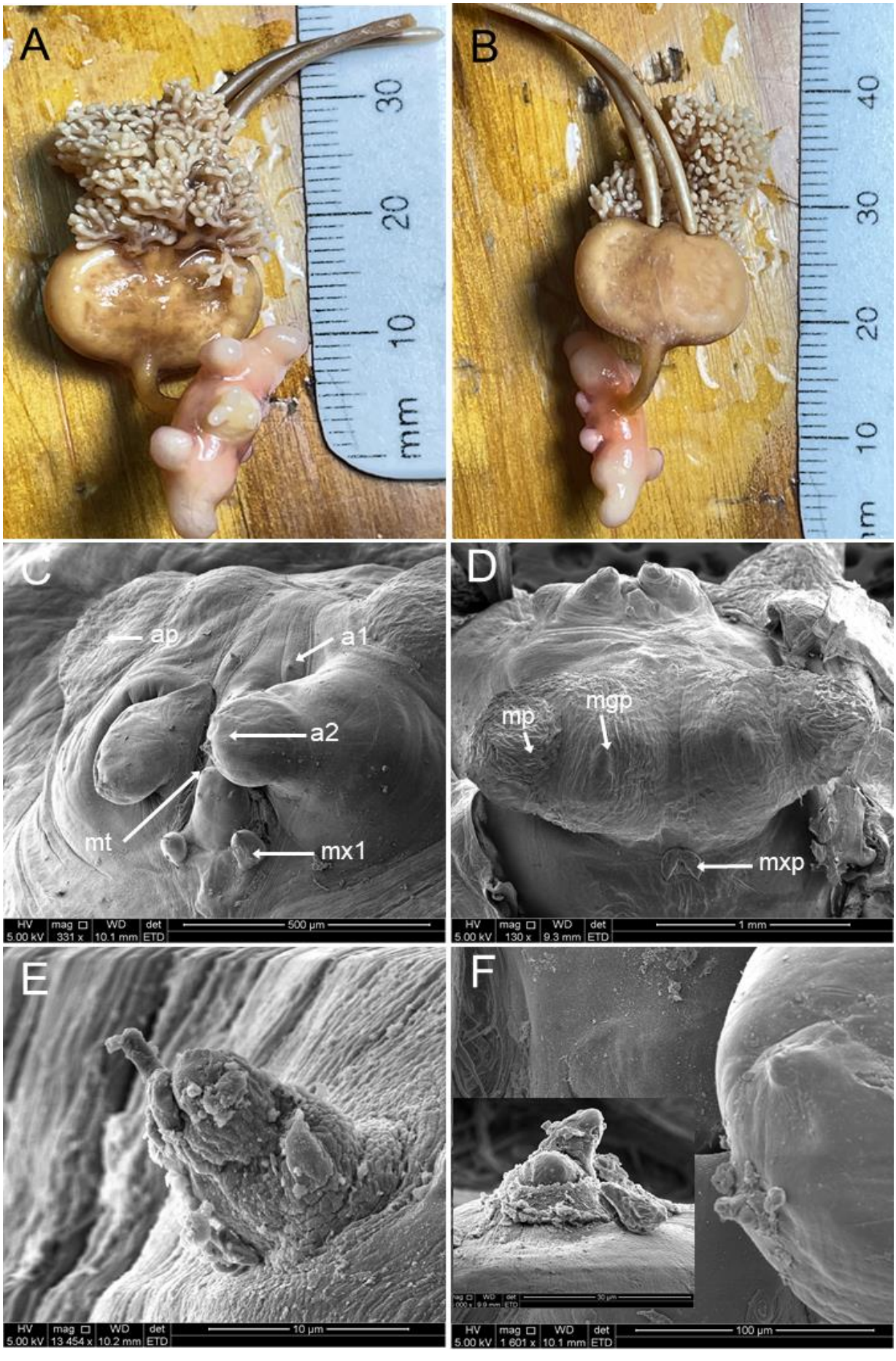

A
B
C
ap
a1
a2
mt
mx1
HV 5.00 kV
mag 331 x
WD 10.1 mm
det ETD
500 µm
D
mp
mgp
mxp
HV 5.00 kV
mag 130 x
WD 9.3 mm
det ETD
1 mm
E
HV 5.00 kV
mag 13 454 x
WD 10.2 mm
det ETD
10 µm
F
HV 5.00 kV
mag 1 601 x
WD 10.1 mm
det ETD
100 µm

Figure 3.3: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) post-metamorphosis female. Scanning electron micrographs: A. maxillule; B. mouth tube; C. labium tubercle; D. maxillary processes with maxillipeds posteromedially; E. maxillary gland pore; F. maxillipeds. Scale bars: A, E = 50 µm; B = 300 µm; C = 10 µm; D = 500 µm; F = 100 µm. (lbr – labrum, lbm – labium).

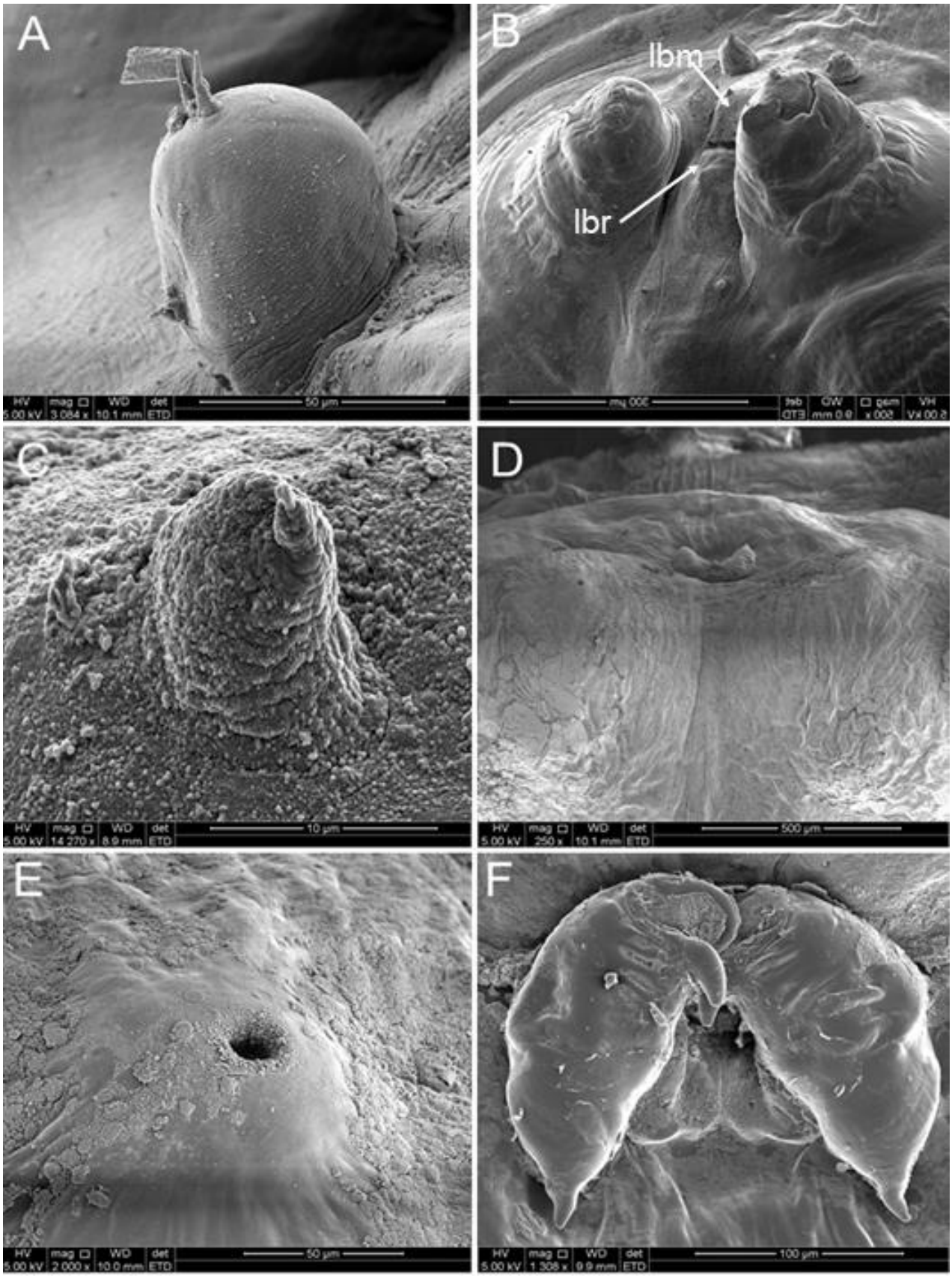

A
B
lbm
lbr
C
D
E
F

Figure 3.4: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) male. A. general habitus, lateral view; B. trunk; C. antennule; D. Antenna; E. maxillule; F. mandible; G. maxilla; H. maxilliped. Scale bars: A – H = 10 µm.

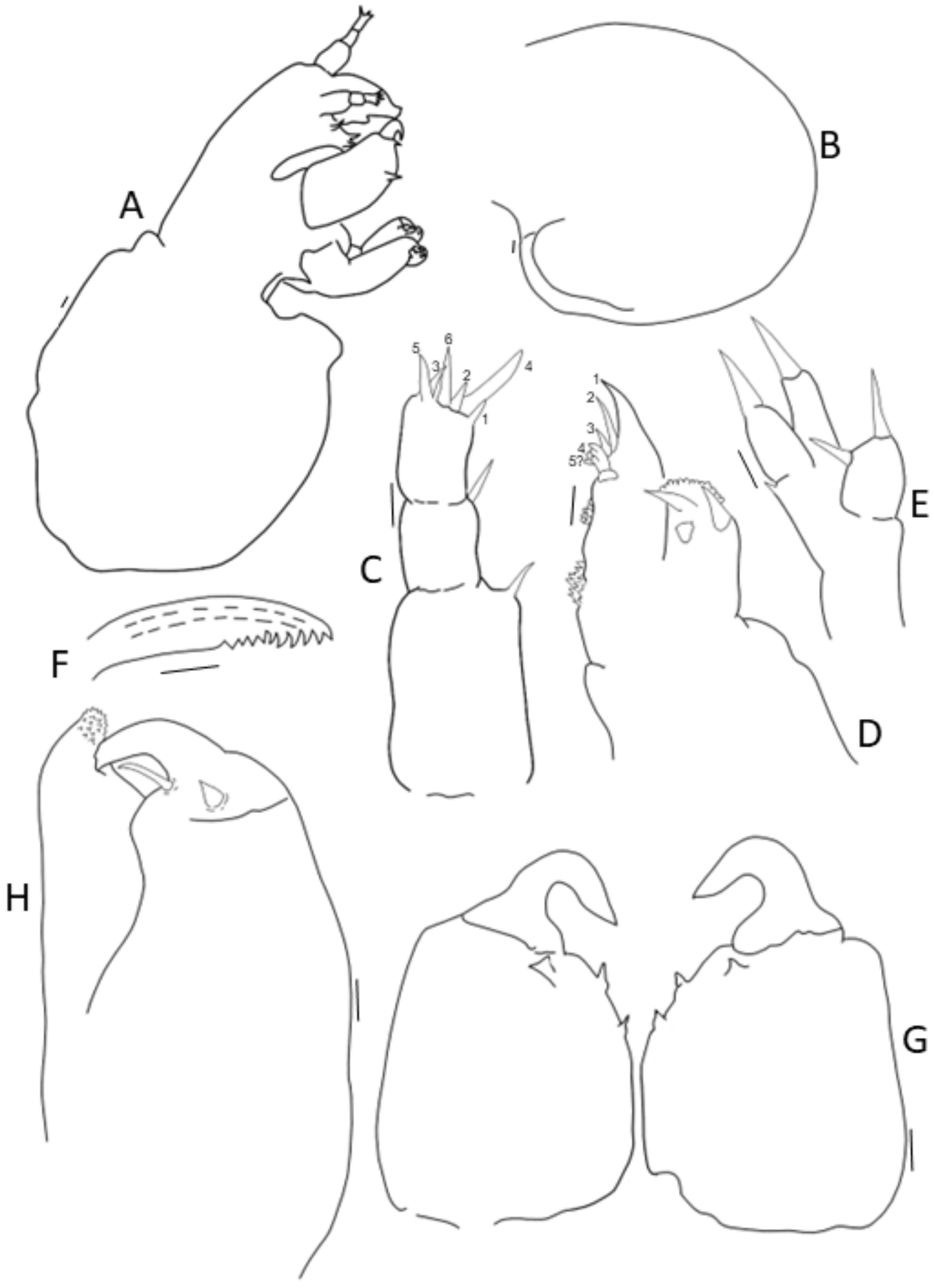

A
B
C
D
E
F
G
H
1
2
3
4
5
6
1
2
3
4
5?

Figure 3.5: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) post-metamorphosis female. A. general habitus, cephalothorax ventral view, trunk dorsal view; B. general habitus, cephalothorax anterodorsal view, trunk ventral view; C. cephalothorax, ventral view. Scale bars: A – C = 1 mm.

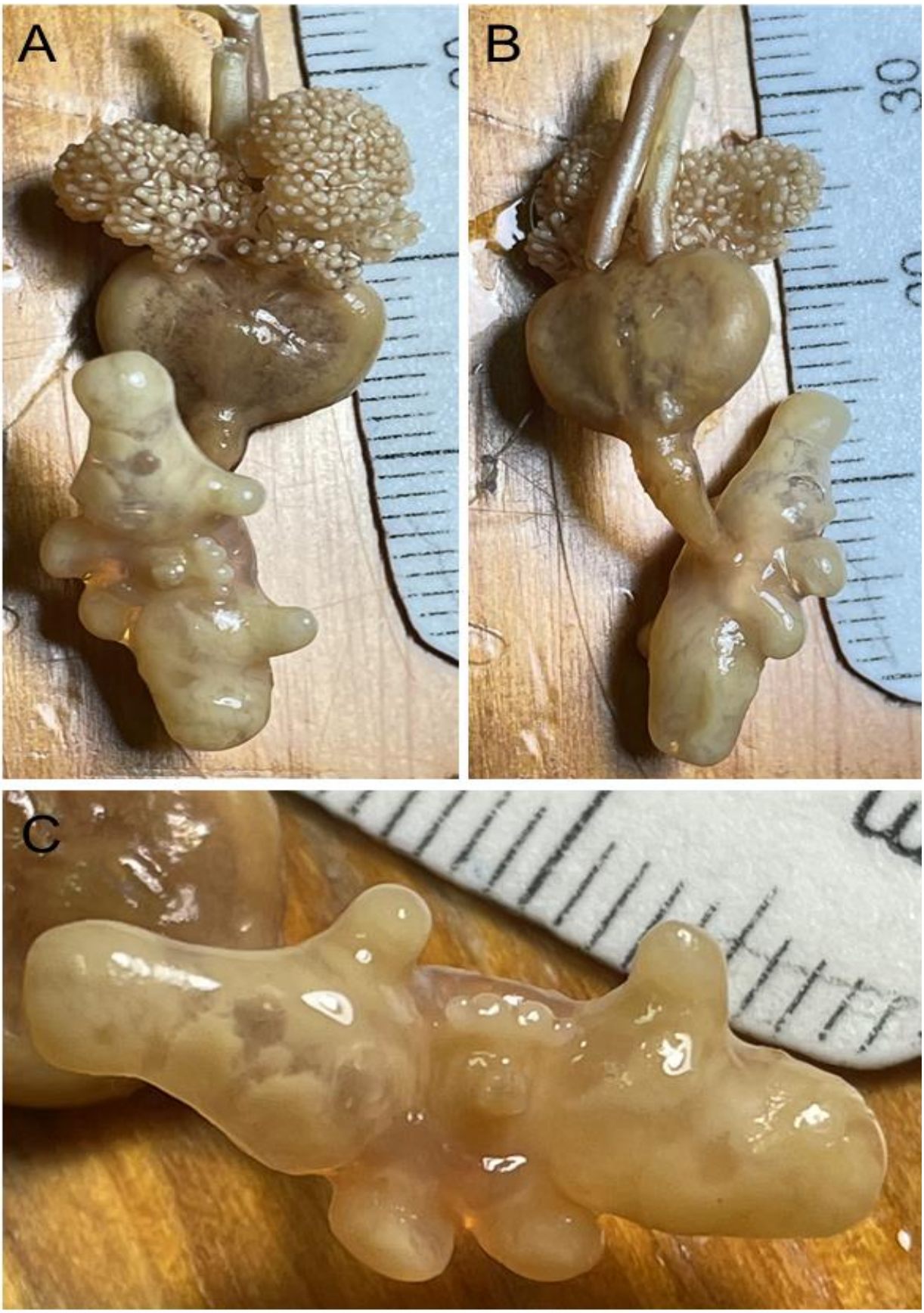

A
B
C
30

Figure 3.6: *Sphyrion laevigatum* (Quoy & Gaimard, 1824) post-metamorphosis female. A. general habitus, cephalothorax ventral view, trunk dorsal view; B. general habitus, cephalothorax dorsal view, trunk ventral view; C. cephalothorax, region with appendages; D. antennule; E. antenna; F. maxillule; G. maxilliped. Scale bars: A – C = 1 mm and E – G = 10 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mx1 – maxillule, mp – maxillary processes, mxp – maxilliped).

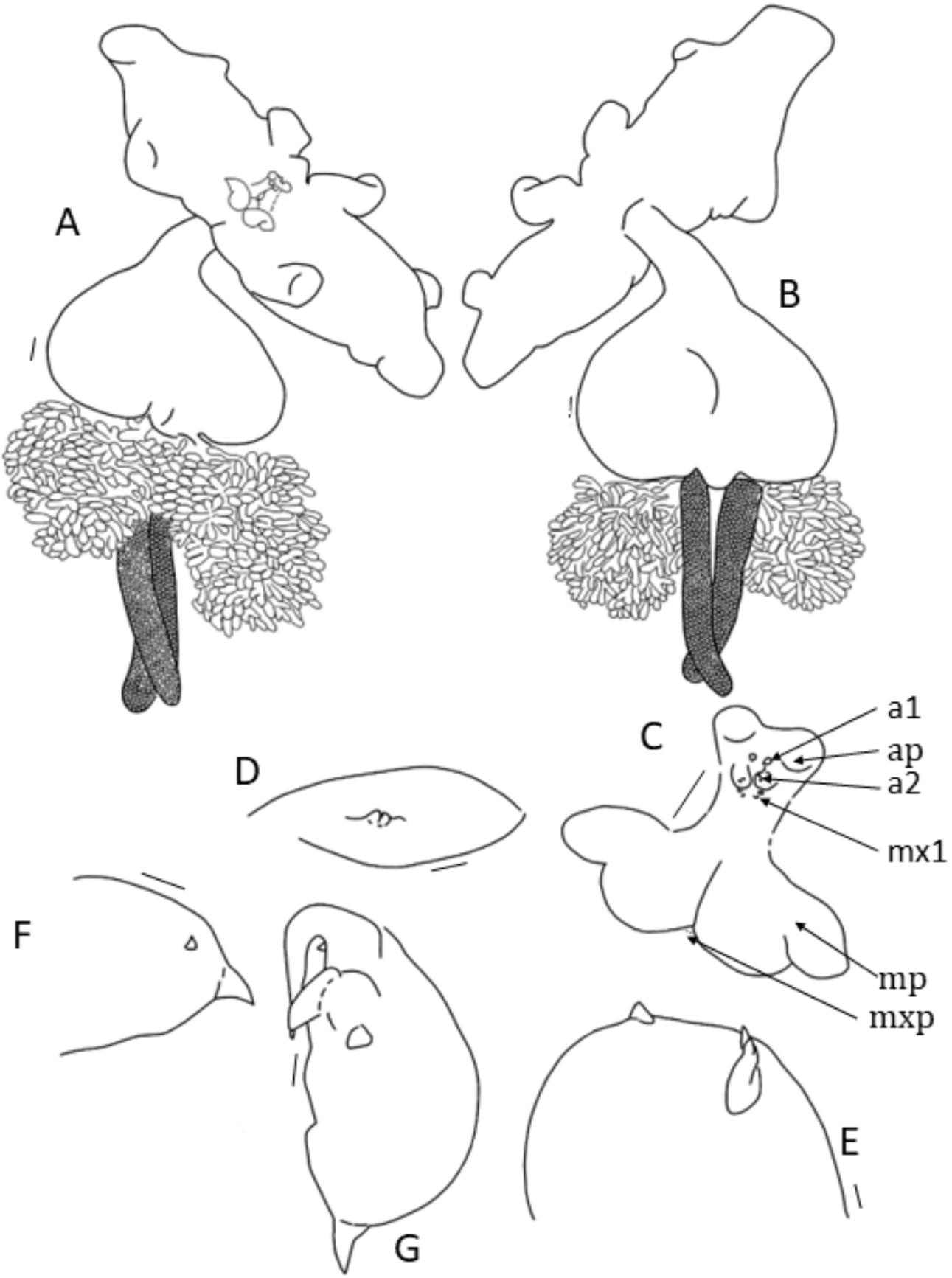

A
B
C
a1
ap
a2
mx1
mp
mxp
D
E
F
G

Figure 3.7: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. A. general habitus, cephalothorax lateral view, trunk ventral view; B. general habitus, cephalothorax ventral view, trunk lateral view; C. general habitus, cephalothorax dorsal view, trunk dorsal view; D. cephalothorax, ventral view; E. cephalothorax, cephalic region with appendages; F. antennule. Scale bars: A – E = 1 mm and F = 10 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mx1 – maxillule, mp – maxillary processes, mxp – maxilliped).

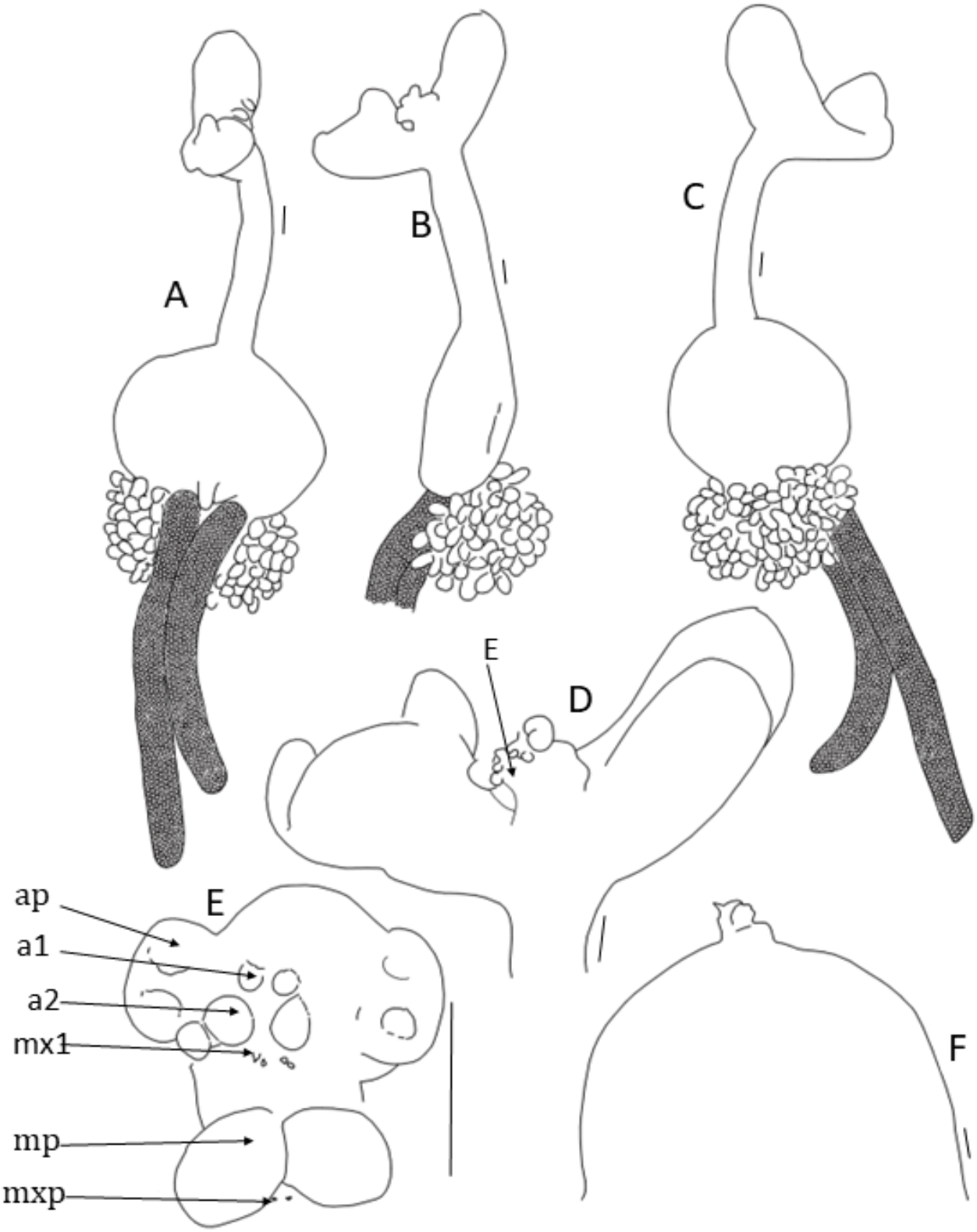

A
B
C
D
E
E
ap
a1
a2
mx1
mp
mxp
F

Figure 3.8: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. A. antenna; B. labium tubercle; C. maxillule; D. maxilliped. Scale bars: A – D = 10 µm.

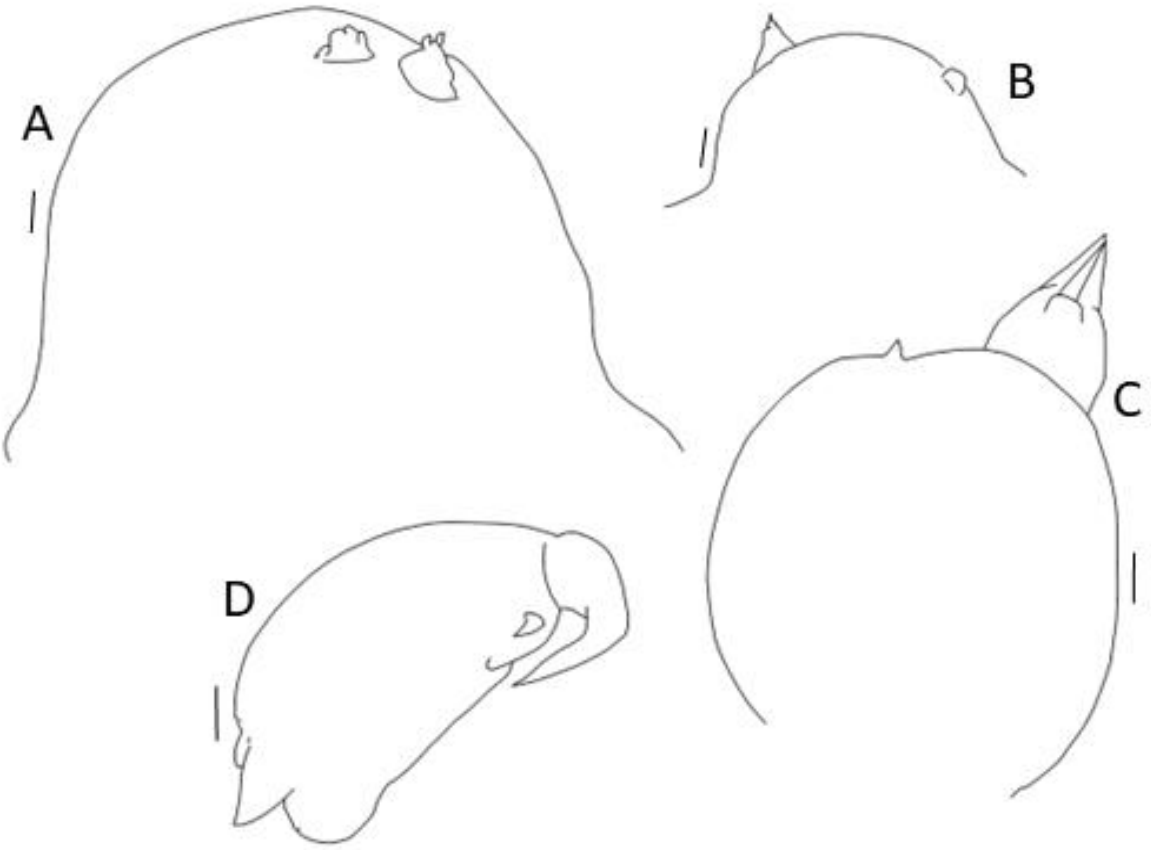

A
B
C
D

Figure 3.9: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. A. general habitus, cephalothorax dorsal view, trunk dorsal view; B. general habitus, cephalothorax ventral view, trunk ventrolateral view; C. general habitus, cephalothorax dorsal view, trunk ventral view; D. general habitus, cephalothorax ventral view, trunk dorsal view. Scanning electron micrographs: E. cephalic region, ventrolateral view; F. cephalic region; E. Scale bars: A – E = 1 mm; F = 300 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mt – mouth tube, mx1 – maxillule, mp – maxillary processes, mgp – maxillary gland pore, mxp – maxilliped).

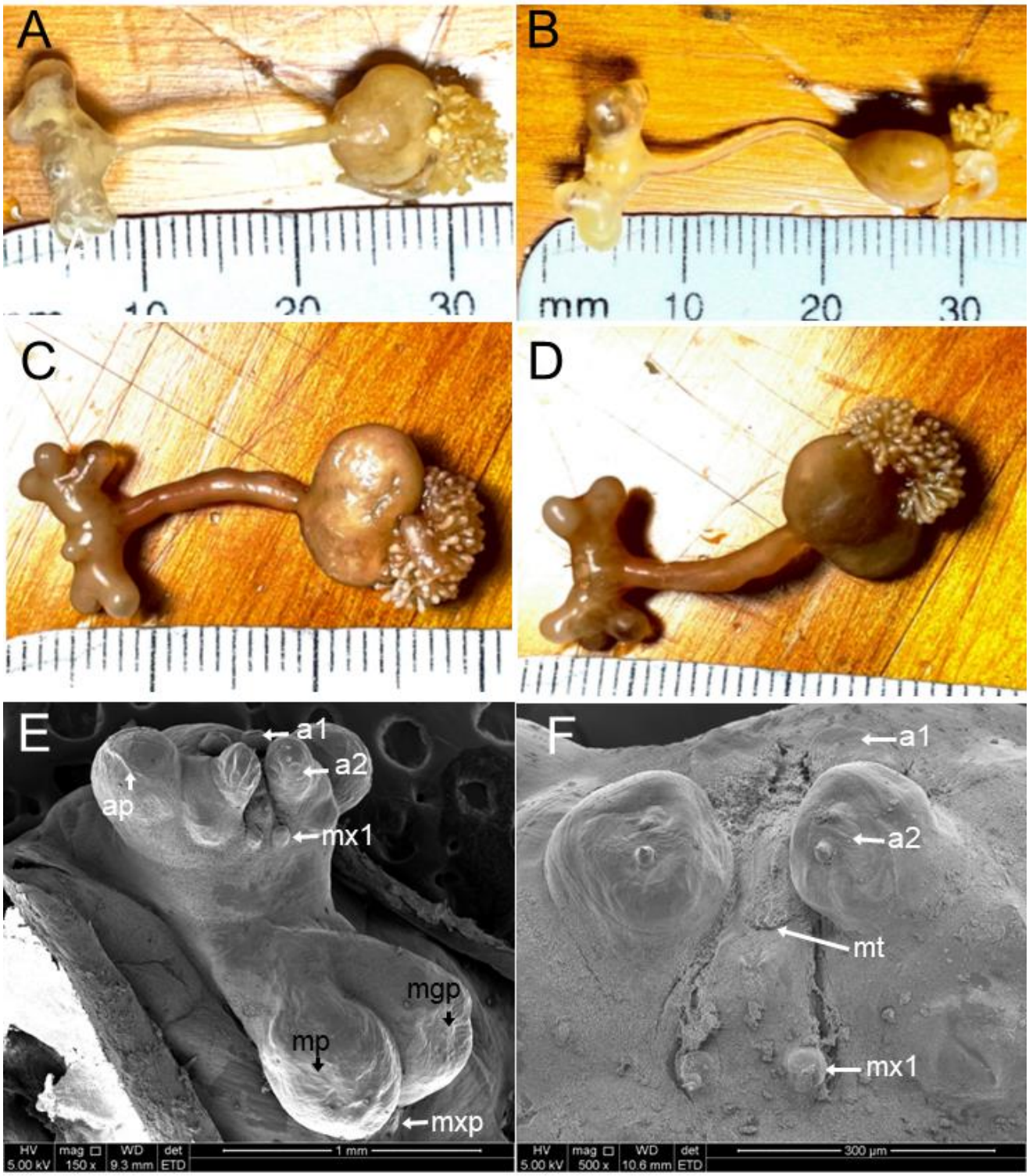

A
B
mm 10 20 30
C
D
E
a1
a2
ap
mx1
mgp
mp
mxp
HV 5.00 kV
mag 150 x
WD 9.3 mm
det ETD
1 mm
F
a1
a2
mt
mx1
HV 5.00 kV
mag 500 x
WD 10.6 mm
det ETD
300 µm

Figure 3.10: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. Scanning electron micrographs: A. antennule; B. antenna; C. mouth tube with labrum tubercle; C. labium tubercle; E. maxillule; F. maxillipeds. Scale bars: A, D = 10 µm; B = 50 µm; C, F = 100 µm; E = 20 µm. (lbr – labrum).

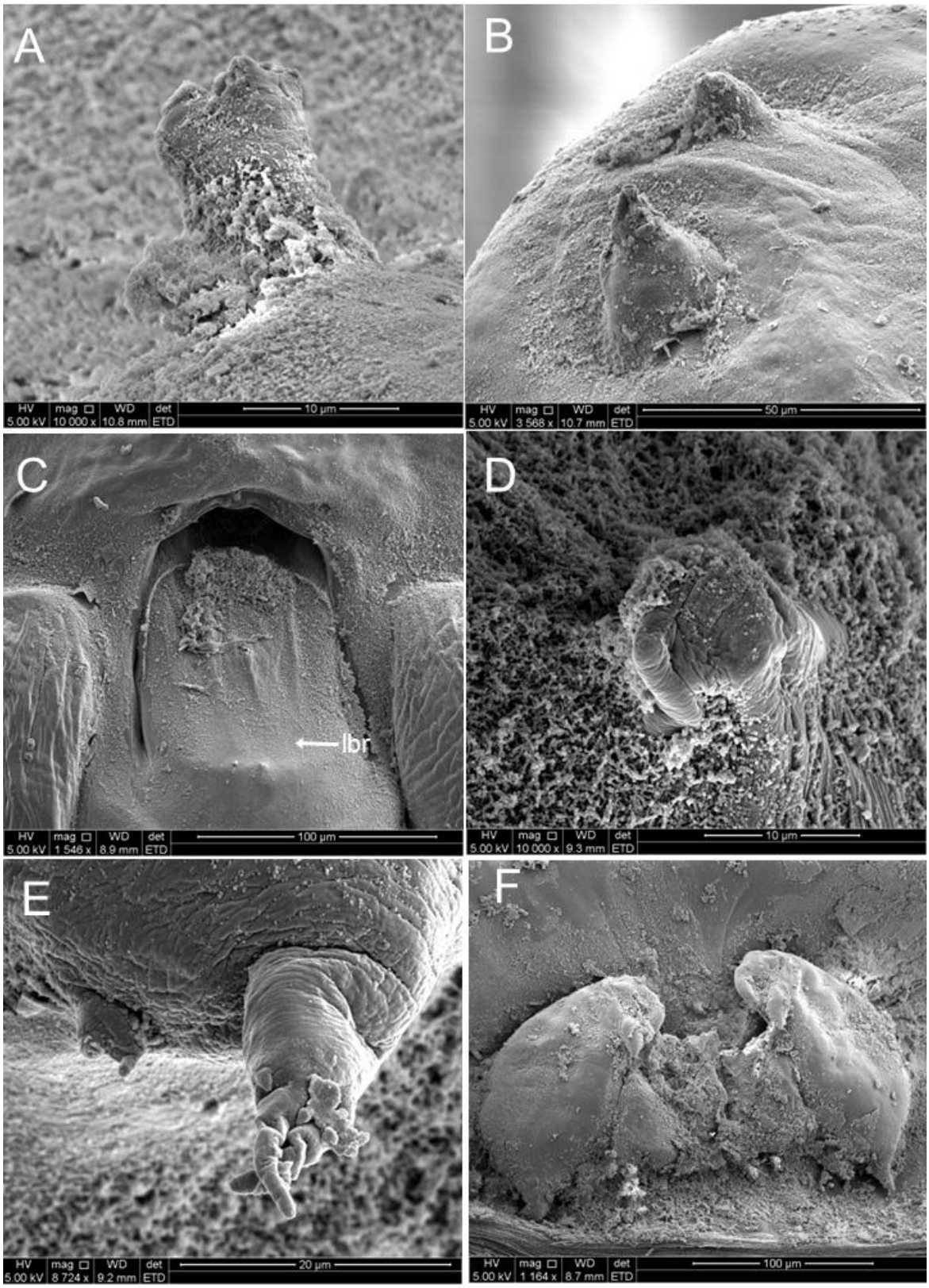

A
HV 5.00 kV
mag 10 000 x
WD 10.8 mm
det ETD
10 µm
B
HV 5.00 kV
mag 3 568 x
WD 10.7 mm
det ETD
50 µm
C
lbr
HV 5.00 kV
mag 1 546 x
WD 8.9 mm
det ETD
100 µm
D
HV 5.00 kV
mag 10 000 x
WD 9.3 mm
det ETD
10 µm
E
HV 5.00 kV
mag 8 724 x
WD 9.2 mm
det ETD
20 µm
F
HV 5.00 kV
mag 1 164 x
WD 8.7 mm
det ETD
100 µm

Figure 3.11: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. A. general habitus, cephalothorax lateral view, trunk ventral view; B. general habitus, cephalothorax lateral view, trunk dorsal view; C. cephalothorax, anterior view; D. cephalothorax, ventral view. Scale bars: A – D = 1 mm.

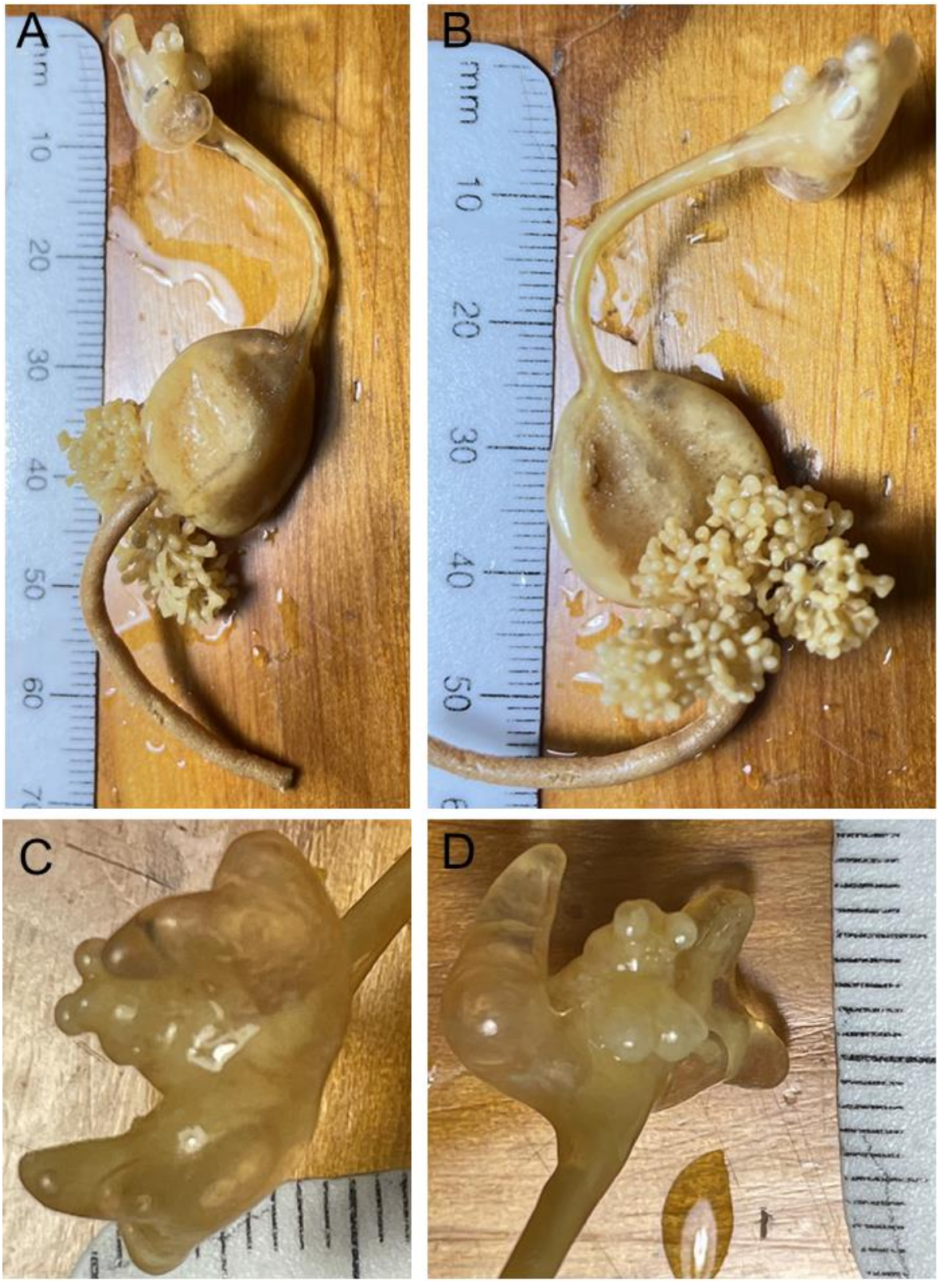

A
B
C
D

Figure 3.12: *Sphyrion lumpi* (Krøyer, 1845) post-metamorphosis female. A. cephalothorax, ventral view; B. cephalic area; C. cephalothorax, dorsal view; D. antennule; E. antenna; F. antenna; G. maxillule; H. maxilliped. Scale bars: A – C =1 mm, D – H = 10 µm. (ap – antennary processes, a2 – antenna, mp – maxillary processes, mxp – maxilliped).

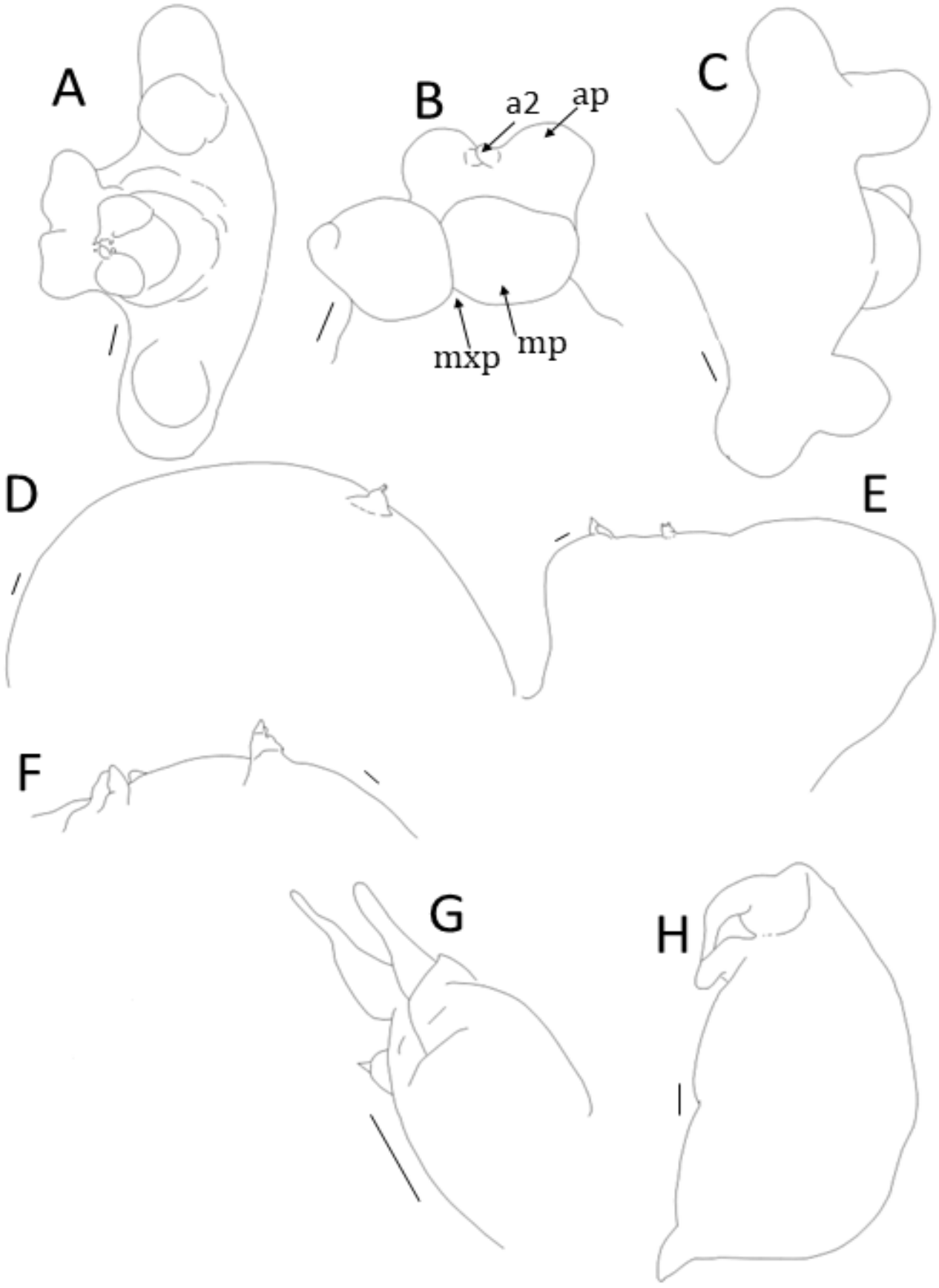

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Figure 3.13: *Sphyrion quadricornis* Gaevskaya & Kovalenva, 1984 post-metamorphosis female. A. general habitus, cephalothorax ventral view, trunk dorsal view; B. cephalothorax, ventral view; C. cephalothorax, dorsal view; D, cephalothorax, cephalic region with appendages; E. antennule; F. antenna; G. labium tubercle; H. maxillule; I. maxilliped. Scale bars: A – D = 1 mm and E – I = 10 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mx1 – maxillule, mp – maxillary processes, mxp – maxilliped).

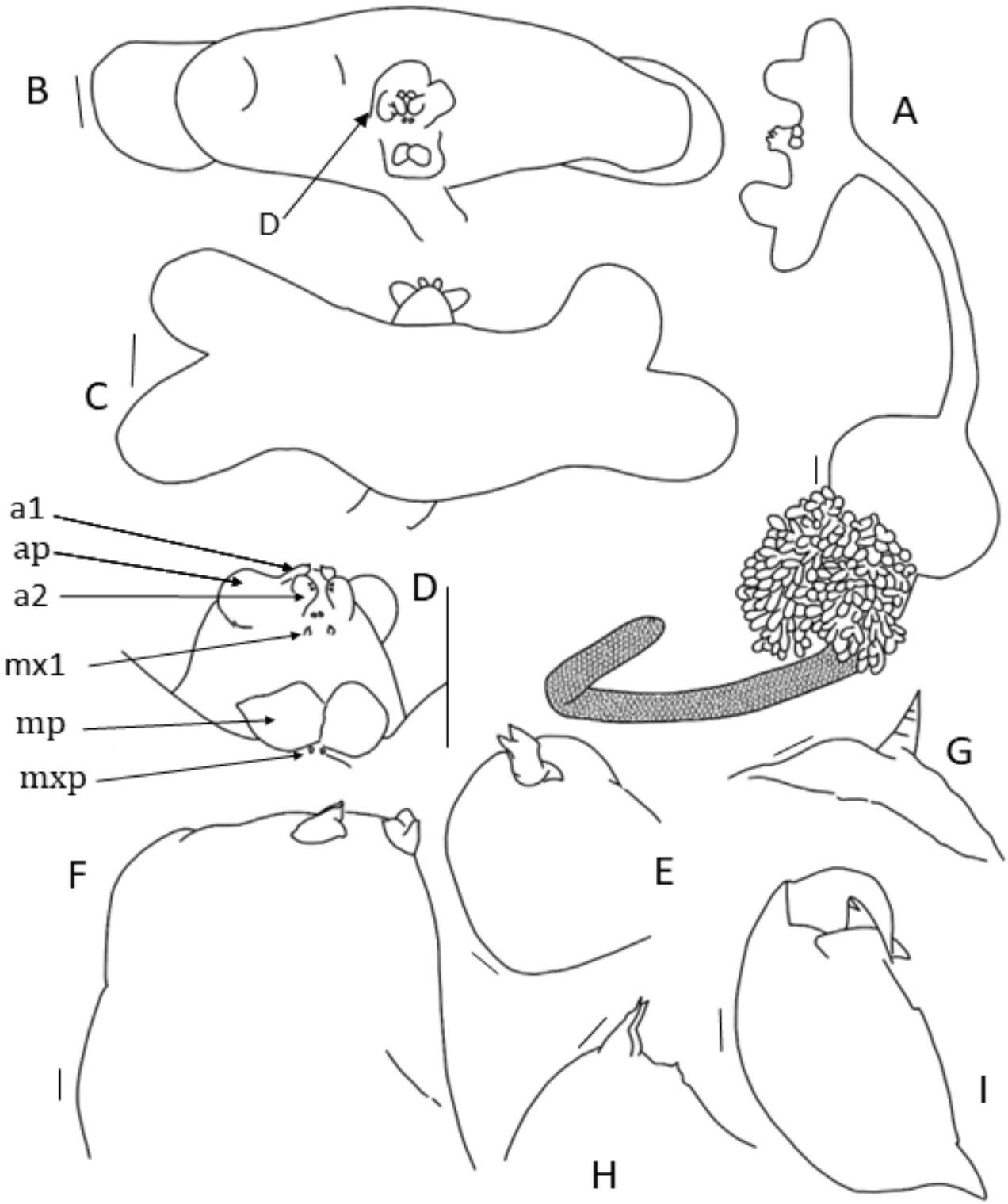
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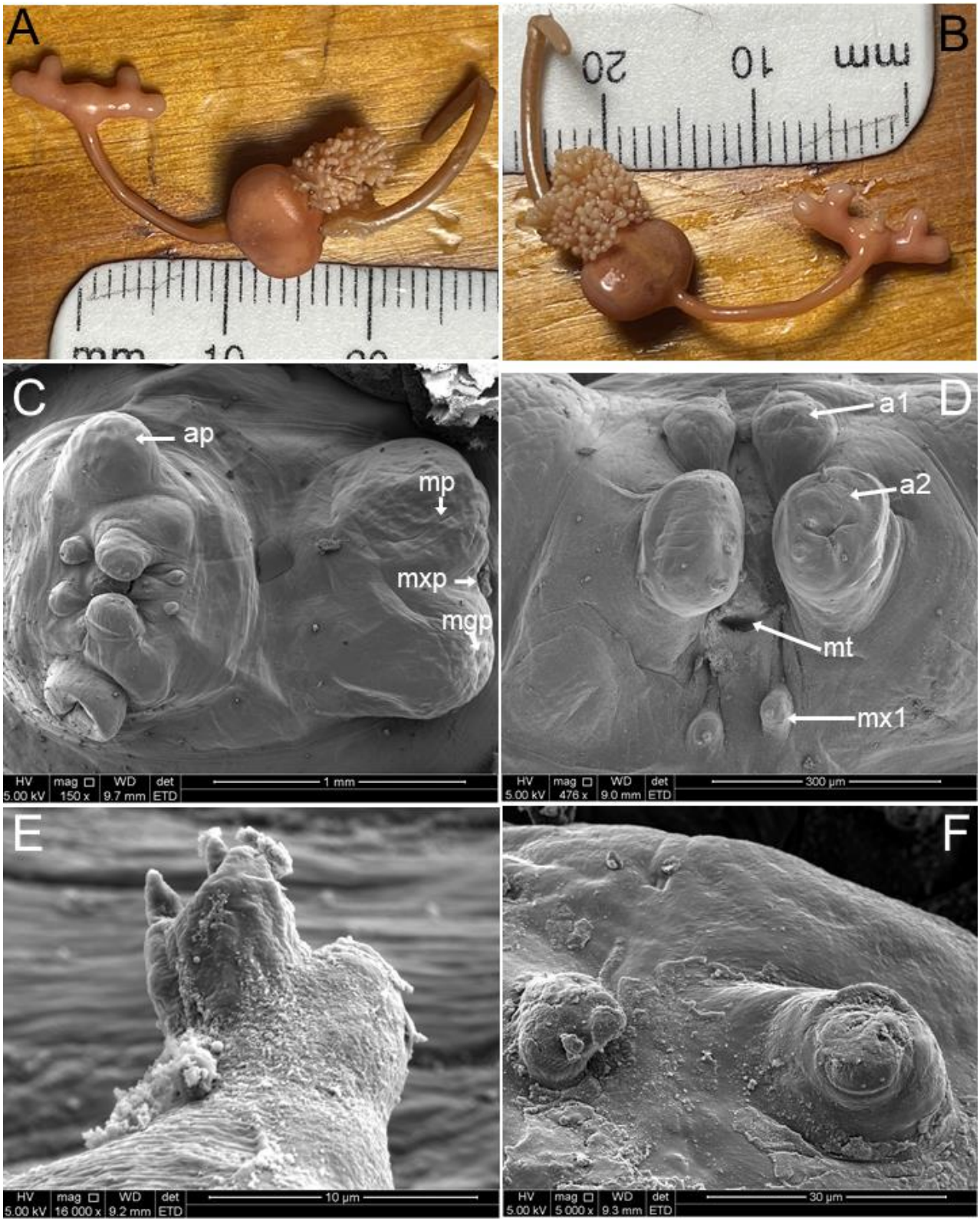
Figure 3.14: *Sphyrion quadricornis* Gaevskaya & Kovalenva, 1984 post-metamorphosis female. A. general habitus, cephalothorax dorsal view, trunk ventral view; B. general habitus, cephalothorax ventral view, trunk dorsal view. Scanning electron micrographs: C. cephalic area, ventral view; D. cephalic area, ventral view; E. antennule; F. antenna. Scale bars: A – C = 1 mm; D = 300 µm; E = 10 µm; F = 30 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mt – mouth tube, mx1 – maxillule, mxp – maxilliped, mp – maxillary processes, mgp – maxillary gland pore).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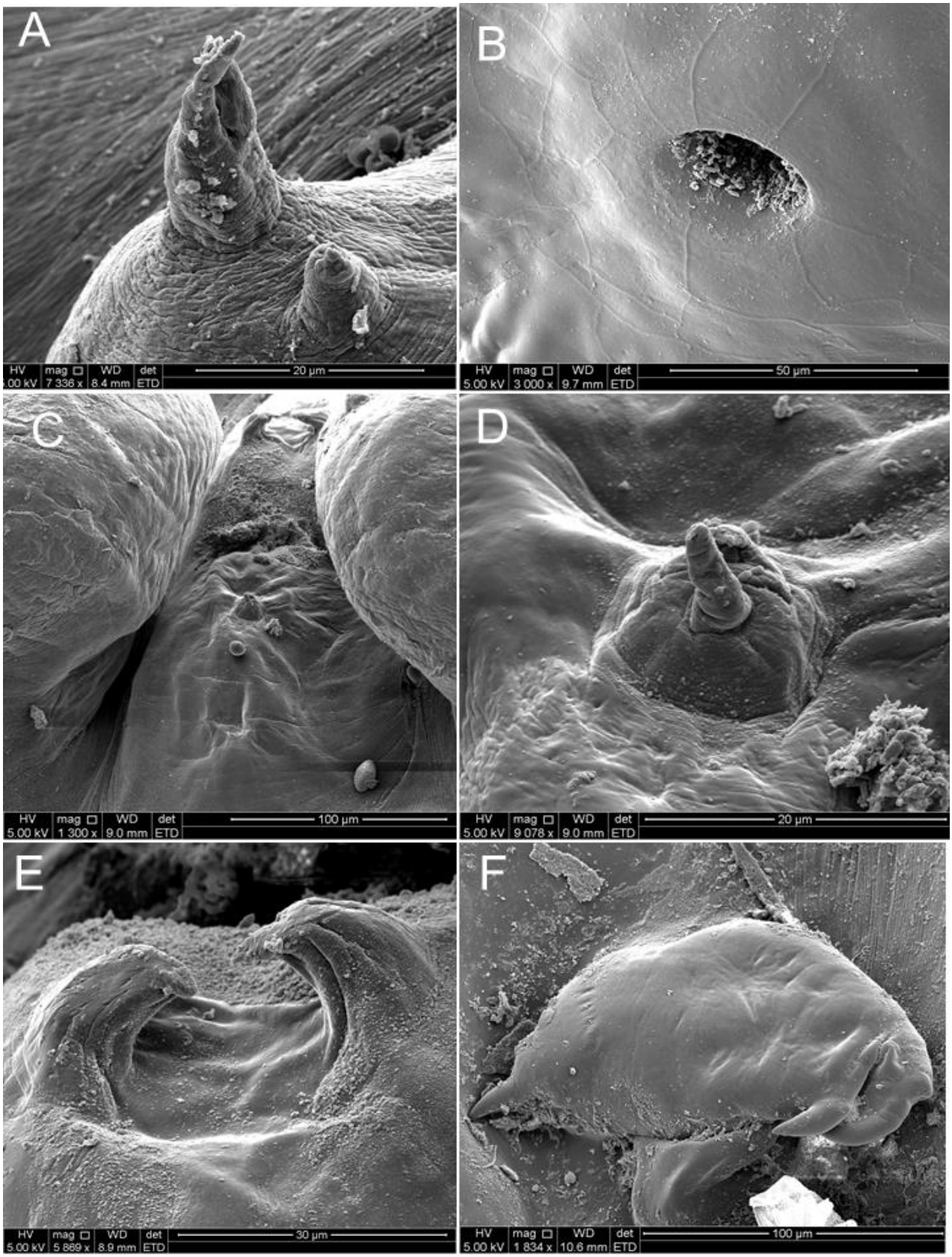
mag 16 000 x
WD 9.2 mm
det ETD
10 µm
F
HV 5.00 kV
mag 5 000 x
WD 9.3 mm
det ETD
30 µm

Figure 3.15: *Sphyrion quadricornis* Gaevskaya & Kovalenva, 1984 post-metamorphosis female. Scanning electron micrographs: A. maxillule; B. maxillary gland pore; C. mouth tube; D. labrum tubercle; E. labium tubercles; F. maxilliped. Scale bars: A, D = 20 µm; B = 50 µm; C, F = 100 µm; E = 30 µm. (ap – antennary processes, a1 – antennule, a2 – antenna, mt – mouth tube, mx1 – maxillule, mxp – maxilliped, mp – maxillary processes, mgp – maxillary gland pore).


A
HV .00 kV | mag 7 336 x | WD 8.4 mm | det ETD | 20 µm
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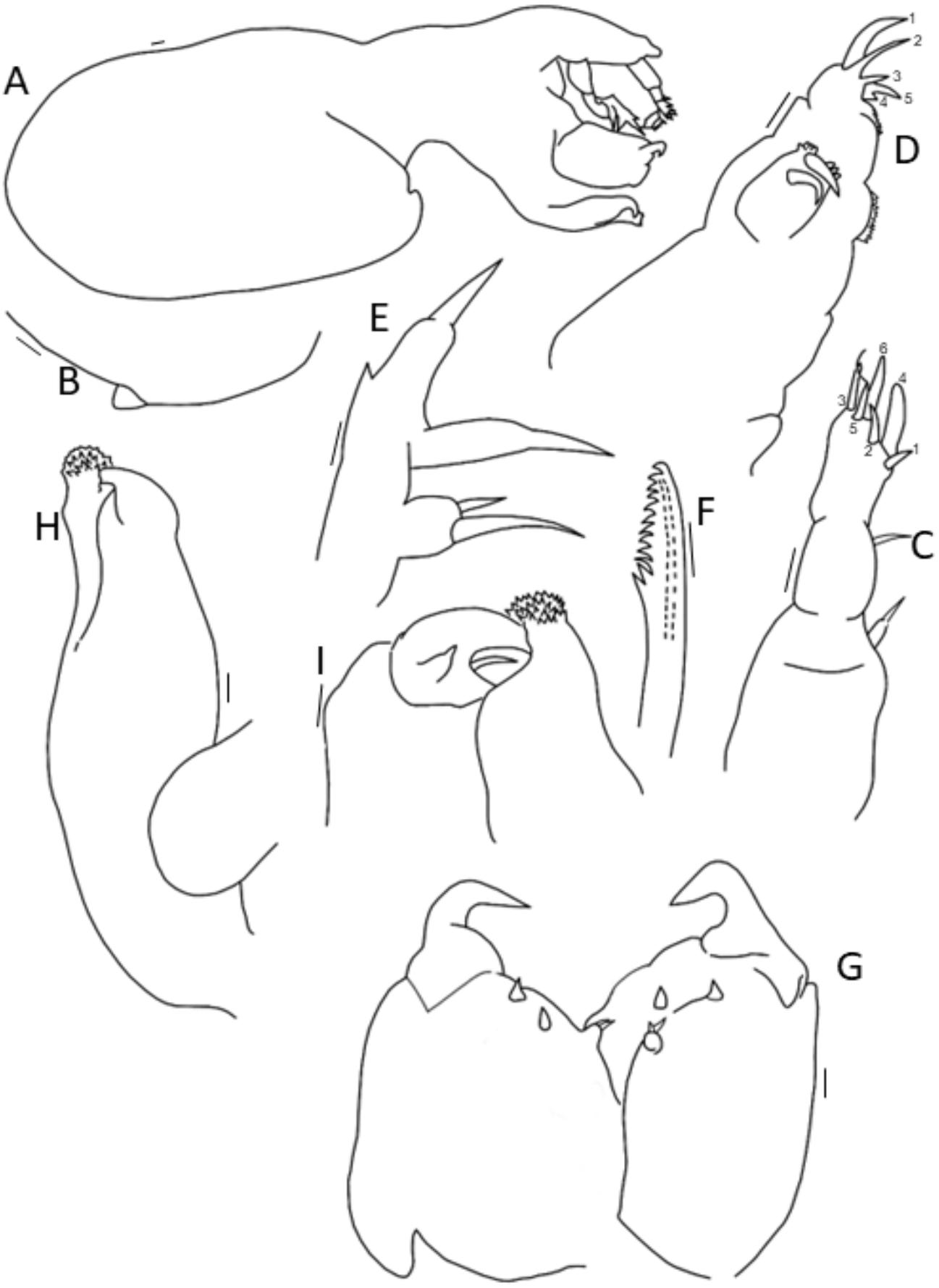
HV 5.00 kV | mag 3 000 x | WD 9.7 mm | det ETD | 50 µm
C
HV 5.00 kV | mag 1 300 x | WD 9.0 mm | det ETD | 100 µm
D
HV 5.00 kV | mag 9 078 x | WD 9.0 mm | det ETD | 20 µm
E
HV 5.00 kV | mag 5 869 x | WD 8.9 mm | det ETD | 30 µm
F
HV 5.00 kV | mag 1 834 x | WD 10.6 mm | det ETD | 100 µm

Figure 3.16: *Sphyrion quadricornis* Gaevskaya & Kovaleva, 1984 male. A. general habitus, lateral view; B. abdomen with caudal rami; C. antennule; D. antenna; E. maxillule; F. mandible; G. maxilla; H. maxilliped; I. maxilliped. Scale bars: A – I = 10 µm.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CHAPTER 4: Genus *Lophoura* Kölliker in Gegenbaur, Kölliker & Müller, 1853

4.1. Introduction

Lophoura is the largest genus in Sphyriidae with 19 accepted species according to World of Copepods (Walter and Boxshall 2022). These species include *Lophoura edwardsi* Kölliker, 1853; *L*. *bouvieri* (Quidor, 1912); *L*. *cornuta* (Wilson C.B., 1919); *L*. *gracilis* Wilson C.B., 1919; *L*. *cardusa* (Leigh-Sharpe, 1934); *L*. *tripartita* (Wilson C.B., 1935); *L*. *caparti* (Nuñes-Ruivo, 1962); *L*. *laticervix* Hewitt, 1964; *L*. *magna* Szidat, 1971; *L*. *elongata* Kensley & Grindley, 1973; *L*. *szidati* Stadler, 1978; *L*. *pentaloba* Ho, 1985; *L*. *tetraphylla* Ho, 1985; *L*. *bipartita* Ho & Kim I.H., 1989; *L*. *tetraloba* Ho & Kim I.H., 1989; *L*. *ventricula* Ho & Kim I.H., 1989; *L*. *simplex* Boxshall, 2000; *L*. *unilobulata* Castro-Romeo & Gonzalez, 2009; *L*. *brevicollum* Gómez, Deets, Kalman & Morales-Serna, 2010). Adult *Lophoura* females possess highly modified bodies, similar to other sphyriid females. The cephalothoraces are longitudinally elongated, with the cephalic appendages on the anterior end, separated from the rest of the cephalothorax by a circular groove. The elongated part of the cephalothorax is sometimes smooth and sometimes transversely wrinkled (Kabata 1979; Ho and Kim 1989). The anterior end of the neck possesses a holdfast organ with the structure and shape varying from one species to another. The elongated neck is heavily chitinized and smooth or has small knobs. The posterior part of the neck expands into the dorsoventrally flattened trunk with shapes varying from almost heart-shaped to sub-quadrangular, with an abdomen at the posterior end (Wilson 1919; Kabata 1979; Ho and Kim 1989). Posterior processes are laterally attached to the abdomen by a porous peduncle, bearing numerous long stalks (Stadler 1978). The egg sacs are long, with multi-seriate eggs. Females of different species are mainly differentiated by the morphology of the holdfast organ on the neck. However, the neck length, the trunk shape, the size of the abdomen and the shape and position of the posterior processes are also considered as distinguishing features of each species (Wilson 1919; Kabata 1979; Ho and Kim 1989) (Table 4.1). *Lophoura* males are dwarf males resembling those of the family Lernaeopodidae (Kabata 1979; Ho and Kim 1989).

Lophoura species are mesoparasites attached in the muscles of teleosts and have been reported from five teleosts families worldwide including Epigonidae, Macrouridae, Moridae, Sparidae and Synaphobranchidae (Gómez et al. 2010)., However, representatives of Macrouridae Bonaparte, 1831 apparently act as major host species (Gómez et al. 2010). Twelve species i.e. *Lophoura tetraloba* from *Nezumia condylura* Jordan & Gilbert, 1904 (Ho and Kim 1989); *L*. *bouvieri* from *Macrourus berglax* Lacepède, 1801 and *Nezumia bairdii* (Goode & Bean, 1877) (Wilson 1919); *L*. *brevicollum* from *Nezumia liolepis* (Gilbert, 1890) (Gómez et al. 2010); *L*. *cardusa* from *Hymenocephalus striatissimus* Jordan & Gilbert, 1904 (Yamaguti 1939)*; L. edwardsi* from *Coelorinchus caelorhincus* (Risso, 1810) (Kabata 1979); *L*. *laticervix* from *Coelorinchus fasciatus* (Günther, 1878) (Hewitt 1964); *L*. *pentaloba* from *Coryphaenoides armatus* (Hector, 1875), *Coryphaenoides filifer* (Gilbert, 1896) and *Nezumia bairdii* (Goode & Bean, 1877) (Ho 1985; Ho and Kim 1989); *L*. *szidati* from *Macrourus holotrachys* Günther, 1878 and *Macrourus whitsoni* (Regan, 1913) (Stadler 1978); *L*. *unilobulata* from *Nezumia stelgidolepis* (Gilbert, 1890) and *Nezumia pulchella* (Pequeño, 1971) (Gómez et al. 2010); *L*. *ventricula* from *Coryphaenoides filifer* (Gilbert, 1896) and *Coryphaenoides nasutus* Günther, 1877 (Ho and Kim 1989); and *L*. *bipartita* from *Coryphaenoides subserrulatus* Makushok, 1976 (Ho and Kim 1989) were reported from macrourids.

Table 4.1: A summary of the distinguishing features of *Lophoura* species based on the morphology of the transformed post-metamorphosis females (compiled from Wilson 1919, 1935; Nuñes Ruivo 1962; Hewitt 1964; Szidat 1971; Kensley and Grindley 1973; Stadler 1978; Kabata 1979; Ho 1985; Hogans and Dadswell 1985; Ho and Kim 1989; Boxshall 1989, 2000; Castro-Romero and Gonzalez 2009; Gómez et al. 2010) with their reported host species and the depths at which the hosts occur (Froese and Pauly 2022).

	Characters	Reported host species and their depths range
Lophoura bipartita	Cephalothorax width diminishing from midway posteriorly; neck shorter than cephalothorax and	*Coryphaenoides subserrulatus* (900 – 1180 m)

	longer than trunk, bearing a holdfast organ with two main processes bearing elongated outgrowths; trunk sub-quadrangular to pyriform; posterior processes extend anteriorly; stalks curled and intertwined.	
Lophoura bouvieri	Neck longer than trunk and cephalothorax, bearing holdfast organ with 3 short, spherical processes; a sub-spherical trunk; posterior processes terminally, with straight stalks.	*Macrourus berglax* (100 – 1000 m); *Nezumia bairdii* (16 – 1000 m)
Lophoura brevicollum	Knobbed neck, shorter than the trunk, and longer than cephalothorax, bearing an irregularly branched holdfast organ with 4 processes (each with or without outgrowths) extending outwards; a quadrangular trunk; posterior processes with straight stalks, some stalks branched into secondary stalks and sometimes tertiary stalks.	*Nezumia liolepis* (581 – 1660 m)
Lophoura caparti	Neck longer than trunk and cephalothorax, bearing holdfast organ consisting of a bulbous process; a pyriform trunk (without longitudinal rows of depressions) bearing posterior processes terminally, with elongated peduncle bearing multiple straight stalks, some diverging into secondary stalks.	*Epigonus telescopus* (75 – 1200 m)
Lophoura cardusa	Neck shorter than trunk and almost same length as cephalothorax, bearing an irregularly shaped holdfast organ, formed by tentacle-like structures, which are not organized into main processes; a pyriform trunk; posterior processes with straight stalks.	*Hymenocephalus striatissimus* (300 – 570 m)
Lophoura cornuta	Neck shorter than trunk, and longer than cephalothorax bearing a holdfast organ with 4 main processes which divide immediately into tentacle-like strips/outgrowths; sub-quadrangular trunk, posterior processes with straight stalks.	*Synaphobranchus brevidorsalis* (900 – 3000 m); *Synaphobranchus affinis* (290 – 2400 m)
Lophoura edwardsi	Neck longer than trunk and cephalothorax, bearing a holdfast organ with 4 short, blunt processes (each with or without outgrowths) extending outwards; a	*Coelorinchus caelorhincus* (90 – 1485 m); *Macrourus* sp

	quadrangular to pyriform trunk, posterior processes with straight stalks.	
Lophoura elongata	Neck longer than trunk and much shorter than cephalothorax, bearing irregularly branched holdfast organ with numerous knob-like processes; trunk pyriform; posterior processes with straight stalks.	*Histiobranchus bathybius* (295 – 5440 m)
Lophoura gracilis	Neck as long as trunk and shorter than cephalothorax, bearing holdfast organ with 3 – 5 irregularly shaped, knoblike chitinous processes; a pyriform trunk; posterior processes with straight stalks.	*Histiobranchus bathybius* (295 – 5440 m)*; Synaphobranchus kaupii (*120 – 4800 m); *Synaphobranchus pinnatus*
Lophoura laticervix	Neck longer than cephalothorax and shorter than trunk, bearing irregularly branched holdfast organ with 2 main processes, each process with 3 outgrowths; trunk pyriform; posterior processes with straight stalks.	*Coelorinchus fasciatus* (73 – 1086 m)
Lophoura magna	Neck longer than cephalothorax and shorter than trunk, bearing irregularly branched holdfast organ with 2 globular processes (an elongated process and a short knob-like process) attached to a circular medial process; trunk pyriform; posterior processes with straight stalks.	*Lepidion ensiferus* (800 – 1000 m)
Lophoura pentaloba	Neck longer than cephalothorax and shorter than trunk, bearing holdfast organ with 5 main processes with or without outgrowths; trunk pyriform; posterior processes extend anteriorly; stalks curled and intertwined.	*Coryphaenoides armatus* (282 – 5180 m); *Coryphaenoides filifer* (1285 – 2904 m); *Nezumia bairdii* (16 – 1000 m)
Lophoura simplex	Neck longer than cephalothorax and trunk, without a holdfast organ; trunk pyriform; posterior processes with straight stalks.	*Histiobranchus bathybius* (295 – 5440 m)
Lophoura szidati	Cephalothorax width diminishing from midway posteriorly; neck longer than trunk and cephalothorax; holdfast organ bipartite, each part with 3 blunt processes; pyriform trunk with 3 – 4 pores; posterior processes with slightly curved short stalks, extending posteriorly.	*Macrourus holotrachys* (300 – 1400 m); *Macrourus whitsoni* (400 – 3185 m)

Lophoura tetraloba	Knobbed neck, longer than trunk and cephalothorax, bearing holdfast organ with 4 processes (each with or without outgrowths) extending outwards; a sub-spherical trunk; posterior processes with straight stalks.	*Nezumia condylura* (200 – 720 m)
Lophoura tetraphylla	Neck shorter than or as long as trunk, and longer than cephalothorax; holdfast organ with 4 processes, each process inflated, with outgrowths; wider than trunk; posterior processes with slightly curved short stalks, extending posteriorly.	*Antimora rostrata* (350 – 3000 m)
Lophoura tripartita	Neck longer than cephalothorax and trunk, bearing a holdfast organ with 3 main processes, bearing outgrowths; trunk pyriform; posterior processes with straight stalks, some diverging into secondary stalks.	*Calamus bajonado* (3 – 200 m)
Lophoura unilobulata	Neck longer than cephalothorax and trunk, bearing holdfast organ with 2 large, spherical processes; trunk pyriform; posterior processes bearing elongated porous peduncle with straight stalks, some diverging into secondary stalks.	*Nezumia stelgidolepis* (277 – 909 m); *Nezumia pulchella* (250 – 960 m)
Lophoura ventricula	Neck longer than cephalothorax and trunk, bearing holdfast organ with 5 main processes bearing tentacle-like long outgrowths; trunk pyriform; posterior processes extend anteriorly; stalks curled and intertwined.	*Coryphaenoides nasutus* (1537 m)

4.2. Material and methods

Refer to Chapter 2.

4.3. Results

4.3.1. Descriptions of *Lophoura* species

4.3.1.1. *Lophoura tetraloba* Ho & Kim I.H., 1989

Host: *Coelorinchus fasciatus* (Gunther, 1878)

Locality: Off the west coast (Atlantic Ocean), South Africa

Material examined: 6♀♀ (2 juveniles and 4 post metamorphosis) from three host specimens and 1♂ (attached to female posterior processes)

Material collected: 24♀♀ and 1♂ from *C*. *fasciatus*,14♀♀ and 1♂ from *Lucigadus ori* and 7♀♀ from unknown hosts; all specimens collected off the west coast (Atlantic Ocean), South Africa

Description of juvenile female (Figs. 4.1A – B).

Body length from tip of cephalothorax to the tip of the abdomen 21.6 mm (*n* = 3; 20.4 – 22.7 mm), cephalothorax length 5.4 mm (*n* = 3; 4.8 – 5.7 mm), width 1.1 mm (*n* = 3; 1 – 1.3 mm); holdfast organ width 4.1 mm (*n* = 3; 3.8 – 4.5 mm); neck length 10.0 mm (*n* = 3; 9.1 – 11.2 mm), width 0.4 mm (*n* = 3; 0.3 – 0.4 mm); trunk length 6.1 mm (*n* = 3; 5.7 – 7 mm), width 4.5 mm (*n* = 3; 3.9 – 5.5 mm); posterior processes length 4.4 mm (*n* = 3; 3.4 – 5.6 mm), width 7.4 mm (*n* = 3; 5.9 – 8.4 mm).

Cephalothorax (Fig. 4.1A) longitudinally elongated, smooth. Neck (Fig. 4.1A) heavily chitinized, elongated and cylindrical, with small knobs on the surface; anteriorly bearing holdfast organ with four short processes extending outwards; each process with or without outgrowths. Trunk (Figs. 4.1A – B) longer than wide with a well-defined abdomen (Figs. 4.1A – B). Posterior processes (Figs. 4.1A – B) attached to the abdomen, comprising of a short peduncle, bearing numerous elongated, straight stalks extending posteriorly.

Remarks:

There are no clear observed significant differences between the juveniles and post-metamorphosis females. However, the width of the holdfast organ processes and the posterior processes seem to mature with age as in the juveniles (non-ovigerous females) the holdfast organ processes (Fig. 4.1A in the current study; also see Figs. 7C – D in Ho and Kim 1989) are shorter with fewer or no outgrowths and the porous peduncle, together with the stalks of the posterior processes are comparatively shorter (Figs. 4.1A – B in the current study; also see Figs. 7A – C in Ho and Kim 1989) while

in the post-metamorphosis (ovigerous) females the holdfast organs processes are longer, some with outgrowths, and the posterior processes (both the porous peduncle, and its stalks) are longer (Figs. 4.1A – B in the current study; also see Figs. 6A – D in Ho and Kim (1989)).

Description of post-metamorphosis female (Figs. 4.2 – 4.5).

Body length from tip of cephalothorax to the tip of the abdomen 26.7 mm (*n* = 4; 25.1 – 29.5 mm), cephalothorax length 7 mm (*n* = 4; 6.5 – 8.1 mm), width 1.7 mm (*n* = 4; 1.4 – 2.1 mm); holdfast organ width 5.5 mm (*n* = 4; 4.2 – 6.3 mm); neck length 12.3 mm (*n* = 4; 8.4 – 15.1 mm), width 0.7 mm (*n* = 4; 0.6 – 0.7 mm); trunk length 8 mm (*n* = 4; 7.6 – 8.7 mm), width 8.5 mm (*n* = 4; 8.3 – 8.7 mm); posterior processes length 7.1 mm (*n* = 4; 5.7 – 8.5 mm), width 10.6 mm (*n* = 3; 10.4 – 10.9 mm) egg-sac length 16.8 mm (*n* = 2; 16.8 mm), width 2.1 mm (*n* = 2; 2 – 2.2 mm).

Cephalothorax (Figs. 4.2A – E; 4.3A – C) longitudinally elongated, smooth (Figs. 4.2C – E) or sometimes transversely wrinkled (Figs. 4.2A – B; 4.3A – C), anterior surface (Figs. 4.3D, 4.4A – B) with two types of enlarged processes medially, i.e., antennary and maxillary processes. Neck (Figs. 4.2A – E; 4.3A – B) heavily chitinized, elongated and cylindrical, with small knobs on surface; anteriorly bearing holdfast organ (Figs. 4.2A – E; 4.3A – C) with four processes extending outwards; each process with or without short outgrowths. Trunk (Figs. 4.2A – B; 4.3A – B) sub-circular, with a well-defined abdomen (Fig. 4.3E); dorsal side (Figs. 4.2B, C, E, 4.3A) and ventral side (Figs. 4.2A, D, 4.3B) with 2 rows of 3 – 4 pores. Posterior processes (Figs. 4.2A – E; 4.3A – B) attached to the abdomen (Figs. 4.3E), comprising of a short peduncle, bearing numerous (more than 30) elongate, straight stalks extending posteriorly. Egg sacs (Figs. 4.3A – B) long, eggs multi-serially arranged.

Cephalic appendages situated on the anterior surface (Figs. 4.3D, 4.4A – B), with a circular groove (Figs. 4.2 A – E; 4.4A – B) separating the cephalic region from the rest of the cephalothorax. Antennule (Figs. 4.4A, C, D) anterior to the antenna, digitiform

with blunt tip. Antenna (Figs. 4.4A, C, E) with three small apical tubercles of different sizes (one curved, large tubercle, with two small tubercles). Mandible not observed. Labrum (Figs. 4.4C, F) with a very small blunt tubercle, with two openings below, forming a triangle (Fig. 4.5A). Maxillule (Fig. 4.4C) posterior to antenna, a flat, rudimentary tubercle. Maxilla represented by the large, bulbous maxillary process (Figs. 4.3D, 4.4A – B, 4.5B) with maxillary gland pore (Figs. 4.4A – B, 4.5B – C). Maxilliped (Figs. 4.3F and 4.5D) posterolateral to maxillary processes (Fig. 4.5B), on the ventral side of the cephalothorax, subchelate, corpus robust, myxa raised; small spine below base of subchela, subchela indistinctly subdivided into shaft and claw, sharply curved with elongated pointed tip.

Remarks:

The studied specimens belong to the *Lophoura* species with four processes on their holdfast organ. These include *L*. *tetraphylla*, *L*. *brevicollum*, *L*. *edwardsi*, *L*. *cornuta* and *L*. *tetraloba*. *Lophoura tetraphylla* (see Fig. 5 in Ho (1985)) differs from *L*. *tetraloba* by the possession of a holdfast organ with four, large, inflated processes with tubercles, a neck shorter or sometimes equal to the trunk and posterior processes with slightly curved stalks. *Lophoura brevicollum* (see Figs. 1 – 6 in Gómez et al. 2010) differs from *L*. *tetraloba* by possessing an irregularly shaped holdfast organ, each process with multiple outgrowths, posterior processes with multiple stalks branched up to tertiary stalks. *Lophoura cornuta* (see Fig. 46 in Wilson (1919)) differs from *L*. *tetraloba* by the possession of a holdfast organ with the four processes each dividing immediately into variable, long, slender branches, irregularly shaped and a neck shorter than the trunk. *Lophoura edwardsi* (see Fig. 1451 in Kabata (1979)) differs from *L*. *tetraloba* by the possession of a neck which is almost as long as the trunk whereas the holdfast organ (see Figs. 1451 – 1452 in Kabata (1979)) resembles that of *L*. *tetraloba*.

Features of *L*. *tetraloba* vary across individuals (see Figs. 4.2A – E, 4.3A – B), e.g. the size of the cephalothorax (shorter cephalothorax in Figs. 4.2A – B and 4.3A – B, in comparison to Figs. 4.2C – E), the neck length, number of knobs on the neck, the

shape of the trunk and the length of posterior processes. Basically, it is distinguished by the holdfast organ with four processes, a neck which is longer than the trunk, and posterior processes with a short porous peduncle bearing straight stalks (which do not diverge). However, it is quite difficult to distinguish between *L*. *tetraloba* (see Fig. 6 in Ho and Kim (1989) and Figs. 4.2A – E, 4.3A – B in the current study) and *L*. *edwardsi* (see Figs. 1451 – 1458 in Kabata (1979)) since they both share similar morphological characters (see Table 4.2). Apparently, *L*. *edwardsi* has a holdfast organ "with two to four rounded or irregular processes of various lengths, but always shorter than the cephalothorax" (Kabata 1979). However, *L*. *edwardsi* was not included in studies that compared *Lophoura* species with four processes on the holdfast organ (see Ho and Kim (1989) and Gómez et al. (2010)).

Description of juvenile male (Figs. 4.6 – 4.8).

Body length from tip of cephalothorax (including mouth tube) to tip of posterior end about 0.633 mm (*n* = 1). Cephalothorax (Figs. 4.6A, B; 4.7A – B) more than halve of the total length. Trunk (Figs. 4.6A, B; 4.7B) segmented, ending with caudal rami posteriorly.

Antennule (Fig. 4.7D) 3-segmented; first segment with distomedial whip; second with distal solus and last segment with 6 long setae (4 possible aesthetasc) on apex. Antenna (Fig. 4.7E) 4-segmented, exopod shorter than endopod, 1-segmented, bulbous, equipped with a long spine-like papilla apically, endopod 2-segmented, basal segment with few denticles anterolaterally, distal segment with strong hook 1, thin long spine 2, and seta 5 emerging from swelling 4 which is covered with denticles (see lernaeopodid males in Kabata (1979)). Mouth tube with labrum and labium fringed by denticles (Figs. 4.6C, 4.8A). Mandible (Fig. 4.8B) with 5 equally sized teeth. Maxillule (Fig. 4.7F) biramous; endite armed with two long truncated apical setae; palp blunt bulbous process at the base of endite. Maxilla (Fig. 4.8C) broad, subchelate, robust corpus with protuberance medially, bearing two pointed tubercles anteriorly; subchela indistinctly separated from claw, tip sharply curved; myxa raised, accommodating the tip of the claw. Maxilliped (Fig. 4.8D) subchelate, corpus with myxal area bearing 3 processes; subchela short and broad with small seta and raised tubercle near the base

of claw; claw short and broad with short seta apically. Caudal rami (Figs. 4.6D; 4.7C) paired, each with bulbous process and elongated spiniform seta.

Remarks:

The morphology of the described adult *Lophoura* males of *L*. *bouvieri* (see fig. 41 in Wilson (1919)), *L*. *cornuta* (see figs. 47 – 48 in Wilson (1919)), *L*. *caparti* (see Fig. 6F in Nuñes-Ruivo (1962)), *L*. *tetraloba* (see Fig. 4.7A) and *L*. *ventricula* (see Fig. 5A in Ho and Kim (1989)) is slightly different from the juvenile *L*. *tetraloba* male studied (Figs. 4.6A – B, D; 4.7B – C). The observed differences include an enlarged, less segmented trunk, inflated posteriorly in the adults (e.g. *L*. *bouvieri* (see fig. 41 in Wilson (1919)), *L*. *cornuta* (see figs. 47 – 48 in Wilson (1919)), *L*. *caparti* (see Fig. 6F in Nuñes-Ruivo (1962)), *L*. *tetraloba* (see Fig. 4.7A) and *L*. *ventricula* (see Fig. 5A in Ho and Kim (1989)) whereas the juvenile possesses a short, slender trunk with distinct segmentation in *L*. *tetraloba* (see Figs. 4.6B, 4.7B). Adults possess paired reduced caudal rami; but the bulbous process, seen in the juvenile *L*. *tetraloba* (see Figs. 4.6B, D, 4.7B – C), was not observed (e.g. *L*. *bouvieri* (see fig. 41 in Wilson (1919)), *L*. *cornuta* (see figs. 47 – 48 in Wilson (1919)), *L. caparti* (see Fig. 6F in Nuñes-Ruivo (1962)), *L*. *tetraloba* (see Fig. 4.7A) and *L*. *ventricula* (see Fig. 5A in Ho and Kim (1989)). Maxillipeds in the adults are less than half of the trunk length (e.g. *L*. *bouvieri* (see fig. 41 in Wilson (1919)), *L. cornuta* (see figs. 47 – 48 in Wilson (1919)), *L. caparti* (see Fig. 6F in Nuñes-Ruivo (1962)), *L*. *tetraloba* (see Fig. 4.7A) and *L*. *ventricula* (see Fig. 5A in Ho and Kim (1989))), while in the juvenile (see Figs. 4.6B, 4.7B) they are almost the same length as the trunk. Thus, it seems there is a certain degree of body modification in maturing *Lophoura* males.

The distal segments of the antennules of *L*. *tetraloba* (Fig. 4.8D) and *L*. *cornuta* (see fig 49 in Wilson (1919)) are armed with six setae, those of *L*. *bouvieri* (see fig 39 in Wilson (1919)) and *L*. *ventricula* (see Fig. 5F in Ho and Kim (1989)) with four setae while *L*. *caparti* (see Fig. 6g in Nuñes-Ruivo (1962)) has three. The mandible of *L*. *tetraloba* (Fig. 4.9B) has about 5 teeth while that of *L*. *ventricula* (see Fig. 5H in Ho and Kim (1989)) has about 7 – 8 equally sized teeth. The maxillule endite is armed

with 2 truncated setae in *L*. *tetraloba* (Fig. 4.8F), *L*. *caparti* (see Fig. 6g in Nuñes-Ruivo (1962)) and *L*. *ventricula* (see Fig. 5I in Ho and Kim (1989)) and a bulbous structured palp was observed at the base of the endite of *L*. *tetraloba* (Fig. 4.8F) which was not observed in the other species. The maxilla of *L*. *tetraloba* (Fig. 4.9C) have a raised tubercle at the base of corpus which was not observed in *L*. *ventricula* (see Fig. 5J in Ho and Kim (1989)). The maxillipeds of *L*. *bouvieri* (see fig. 40 in Wilson (1919)), *L*. *cornuta* (see fig. 53 in Wilson (1919)) and *L*. *tetraloba* (Fig. 4.9D) are mostly similar.

4.3.1.1.b. *Lophoura cf edwardsi* Kölliker, 1853

Host: *Coelorinchus fasciatus* (Gunther, 1878)

Locality: Off the west coast (Atlantic Ocean), South Africa

Material examined: 3♀♀ (2 post-metamorphosis and 1 juvenile)

Material collected: 6♀♀ from *C*. *fasciatus* and 2♀♀ from *Lucigadus ori* collected off the west coast (Atlantic Ocean), South Africa

Description of juvenile female (Figs. 4.1C – D).

Body length from tip of cephalothorax to the tip of the abdomen 21.9 mm (*n* = 2; 21 – 22.8 mm), cephalothorax length 5.6 mm (*n* = 2; 5.2– 5.9 mm), width 1.2 mm (*n* = 2; 1.1 – 1.3 mm); holdfast organ width 2.6 mm (*n* = 2; 1.8 – 3.4 mm); neck length 10.7 mm (*n* = 2; 8.8 – 12.5 mm), width 0.5 mm (*n* = 2; 0.4 – 0.6 mm); trunk length 5.8 mm (*n* = 2; 5.2 – 6.3 mm), width 3.9 mm (*n* = 2; 2.9 – 4.9 mm); posterior processes not observed.

Cephalothorax (Fig. 4.1C) smooth, longitudinally elongated. Neck (Fig. 4.1C) elongated and cylindrical, with short knobs on the surface; anterior part bearing a holdfast organ with four short processes without outgrowths, extending laterally. Trunk (Fig. 4.1C – D), longer than wide with a well-defined abdomen; both dorsal and ventral side with 2 rows of 3 pores. Posterior processes not observed.

Description of post-metamorphosis female (Figs. 4.9 – 4.11).

Body length from tip of cephalothorax to the tip of the abdomen 25 mm (*n* = 2; 19.5 – 30.4 mm), cephalothorax length 6.3 mm (*n* = 2; 6 – 6.6 mm), width 1.1 mm (*n* = 2; 1 – 1.2 mm); holdfast organ width 4 mm (*n* = 2; 3.8 – 4.2 mm); neck length 8.1 mm (*n* = 2; 6.7 – 9.4 mm), width 0.4 mm (*n* = 2); trunk length 6.9 mm (*n* = 2; 6.7 – 7 mm), width 7.1 mm (*n* = 2; 6.7 – 7.4 mm); posterior process length 4.9 mm (*n* = 2; 4.6 – 5.1 mm); egg-sac length 14 mm (*n* = 2; 11.2 – 16.8 mm), width 2.1 mm (*n* = 2; 2 – 2.2 mm).

Cephalothorax (Figs. 4.9A – C; 4.10A – B) smooth, longitudinally elongated; anterior surface (Figs. 4.10C; 4.11A – B) with enlarged processes anteriorly, i.e., antennary and maxillary processes. Neck (Figs. 4.9A – C; 4.10A) elongated and cylindrical, with short knobs on the surface; anterior part bearing holdfast organ with four short, blunt processes extending laterally. Trunk (Figs. 4.9A – C; 4.10A) sub-circular, dorsal and ventral side with 2 rows of 3 – 4 pores; with a well-defined abdomen (Fig. 4.10D) posteriorly. Posterior processes (Figs. 4.9A – C; 4.10A) attached to abdomen, with a short porous peduncle, bearing numerous (more than 30) stalks. Egg sacs (Figs. 4.9C; 4.10A) long, eggs multi-serially arranged.

Cephalic appendages situated on anterior surface, with circular groove separating cephalic region from posterior part. Antennule (Figs. 4.10E; 4.11C) digitiform. Antenna (Figs. 4.10F; 4.11D) with three apical tubercles of different sizes. Mandible not observed. Maxilla, represented by large bulbous maxillary processes (Figs. 4.10C; 4.11A – B) with maxillary gland pores (Fig. 4.11A). Maxilliped (Figs. 4.10G; 4.11F) posterolateral to maxillary processes (Fig. 4.11E), on the ventral side of the cephalothorax, corpus broad, myxa slanted, facing posteriorly, small spine at base of subchela; subchela not distinctly subdivided into shaft and claw, claw sharply curved with pointed tip.

Remarks:

The studied species (Figs. 4.9 – 4.11) resembles both *L*. *edwardsi* and *L*. *tetraloba* but differs slightly from *L*. *tetraloba* (Ho and Kim 1989) by possessing a relatively shorter neck as well as short, blunt processes of the holdfast organ (with few or no outgrowths) which is why it is described as *L*. *cf edwardsi* in the current study. According to descriptions and illustrations of *L*. *edwardsi* (Candeais (1952) and Kabata (1979)) this species is characterised by a holdfast organ with four rounded or irregular processes, with or without outgrowths (see Fig. 2 in Candeais (1952) and Fig. 1451 in Kabata (1979)); sub-circular to sub-quadrangular trunk, with 2 rows of 4 – 7 pores on dorsal side (see Figs. 1451, 1456 in Kabata (1979)); and posterior processes with 16 – 18 stalks according to Fig. 1451 in Kabata (1979) and 44 stalks according to Fig. 1 in Candeais (1952)) on each peduncle. However, this is similar to the features of *L*. *tetraloba* e.g. a holdfast organ with four processes, with or without outgrowths (see Figs. 4.2A – E, 4.3A – B and Figs. 6B – C in Ho & Kim (1989)), a sub-circular to sub-quadrangular trunk, with 2 rows of 3 – 5 pores on dorsal and ventral side (see Figs. 4.2A – E, 4.3A – B and Figs. 6A, D in Ho & Kim (1989)); and posterior processes with stalks (more than 30) on each peduncle (see Figs. 4.2A – E, 4.3A – B and Figs. 6A, D in Ho & Kim (1989)). Currently the identity of the studied species could not be confirmed based on the descriptions of *L*. *edwardsi*.

The morphology of the post metamorphosis females of *L*. *edwardsi* (See Kabata 1979) and *L*. *tetraloba* (Ho and Kim 1989) are highly similar both in the illustrations and descriptions in the literature, as well as in the currently examined species identified as *Lophoura cf edwardsi* (Figs. 4.9A – C; 4.10A – B) and *L*. *tetraloba* (see Figs. 4.2A – E, 4.3A – B) (Table 4.2. Apparently, Ho and Kim (1989) described *L*. *tetraloba* as a new species without considering previous descriptions of *L*. *edwardsi*. Thus, there is a possibility that *L*. *edwardsi* and *L*. *tetraloba* are synonymies. This is supported by their reported characteristic features that are applicable to both *L*. *edwardsi* and *L*. *tetraloba*. *Lophoura edwardsi* and *L*. *tetraloba* are distinguished based on the length of the neck with *L*. *edwardsi* having a shorter neck (Kabata 1979), which is slightly longer or as long as the trunk and *L*. *tetraloba* with a neck which is clearly longer than the trunk (Ho and Kim 1989). However, neck length varied considerably amongst the collected specimens (Figs. 4.2A – E, 4.3A – B). Additionally, the difference in neck lengths may be due to the attachment site of the specimen on its host, e.g., attachment

to host musculature may require a shorter neck than attachment near the visceral cavity (e.g., *L*. *edwardsi* according to Kabata (1979)) or the host's liver (e.g. *L*. *tetraloba* according to Ho and Kim (1989)). Furthermore, neck length may be related to the age of the specimen and whether the final attachment site has been reached. Host species (*C*. *fasciatus* and *L*. *ori*) of both *L*. *tetraloba* and *L*. *cf edwardsi* share a similar vertical distribution as *C*. *caelorinchus* (host species reported multiple times for *L*. *edwardsi*), while *N*. *condylura* (host species reported for *L*. *tetraloba*) comparably, inhabits shallower waters.

Lophoura tetraloba has only been reported once, from *Nezumia condylura*, whereas *L*. *edwardsi* has been reported multiple times from *Coelorinchus caelorinchus* and *Macrourus* sp. (only once). In this study both *Lophoura cf edwardsi* and *L*. *tetraloba* were collected from *Lucigadus ori* and *Coelorinchus fasciatus* (Table 4.2). According to Ho and Kim (1989), *L*. *tetraloba* is the only *Lophoura* species to penetrate the host liver, although Kabata (1979) mentioned that *L*. *edwardsi* attach near the visceral cavity, which is close to the liver.

On the cephalic area of *L*. *cf edwardsi* and *L*. *tetraloba*, minor morphological differences were observed, which may be related to the age or the preservation of the specimens. The antennary process of *L*. *cf edwardsi* (Figs. 4.11A – B) is protruding while that of *L*. *tetraloba* (Figs. 4.4A – B) is flattened. Posteriorly to maxillary spheres, there are two pronounced bulbous tubercles in *L*. *cf edwardsi* (see Figs. 4.11B, E) while those in *L*. *tetraloba* (see Fig. 4.5B) are flattened.

4.3.1.2. *Lophoura caparti* (Nuñes-Ruivo, 1962)

Host: *Epigonus denticulatus* Dieuzeide, 1950

Locality: Off the west coast (Atlantic Ocean), South Africa

Material examined: 3♀♀ from three host specimens

Material collected: 4♀♀ from *E*. *denticulatus* hosts collected off the west coast (Atlantic Ocean), South Africa; 1♀♀ from *E*. *telescopus* (Risso, 1810) collected off the east coast (Indian Ocean), South Africa

Description of post-metamorphosis female (Figs. 4.12 – 4.14).

Body length from tip of cephalothorax to the tip of the abdomen 58.7 mm (*n* = 3; 57.1 – 60.8 mm), cephalothorax length 14.3 mm (*n* = 3; 11.2 – 16.8 mm), width 1.6 mm (*n* = 3; 1.3 – 2 mm); holdfast organ length 2.6 mm (*n* = 3; 2.2 – 2.8 mm), width 4.1 mm (*n* = 3; 3.9 – 4.2 mm); neck length 28.3 mm (*n* = 3; 28 – 28.6 mm), width 0.9 mm (*n* = 3; 0.8 – 1 mm); trunk length 16.8 mm (*n* = 3; 15.4 – 17.9 mm), width 8.1 mm (*n* = 3; 6.3 – 9.2 mm); abdomen length 1.3 mm (*n* = 3; 1.3 mm), width 1.4 mm (*n* = 3; 1.3 – 1.4 mm); posterior process length 17.3 mm (*n* = 3; 15.8 – 18.5 mm), width 8.7 mm (*n* = 3; 7 – 9.8 mm); egg-sac width 2 mm (*n* = 1).

Cephalothorax (Figs. 4.12A; 4.13A) smooth, longitudinally elongated; anterior surface (Figs. 4.13B – C) with enlarged processes i.e. antennary processes and maxillary processes; circular groove separating the anterior part of cephalothorax from elongated posterior part. Neck (Figs. 4.12A; 4.13A) elongated, smooth; anterior part bearing bulbous, rounded holdfast organ (Figs. 4.12A – B; 4.13A). Trunk (Figs. 4.12A; 4.13A) elongated, pyriform, longer than wide with a well-defined abdomen (Figs. 4.12C; 4.13D). Posterior processes (Figs. 4.12C; 4.13A) attached lateral to abdomen, consisting of elongated porous peduncle, bearing numerous straight stalks (sometimes diverging into secondary stalks) extending posteriorly, resembling “bananas on a tree”.

Cephalic appendages (Fig. 4.14A) on the anterior surface, with a circular groove separating the cephalic region from the posterior part. Antennary process raised, accommodating antennules and antennae. Antennule (Figs. 4.13E; 4.14B) bifid tubercle, anterolateral to antennae. Antenna (Figs. 4.13F; 4.14C) anteromedial to maxillary processes (see Fig. 4.14A), with two apical tubercles. Maxillule (Fig. 4.14D) situated between maxillary processes, digitiform, endite with pointed apical seta, palp not observed. Maxilla represented by large, bulbous maxillary processes (Figs. 4.13B

– C; 4.14A) with maxillary gland pores. Maxilliped (Figs. 4.13G, 4.14E) posterior to maxillary processes (see Fig. 4.13B), ventrally on cephalothorax, subchelate, corpus broad, myxa raised; subchela not distinctly subdivided into shaft and claw, claw sharply curved with pointed tip.

Remarks:

The studied specimens belong to the *Lophoura* species with simple holdfast organs, bearing bulbous processes, which include *L*. *caparti*, *L*. *unilobulata* and *L*. *bouvieri*. *Lophoura bouvieri* possesses three large, bulbous processes on the holdfast organ (see Figs. 34 – 35 in Wilson (1919)), *L*. *unilobulata* (Figs. 22 – 25 in Castro-Romero and Gonzalez (2009)) possesses of a holdfast organ with two large, bulbous processes while *L*. *caparti* (see Figs. 4.12A – B, 4.13A) possesses a holdfast organ consisting of one bulbous process. Posterior processes of *L*. *bouvieri* (see Fig. 34 in Wilson (1919)) possesses a short porous peduncle with short stalks while those of *L*. *caparti* (Figs. 4.12A, C, 4.13A) and *L*. *unilobulata* (Fig. 22 in Castro-Romero and Gonzalez (2009)) bear an elongated porous peduncle bearing multiple stalks. *Lophoura caparti* (Figs. 4.12A, 4.13A) lacks longitudinal rows of depressions on the trunk while *L*. *unilobulata* (Fig. 22 in Castro-Romero and Gonzalez (2009)) and *L*. *bouvieri* (see Fig. 34 in Wilson (1919)) possess the longitudinal rows of depressions on the trunk.

4.3.1.3. *Lophoura cornuta* (Wilson C.B., 1919)

Host: *Bassanago albescens* (Barnard, 1923)

Locality: Off the south coast, South Africa

Material examined: 1♀ from one host specimen

Material collected: 1♀ from *B*. *albescens* collected off the south coast, South Africa

Description of post-metamorphosis female (Figs. 4.15 – 4.16).

Body length from tip of cephalothorax to the tip of the abdomen 28.4 mm (*n* = 1), cephalothorax length 8.4 mm, width 1.5 mm; holdfast organ length 5.6 mm, width 5.6

mm; neck length 9.8 mm, width 1.1 mm; trunk length 10.2 mm, width 7 mm; abdomen length 1.3 mm, width 1.4 mm; posterior processes length 5.6 mm, width 9.8 mm.

Cephalothorax (Figs. 4.15A; 4.16A – C) longitudinally elongated, transversely wrinkled, anterior surface (Fig. 4.16D) with two pairs of enlarged processes anteriorly, i.e. antennary and maxillary processes. Neck (Figs. 4.15A; 4.16A – B) cylindrical; anteriorly with irregularly shaped holdfast organ with four main processes, immediately dividing into numerous elongated thin strips (Figs. 4.15B; 4.16A – B). Trunk (Figs. 4.15A; 4.16A – B, E) sub-quadrangular, longer than wide, dorsally and ventrally with 2 rows of 4 pores; posterior with enlarged abdomen. Posterior processes (Figs. 4.15A; 4.16A – B) attached laterally to the abdomen, each comprising a short peduncle, bearing numerous straight stalks extending posteriorly.

Remarks:

Lophoura cornuta bears a close resemblance to *L*. *cardusa*. *Lophoura cornuta* (Figs. 4.15 – 4.16) differs from *L*. *cardusa* (see Fig. 1 in Ho and Kim (1989)) by possession of four main processes, which divides immediately into tentacle-like strips/outgrowths (Figs. 4.15A – B, 4.16 A – B) while *L*. *cardusa* has an irregularly shaped holdfast organ, formed by tentacle-like structures, which are not organized into main processes (see Fig. 1 in Ho and Kim (1989)).

4.3.1.4. *Lophoura* sp.

Host: *Nezumia umbracincta* Iwamoto & Anderson, 1994

Locality: Off the west coast (Atlantic Ocean), South Africa

Material examined: 1♀ (broken) from one host specimen

Material collected:1♀ (broken) from *N*. *umbracincta* collected off the west coast (Atlantic Ocean), South Africa

Descriptions of post-metamorphosis female (Figs. 4.17 – 4.18).

Body length from tip of cephalothorax to the tip of abdomen unknown, cephalothorax length 5.6 mm (n = 1), width 1.4 mm; holdfast organ length 1.4 mm, width 6.3 mm; trunk length 11.5 mm, width 11.9 mm; abdomen length 1.7 mm, width 1.7 mm; posterior process length 16.8 mm.

Cephalothorax (Figs. 4.17A – B; 4.18A) longitudinally elongated, anterior surface (Figs. 4.18B – C) with two pairs of enlarged processes i.e. antennary and maxillary processes; cephalic part separated from posterior part by circular groove. Neck (Fig 4.18A), heavily chitinized, knobbed and cylindrical; anterior part bearing holdfast organ with five elongated processes extending laterally. Trunk (Figs. 4.17 C – D; 4.18D) circular, slightly wider than long, dorsally with 2 rows of 5 depressions, ventrally with 2 rows of 4 depressions; abdomen (Figs. 4.17E; 4.18E) terminally. Posterior processes (Figs. 4.17F; 4.18D) attached to abdomen, consisting of short porous peduncle bearing long, elongated straight stalks, extending posteriorly, longer than trunk.

Remarks:

The studied specimen has five processes on the holdfast organ. There are only two known species with five processes, i.e. *L*. *pentaloba* and *L*. *ventricula.* Both *L*. *ventricula* (see Fig. 4 in Ho and Kim (1989)) and *L*. *pentaloba* (see Fig. 2 in Ho and Kim (1989)) differ from the studied species (see Figs. 4.17 – 4.18) by possession of a pyriform trunk and posterior processes extending anteriorly, well curved and intertwined across the trunk's dorsal surface. *Lophoura ventricula* also possesses a holdfast organ with long tentacle-like processes (see Figs. 4A – F in Ho and Kim (1989)). The studied *Lophoura* sp. differs from other *Lophoura* species by possessing a holdfast organ with five slender processes, without outgrowths; a circular trunk with posterior processes extending posteriorly; and a short peduncle bearing elongated, straight stalks (Table 4.2).

Table 4.2: Comparisons of *Lophoura* species, with reported hosts and distribution in the current study.

	Lophoura caparti	*Lophoura cornuta*	*Lophoura cf edwardsi*	*Lophoura tetraloba*	*Lophoura* sp.
Host species	*Epigonus denticulatus Epigonus telescopus*	*Bassanago albescens*	*Coelorinchus fasciatus Lucigadus ori*	*Coelorinchus fasciatus Lucigadus ori*	*Nezumia umbrancincta*
Geographical distribution	Atlantic Ocean (off South Africa) and Indian Ocean (off South Africa)	Atlantic Ocean (off South Africa)	Atlantic Ocean (off South Africa)	Atlantic Ocean (off South Africa)	Atlantic Ocean (off South Africa)
Cephalothorax	Elongate, 0.9 times as long as trunk	Elongate, 0.8 times as long as trunk	Elongate, 0.9 times as long as trunk	Elongate, 0.9 times as long as trunk	Elongate, 0.5 times as long as trunk
Holdfast organ	a bulbous holdfast organ	irregular shaped holdfast organ with numerous thin strips	4 blunt processes on the holdfast organ, extending laterally	4 pointed processes on the holdfast organ, extending laterally	Five processes on the holdfast organ, extending laterally
Neck	Elongate, smooth, 1.7 times longer than wide	Elongate, 1 time as long as trunk	Elongate, with knobs on surface, elongate, 1.2 times as long as trunk	Elongate, with knobs on surface, 1.5 times as long as trunk	Elongate, with knobs on surface
Trunk	Pyriform, 2.1 times longer than wide	Pyriform, 1.5 times as long as wide	Sub-circular to sub-quadrangular, 1 time as long as wide	Sub-circular to sub-quadrangular, elongate, 0.9 times as long as wide	Circular, 1 time as long as wide

Abdomen	Knob-like peri anal lobe	Enlarged perianal lobe	Enlarged perianal lobe	Enlarged perianal lobe	Flat perianal lobe
Posterior processes	With long porous peduncle, bearing multiple straight stalks; some stalks diverge into secondary stalks; stalks shorter than the trunk	With short porous peduncle, bearing multiple straight stalks, which do not diverge; stalks shorter than the trunk	With short porous peduncle, bearing multiple straight stalks, which do not diverge; stalks shorter than the trunk	With short porous peduncle, bearing multiple straight stalks, which do not diverge; stalks shorter than the trunk	With short porous peduncle, bearing straight stalks, which do not diverge; stalks longer than the trunk.

4.3.2. Identification key to all the adult female *Lophoura* species

(Compiled from current study as well as Wilson 1919, 1935; Nuñes Ruivo 1962; Hewitt 1964; Szidat 1971; Kensley and Grindley 1973; Stadler 1978; Kabata 1979; Ho 1985; Hogans and Dadswell 1985; Ho and Kim 1989; Boxshall 1989, 2000; Castro-Romero and Gonzalez 2009; Gómez et al. 2010).

1a. Without holdfast organ……..…*L*. *simplex*

1b. Irregularly shaped holdfast organ without main processes ……..….*L*. *cardusa*

1c. Holdfast organ with main processes…….....2

2a. Cephalothorax length longer or as long as trunk length……..……..….3

2b. Cephalothorax shorter than neck……..……..……..5

3a. Cephalothorax 2 times longer than neck; holdfast organ with short, knob-like outgrowths; posterior processes with short straight stalks…..…….*L*. *elongata*

3b. Cephalothorax less than 2 times length of the neck……..…4

4a. Holfast organ with a single bulbous process; posterior processes bearing elongated porous peduncle with long stalks diverging into secondary stalks……..……..……..*L*. *caparti*

4b. Small holdfast organ, width slightly wider than neck; straight stalks of posterior processes, shorter than half trunk length ……..……...*L*. *gracilis*

4c. Cephalothorax (baton/ club shaped) increasing in width anteriorly; holdfast organ with short rounded outgrowths (bowtie-shaped); posterior process shorter than half of trunk length with slightly curved stalks…..……..….*L. szidati*

4d. Cephalothorax slightly increasing in width; holdfast organ with 2 main processes with finger-like outgrowths; stalks of posterior processes curved and heavily intertwined……...……..…..……...*L. bipartita*

5a. Holdfast organ simple, without complex outgrowths..……..……..…6

5b. Holdfast organ complex, with several outgrowths……7

6a. Holdfast organ bulbous, with 3 rounded processes; posterior processes length shorter than trunk length, with straight stalk..……...*L. bouvieri*

6b. Holdfast organ with 4 – 5 main processes, barely wider than cephalothorax; posterior processes almost the same length as trunk, with straight stalks …….....*L. edwardsi*

6c. Holdfast organ consisting of 2 bulbous processes, wider than cephalothorax; length of posterior processes longer than trunk length, elongated porous peduncle, stalks diverge into secondary stalks…….……..*L. unilobulata*

6d. Holdfast organ with 2 main processes, each with 3 short knob-like outgrowths; neck increasing width anteriorly; posterior processes length shorter than trunk length, with straight stalks……..……..…….*L. laticervix*

6e. Irregular holdfast organ with globular and diverging extended outgrowths; posterior processes length shorter than trunk length, with straight stalk ……..*L. magna*

7a. Holdfast organ with 4 inflated main processes, with outgrowths; spherical trunk; stalks of posterior processes slightly curved……..……..*L. tetraphylla*

7b. Posterior processes heavily intertwined……..……..8

7c. Posterior processes with straight stalks……..……..……9

8a. Holdfast organ with 5 main processes, with knob-like outgrowths ……...……..……..…….*L. pentaloba*

8b. Holdfast organ with 5 main processes, with finger-like outgrowths extending anteriorly……..……..*L. ventricula*

9a. Holdfast organ with elongated outgrowths of varying lengths…….……10

9b. Holdfast organ with knob-like outgrowths of varying lengths …….……11

10a. Holdfast organ with 3 main processes, with irregular outgrowths; posterior processes with straight stalks that diverge into secondary stalks.….*L*. *tripartita*

10b. Holdfast organ with 4 main processes, with elongated tentacle-like outgrowths; posterior processes longer than trunk ……..….*L*. *cornuta*

11a. Holdfast organ with 4 main processes, with irregular knob-like outgrowths; cephalothorax wider at base; neck as long as trunk; stalks of posterior processes diverge into tertiary stalks……..……..….*L*. *brevicollum*

11b. Holdfast organ with 4 main processes, with irregular knob-like outgrowths; cephalothorax of constant width; neck longer than trunk; posterior processes with single straight stalks……..……..……*L*. *tetraloba*

4.3.3. Cladistic analysis

Parsimony analysis was performed based on 13 characters (see Table 2.3) of which 12 were parsimony informative and only one character was parsimony uninformative. The analysis yielded two most parsimonious trees, with tree length of 42 (number of transformations) and consistency index (CI) of 0.5238, retention index (RI) of 0.6078 and rescaled consistency index (RCI) of 0.3184. The consistency index measures the amount of homoplasy exhibited by a tree (value of 1 shows no homoplasy, whereas value less than 1 shows homoplasy which increases as the value decreases). Retention index measures the synapomorphies exhibited by a character (or overall characters) in parsimonious tree (value of 1 represents synapomorphy and the value of 0 represents autapomorphy and increase in homoplasy). Rescaled consistency index indicates the amount of synapomorphy and homology of the characters used to estimate a parsimonious tree (Lipscomb 1998).

Lophoura species form a monophyletic grouping in the 50% majority rule consensus tree (Fig. 4.19). Node A leads to *L*. *simplex* which is basal to a monophyletic grouping of all the other *Lophoura* species. Node B leads to two sister groupings with *L*. *laticervix* basal to the rest of the species in the first sister grouping consisting of a clade comprising *L*. *cardusa* and *L*. *magna* and a clade comprising of *L*. *cornuta*, basal to *L*. *pentaloba* and *L*. *ventricula*. Node F also leads to sister groups i.e. node G, with *L*. *bouvieri* basal to the unresolved grouping between *L*. *tetraloba*, *L*. *edwardsi* and a

sister grouping comprised of *L*. *brevicollum* and *L*. *tetraphylla*. Node I leads to a monophyletic grouping i.e. node J with *L*. *tripartita* basal to the sister grouping of *L*. *unilobulata* and *L*. *caparti* while node K with *L*. *szidati* basal to a clade comprising of *L*. *bipartita* basal to the sister grouping of *L*. *gracilis* and *L*. *elongata*.

Table 4.3: The consistency index (ci), retention index (ri) and rescaled consistency index (rci) values calculated for each character used in the cladistic analysis of all *Lophoura* species with *Tripaphylus elongatus* as the outgroup taxon.

Character	ci	ri	rci
1	1.000	0/0	0/0
2	1.000	1.000	1.000
3	0.333	0.667	0.222
4	0.500	0.333	0.167
5	0.400	0.500	0.200
6	0.600	0.600	0.360
7	0.500	0.778	0.389
8	0.500	0.600	0.300
9	0.500	0.800	0.400
10	0.500	0.000	0.000
11	0.500	0.500	0.250
12	0.600	0.333	0.200
13	0.500	0.500	0.250

Only characters 1 and 2 (with ci = 1; Table 4.3) are not homoplaciously repeated in the two parsimonious trees while the rest of the characters exhibit low ci (0.3 – 0.6; see Table 4.3) values, hence consisting of multiple homoplacious repetitions illustrated

on the 50% majority rule consensus tree (Fig. 4.19). Character 1 (cephalothorax elongated) is the only synapomorphy separating the ingroup from the outgroup taxa. Character 2 (cephalothorax length longer than neck) is a synapomorphy that defines a monophyletic grouping of *L*. *bipartita* basal to the sister group between *L*. *elongata* and *L*. *gracilis* (Node L, Fig. 4.19). The sister grouping of *L*. *cardusa* and *L*. *magna* is defined by the synapomorphic character 6 (holdfast organ complex, processes irregular/ without uniform shape). Although, some states of character 6 exhibit homoplasy (hence ci = 0.6, ri = 0.6, hi = 0.4; see Table 4.3). Most characters used exhibit a high amount of homoplasy and thus low amounts of synapomorphy and homology and thus many groupings indicated on 50% majority rule consensus tree (Fig. 4.19) resulted from homoplaciously repeated characters.

4.4. Discussion

Although there are multiple variations among post-metamorphosis females of *Lophoura*, the shape, size and number of processes on the holdfast organ is the primary character which differentiate these species (see identification key (4.3.2)). Additionally, the position and branching of the posterior processes also distinguish species. Other characters such as the trunk shape, seem to vary among individuals of the same species.

Of the 19 accepted *Lophoura* species, only one species does not possess any holdfast organ, i.e. *L*. *simplex* (see Fig. 3 in Boxshall (2000)), hence basal to all other *Lophoura* species (see Fig. 4.19), while *L*. *caparti* differs from the remaining *Lophoura* species by possession of only one bulbous process on the holdfast organ (see Fig. 6a in Nuñes-Ruivo (1962)).

Several *Lophoura* species possess two main processes on the holdfast organ, including, *L*. *bipartita*, *L*. *laticervix*, *L*. *szidati* and *L*. *unilobulata*. The holdfast organ of *L*. *bipartita* is bipartite, with each process on either side of the neck dividing into slender, elongated outgrowths, extending outwards. The holdfast organ of *L*. *unilobulata* differs from the remaining *Lophoura* species with two main processes by

possessing two bulbous processes, without outgrowths. The holdfast organs of *L*. *laticervix* (Figs. 1, 3 in Hewitt (1964)) and *L*. *szidati* (Figs. 3, 5 – 7 in Stadler (1978)) are more similar with both holdfast organs being bipartite, with each process bearing three knobs, although irregularly shaped in *L*. *larticervix* (Figs. 1, 3 in Hewitt (1964)) and uniformly shaped in *L*. *szidati* (Figs. 3, 5 – 7 in Stadler (1978)). Other differences between *L*. *szidati* and *L*. *laticervix* include the neck length which is shorter than the trunk length in *L*. *laticervix* while longer than the trunk in *L*. *szidati* and the stalks of the posterior processes that are straight in *L*. *laticervix* but slightly curved in *L*. *szidati*. *Lophoura unilobulata* resembles *L*. *caparti* (see Fig. 6a from Nuñes-Ruivo (1962)), but mainly differs by the number of processes on their holdfast organs (*L*. *unilobulata* with two processes and *L*. *caparti* with one process), hence they form a sister group in the 50% majority rule tree (Fig. 4.19). However, having a simple shaped holdfast organ (character 6, see Table 2.3) is not a synapomorphy as it also occurs in *L*. *szidati* and *L*. *bouvieri* on the 50% majority rule consensus tree (see Fig. 4.19), thus, it is a homoplaciously repeated character amongst some *Lophoura* species as a result of convergent evolution.

Lophoura species that possess three main processes on the holdfast organ, include *L*. *bouvieri*, *L*. *tripartita*, *L*. *magna*. The holdfast organ of *L*. *bouvieri* consists of three bulbous processes and that of *L. magna* (see Figs. 2 – 3 in Szidat (1971)) consists of two globular processes (an elongated process and a short knob-like process) attached to a circular medial process, while that of *L*. *tripartita* is composed of three main processes extending outwards, each bearing outgrowths.

Lophoura species that possess four main processes on the holdfast organ include *L*. *tetraphylla*, *L*. *brevicollum*, *L*. *edwardsi*, *L*. *cornuta* and *L*. *tetraloba*. The holdfast organ of *L*. *tetraphylla* (see Fig. 5 in Ho (1985)) is wider than the trunk, and possess four, large, inflated processes with tubercles, while that of *L*. *brevicollum* (see Figs. 1 – 6 in Gómez et al. (2010)) is irregularly shaped and each process has multiple outgrowths. *Lophoura cornuta* possesses a holdfast with each process dividing immediately into variable, long, slender outgrowths, irregularly shaped and branched (see Fig. 46 in Wilson (1919) and Figs. 4.15A – B, 4.16A – B). The holdfast organs of both *L*. *edwardsi*

and *L*. *tetraloba* also possess four processes (see Figs. 1451 – 1452 in Kabata (1979) and Figs. 6A – C in Ho and Kim (1989), respectively), with or without outgrowths (Table 4.2). It seems they only differ in the length of the neck with that of *L*. *edwardsi* almost as long as the trunk (Fig. 1451 in Kabata (1979)) and that of *L*. *tetraloba* clearly longer than the trunk (Fig. 6A in Ho and Kim (1989)). As expected, the relationship between *L*. *edwardsi* and *L*. *tetraloba* is unresolved in the 50% majority rule consensus tree (Fig. 4.19). Species with four processes on the holdfast organ are related and form a monophyletic grouping between an unresolved relationship of *L*. *edwardsi* and *L*. *tetraloba* basal to the sister grouping of *L*. *brevicollum* and *L*. *tetraphylla* estimated on the 50% majority rule consensus tree (see Fig. 4.19) except for *L*. *cornuta* estimated to be the sister group of the *L*. *pentaloba* and *L*. *ventricula* clade. However, having a holdfast of 4-5 processes (character 7, Table 2.3) is not a synapomorphy as it is shared by the sister groupings consisting of *L*. *elongata* and *L*. *gracilis* as well as *L*. *pentaloba* and *L*. *ventricula*, meaning this is a homoplacious character that developed simultaneously in the evolution of *Lophoura* species according to the estimated topology (Fig. 4.19). Additionally, the sister grouping of *L*. *brevicollum* and *L*. *tetraphylla* (see Fig. 4.19) possesses a neck shorter than the trunk (character 5, Table 2.3), which is not a synapormorphy as it is shared with taxa from node B (excluding *L*. *ventricula* which possess a neck longer than the trunk (character 5, Table 2.3)). Thus, it is a homoplaciously repeated character amongst some *Lophoura* species as a result of convergent evolution.

Lophoura species that possess five main processes on the holdfast organ, include *L*. *pentaloba* and *L*. *ventricula.* The holdfast organ of *L*. *ventricula* possesses five main processes, with each dividing immediately into long tentacle-like outgrowths (see Figs. 4A – E in Ho and Kim (1989)) while that of *L*. *pentaloba* (see Figs. 2A – C in Ho and Kim (1989)) possesses five main processes with each dividing immediately into short outgrowths. Both *L*. *pentaloba* and *L*. *ventricula* possess posterior processes extending anteriorly on the dorsal side of the trunk, with stalks curved and intertwined (Ho and Kim 1989). However, from the 50% majority rule consensus tree (Fig. 4.19), both characters 11 (posterior processes extending anteriorly on the dorsal side of the trunk, see Table 2.3) and 12 (stalks of posterior processes are curved and intertwined, see Table 2.3) are shared between the sister group of *L*. *pentaloba* and *L*. *ventricula*,

and *L*. *bipartita*, which are homoplaciously repeated characters developed through convergent evolution across *Lophoura* taxa.

Some *Lophoura* species have an uncertain number of processes on the holdfast organ due to incomplete descriptions or irregularly shaped holdfast organs including *L*. *cardusa*, *L*. *gracilis* and *L*. *elongata*. *Lophoura gracilis* is known to possess 3 – 5 irregularly shaped knob-like processes (see Fig. 1 in Hogans and Dadswell (1985)), whereas *L*. *cardusa* possesses an irregularly shaped holdfast organ, formed by tentacle-like structures, which are not organized into main processes (see Fig. 1 in Ho and Kim (1989)) and *L*. *elongata* with an irregularly branched holdfast organ with numerous knob-like processes (see Figs. 35a – d from Kensley and Grindley (1973)). Apart from the holdfast organ, *L*. *elongata* and *L*. *gracilis* differ from other *Lophoura* species by possession of a cephalothorax which is longer than the neck length (Kensley and Grindley 1973; Hogans and Dadswell 1985), but that of *L*. *elongata* is 2 times as long as the neck (see Fig. 35a in Kensley and Grindley (1973)) while that of *L*. *gracilis* is only 1.1 times as long as the neck. The monophyletic grouping of *L*. *bipartita* basal to the sister group of *L*. *elongata* and *L*. *gracilis* is based on a synapomorphy (character 2, see Table 2.3) of a cephalothorax length longer than neck as observed on the 50% majority rule consensus tree (Fig. 4.19). In addition to being sister taxa (Fig. 4.19), *L*. *elongata* and *L*. *gracilis* share a common host family i.e. Synaphobranchidae, with a common host species of *Histiobranchus bathybius* (see Kensley and Grindley (1973) and Wilson (1919) respectively). Thus, it is possible that *L*. *elongata* and *L*. *gracilis* were derived from a common ancestor through allopatric speciation, influenced by insufficient oxygen and hydraulic pressure (see Gómez et al. 2010). Furthermore, the sister grouping between *L*. *cardusa* and *L*. *magna* (Fig. 4.19) is defined by a synapomorphy of a complex holdfast organ, with irregular processes/without uniform shape (character 6, see Table 2.3).

The CI value (0.5238) less than 1, indicates that there is an amount of homoplasy exhibited by the most parsimonious trees. The RI value (0.6078) indicates that there are more synapomorphies than autapomorphies in the selected characters. The RCI value (0.3184) is very low, which indicates that there is a high amount of homoplasy

and low amount of synapomorphy and homology in the characters used to estimate the parsimonious tree.

Lophoura caparti has been reported from the family Epigonidae (Nuñes-Ruivo 1962). In addition to the known host i.e. *E*. *telescopus* from a new geographical location (south coast (Indian Ocean), South Africa), it was also collected from *E*. *denticulatus* which constitutes a new host record as well as a new geographical record (west coast (Atlantic Ocean), South Africa). *Lophoura cornuta* has previously been reported from members of Synaphobranchidae (Wilson 1919; Boxshall 1989). However, it has been collected from *B*. *albescens* in the current study, thus, a new host record and the first *Lophoura* species to be reported from Congridae which also constitutes a new geographical record (south coast (Indian Ocean), South Africa). *Coelorinchus fasciatus*; *L*. *ori* and *N*. *umbracincta* are new host records from the family Macrouridae, while *L*. *tetraloba* and *Lophoura* sp. are new geographical records from the Atlantic Ocean off South Africa. All the host species reported in this study are deep-water fish, hence, confirming that *Lophoura* species are mesoparasites of deep-water teleosts (Kabata 1979; Ho and Kim 1989).

To date, only four *Lophoura* males have been reported including *L*. *bouvieri*, *L*. *cornuta* (see Wilson 1919), *L*. *caparti* (see Nuñes-Ruivo 1962) and *L*. *ventricula* (see Ho and Kim 1989). This is the first description of the male of *L*. *tetraloba*. *Lophoura tetraloba* male also resemble Lernaeopodidae males (Kabata 1979). The habitus of *Lophoura* males are similar, but the armature of the appendages differ slightly. However, complete comparisons could not be done for *L*. *tetraloba*, *L*. *bouvieri*, *L*. *cornuta* and *L*. *caparti* as previous descriptions and illustrations are incomplete.

The cephalothorax of *Lophoura* species is soft, non-chitinized, thus its shape may change due to increased pressure. From previous reports of *Lophoura* species, some species were described with a transversely wrinkled cephalothorax, including *L*. *tetraloba* (Ho and Kim 1989). However, the examined *L*. *tetraloba* specimens appeared to have different cephalothorax features with some specimens having

transversely wrinkled cephalothoraces (see Figs. 4.3A – B, 4.4A – B), in comparison to others with smooth cephalothoraces (Figs. 4.3C – D). Possibly all cephalothoraces are initially smooth, but then wrinkles and shrinks once subjected to pressure (perhaps pressure from host musculature) which may depend on the attachment site and feeding site of the species on the host. Additionally, the occurrence of these species on deep-water hosts (Ho and Kim 1989) may also have an influence on their morphological and physiological adaptations (Gómez et al. 2010). However, more studies are needed to confirm these possible effects. Furthermore, more specimens are needed to re-described *L*. *edwardsi* in order to determine the validity of this species and the morphological differences (if any) between *L*. *edwardsi* and *L*. *tetraloba*. Additionally, more deep-water fish species should be examined to find *Lophoura* species to re-describe incomplete described species and determine the number of valid species and thus the diversity of this genus as well as the extend of hosts infected.

Figure 4.1: *Lophoura tetraloba* Ho & Kim I.H., 1989 juvenile female. A. general habitus, trunk ventral view, cephalothorax lateral view; B. trunk, lateral view. *Lophoura edwardsi* Kölliker, 1853, juvenile female. C. general habitus, trunk ventrolateral view, cephalothorax lateral view; D. trunk, dorsal view. Scale bars: A – C = 1 mm

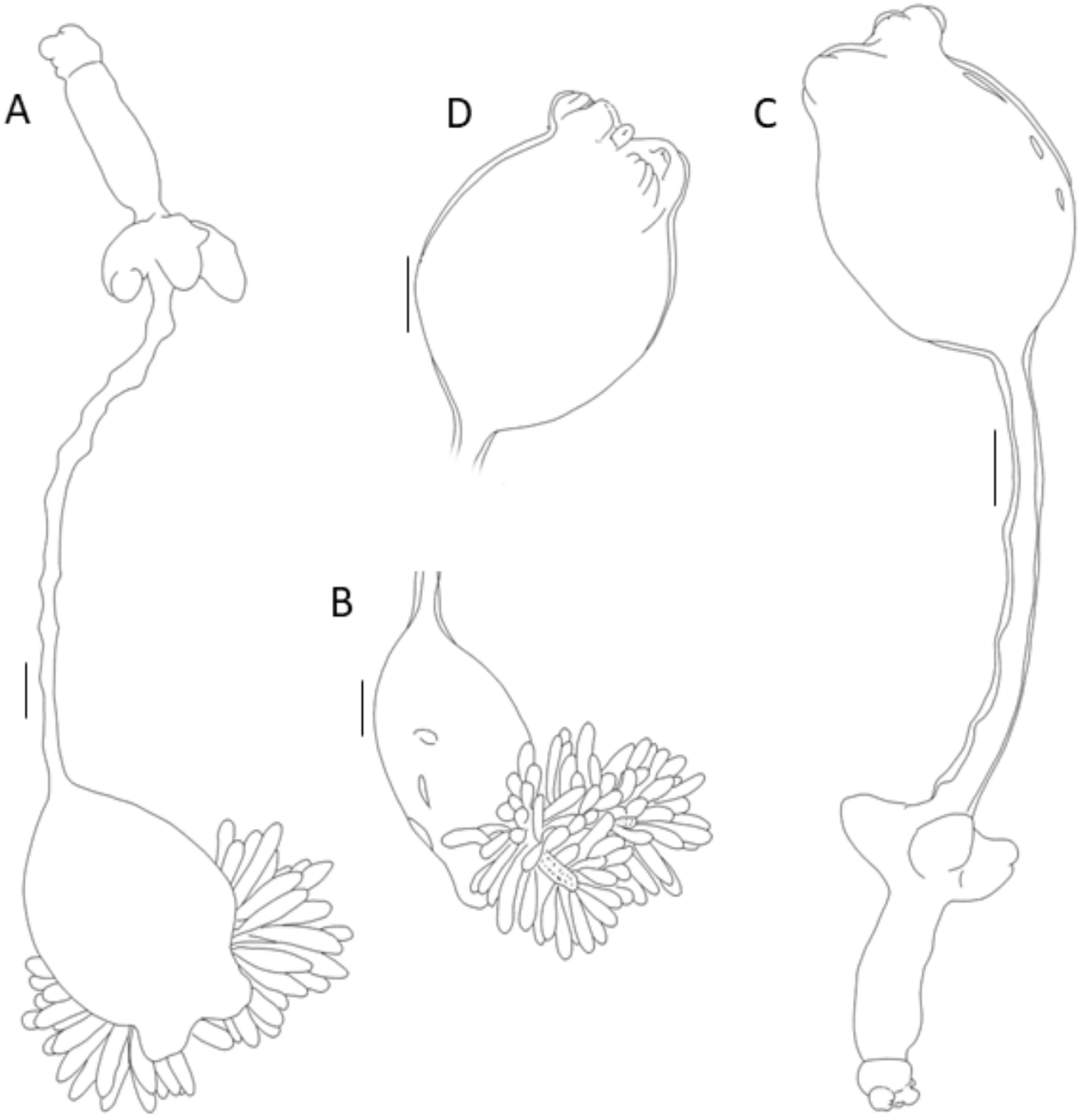

A
D
C
B

Figure 4.2: *Lophoura tetraloba* Ho & Kim I.H., 1989 post-metamorphosis female. A. general habitus, trunk ventral view, cephalothorax dorsolateral view; B. general habitus, dorsal view, cephalothorax ventrolateral view; C. general habitus, trunk dorsal view, cephalothorax ventral view; D. general habitus, trunk ventral view, cephalothorax dorsolateral view; E. general habitus, dorsal view, cephalothorax lateral view. Scale bars: A – E = 1 mm.

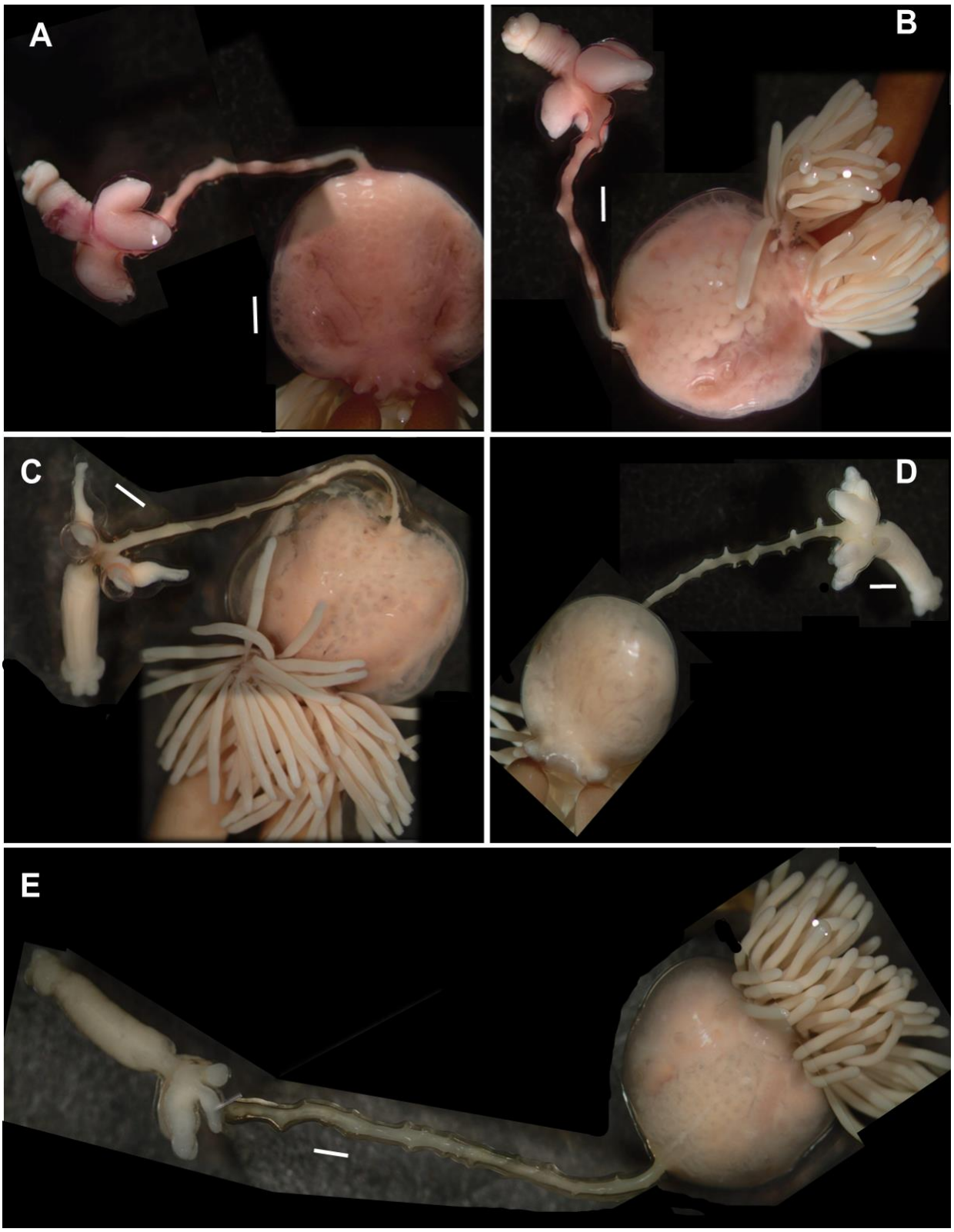

A
B
C
D
E

Figure 4.3: *Lophoura tetraloba* Ho & Kim I.H., 1989 post-metamorphosis female. A. general habitus, trunk dorsal view, cephalothorax ventral view; B. general habitus, trunk ventral view, cephalothorax dorsal view; C. cephalothorax, anterodorsal view; D. cephalothorax, anterodorsal view; E. abdomen; F. maxilliped. Scale bars: A – E = 1 mm and F = 10 µm. (mp – maxillary process, ap – antennary process).

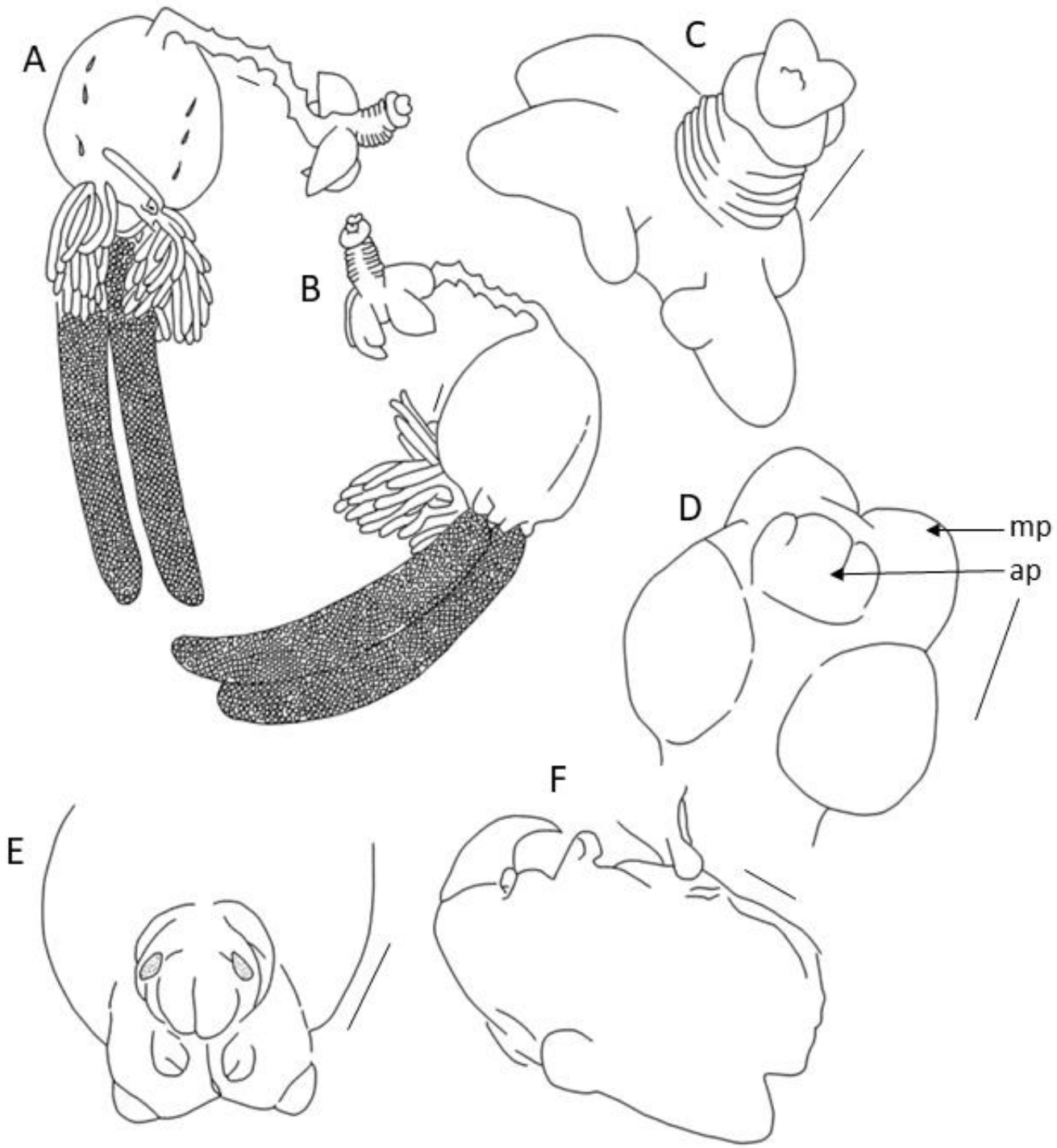

A
B
C
D
mp
ap
E
F

Figure 4.4: *Lophoura tetraloba* Ho & Kim I.H., 1989 post-metamorphosis female. Scanning electron micrographs: A. cephalothorax, cephalic region, anterior view; B. cephalothorax anterolateral view; C. cephalothorax, cephalic region; D. antennule; E. antenna; F. mouth tube. Scale bars: A – B = 500 µm; C = 300 µm; D – E = 10 µm; F = 50 µm. (ap – antennary process, a1 – antennule, a2 – antenna, mt – mouth tube, lbr – labrum, mx1 – maxillule, mp – maxillary processes, mgp – maxillary gland pore, cg – circular groove).

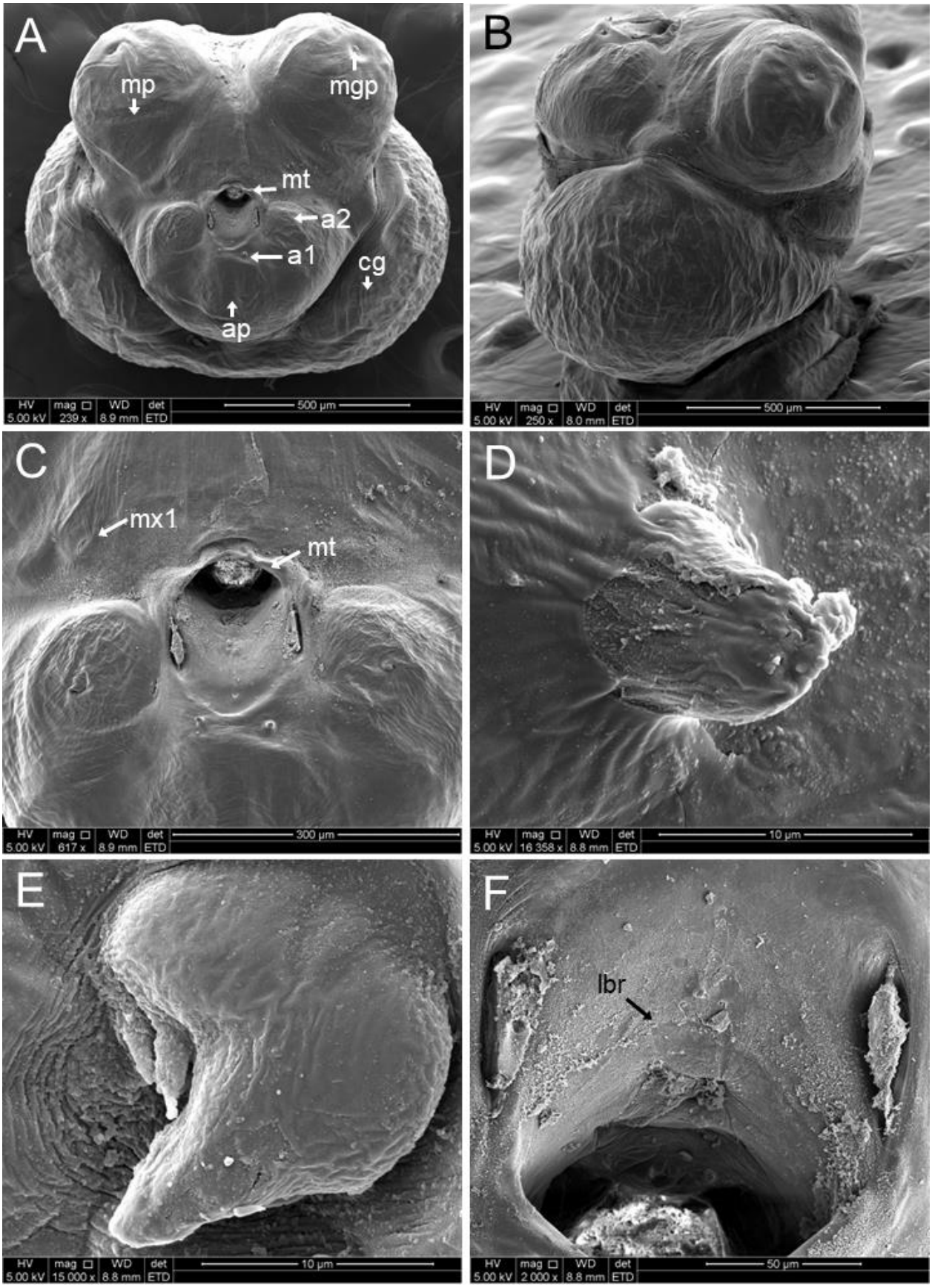

A
mp
mgp
mt
a2
a1
cg
ap
HV 5.00 kV
mag 239 x
WD 8.9 mm
det ETD
500 µm
B
HV 5.00 kV
mag 250 x
WD 8.0 mm
det ETD
500 µm
C
mx1
mt
HV 5.00 kV
mag 617 x
WD 8.9 mm
det ETD
300 µm
D
HV 5.00 kV
mag 16 358 x
WD 8.8 mm
det ETD
10 µm
E
HV 5.00 kV
mag 15 000 x
WD 8.8 mm
det ETD
10 µm
F
lbr
HV 5.00 kV
mag 2 000 x
WD 8.8 mm
det ETD
50 µm

Figure 4.5: *Lophoura tetraloba* Ho & Kim I.H., 1989 post-metamorphosis female. Scanning electron micrographs: A. labium tubercle; B. maxillary process; C. maxillary pore; D. maxilliped. Scale bars: A = 10 µm; B = 500 µm; C = 20 µm; D = 30 µm. (mxp – maxilliped).

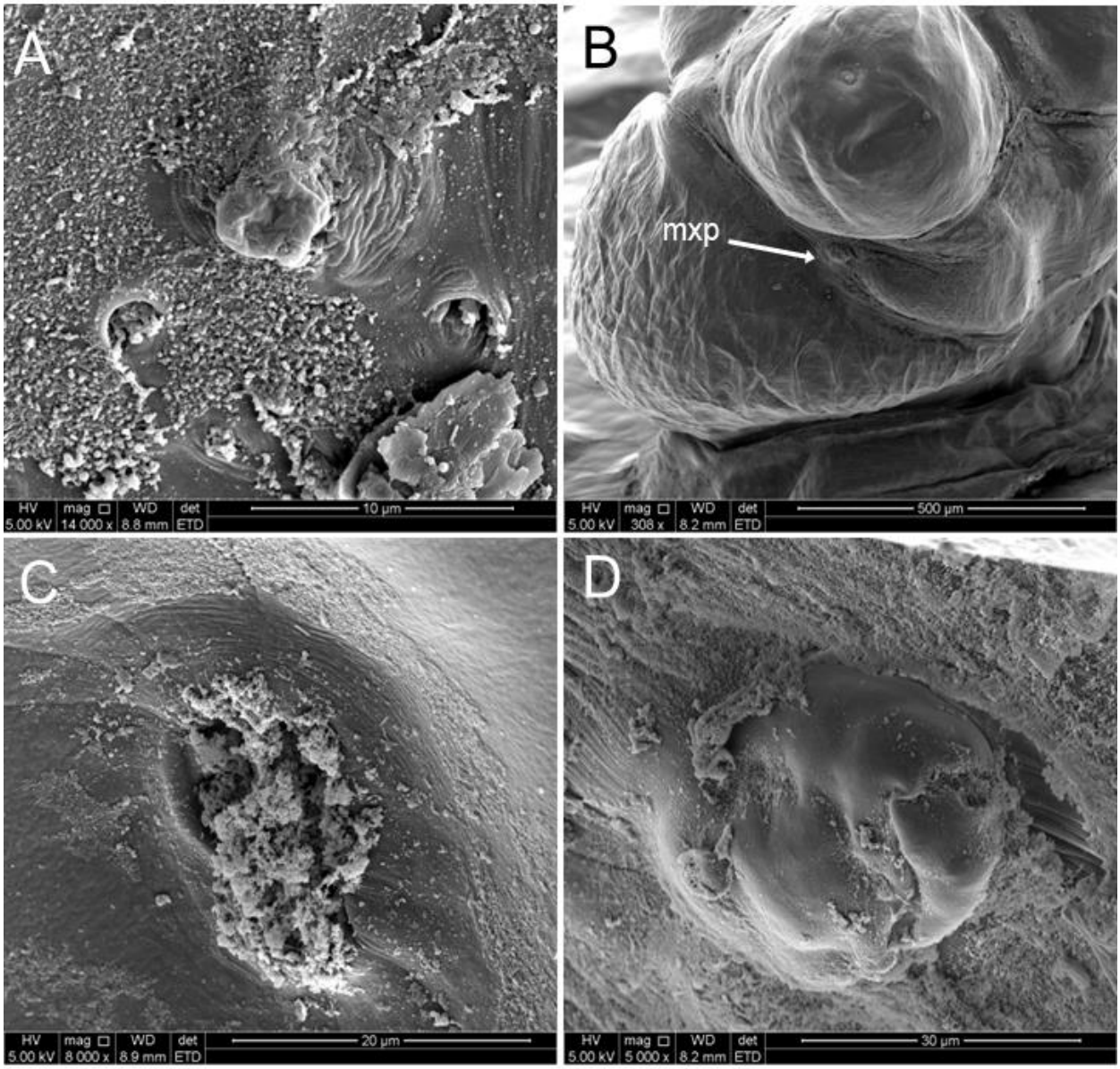

A
HV 5.00 kV
mag 14 000 x
WD 8.8 mm
det ETD
10 µm
B
mxp
HV 5.00 kV
mag 308 x
WD 8.2 mm
det ETD
500 µm
C
HV 5.00 kV
mag 8 000 x
WD 8.9 mm
det ETD
20 µm
D
HV 5.00 kV
mag 5 000 x
WD 8.2 mm
det ETD
30 µm

Figure 4.6. *Lophoura tetraloba* Ho & Kim I.H., 1989 male. A. male amongst posterior processes of female; B. general habitus, lateral view; C. mouth tube; D. caudal rami, lateral view. Scale bars: B = 50 µm; C = 20 µm; D = 10 µm.

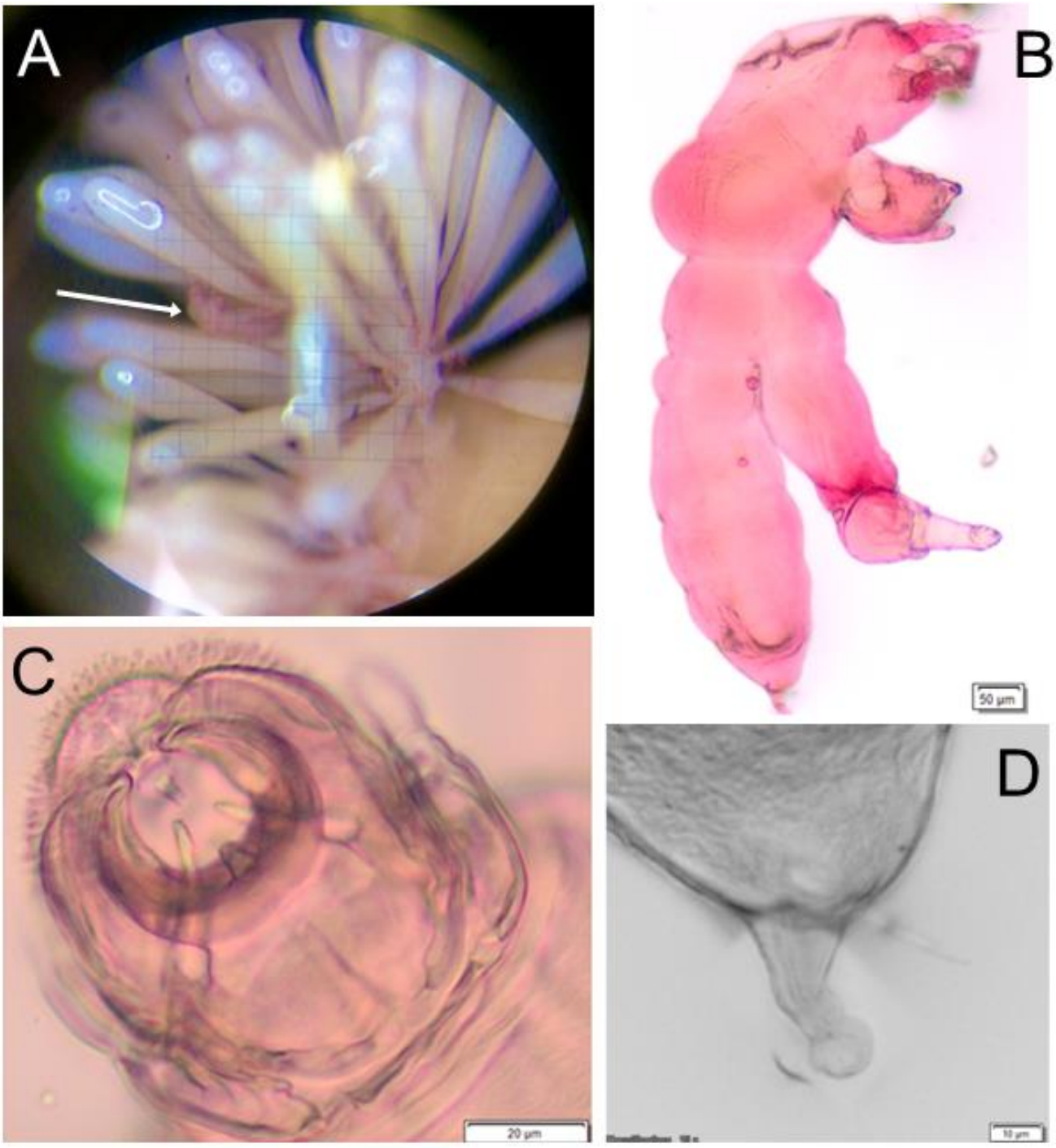

A
B
50 µm
C
20 µm
D

Figure 4.7*. Lophoura tetraloba* Ho & Kim I.H., 1989 male. A. adult male general habitus, lateral view; B. juvenile male general habitus, lateral view; C. caudal rami, ventrolateral view; D. antennule; E. antenna; F. maxillule. Scale bars: A = 1 mm; B = 50 µm; C – F = 10 µm.

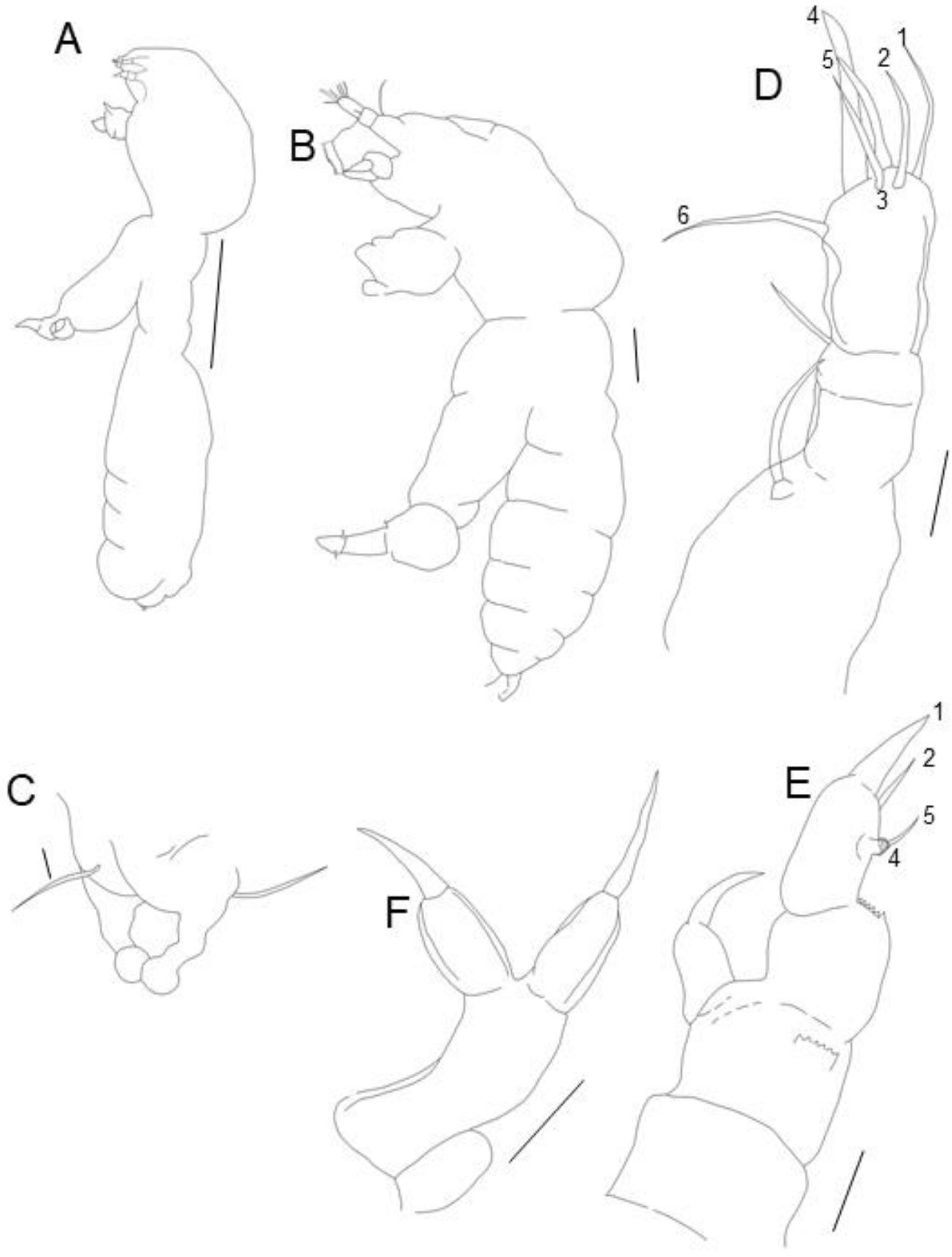

A
B
C
D
4
1
5
2
3
6
E
1
2
5
4
F

Figure 4.8*. Lophoura tetraloba* Ho & Kim I.H., 1989 male. A. mouth tube; B. mandible; C. maxilla; D. maxilliped. Scale bars: A – B = 5 µm; C – D = 10 µm.

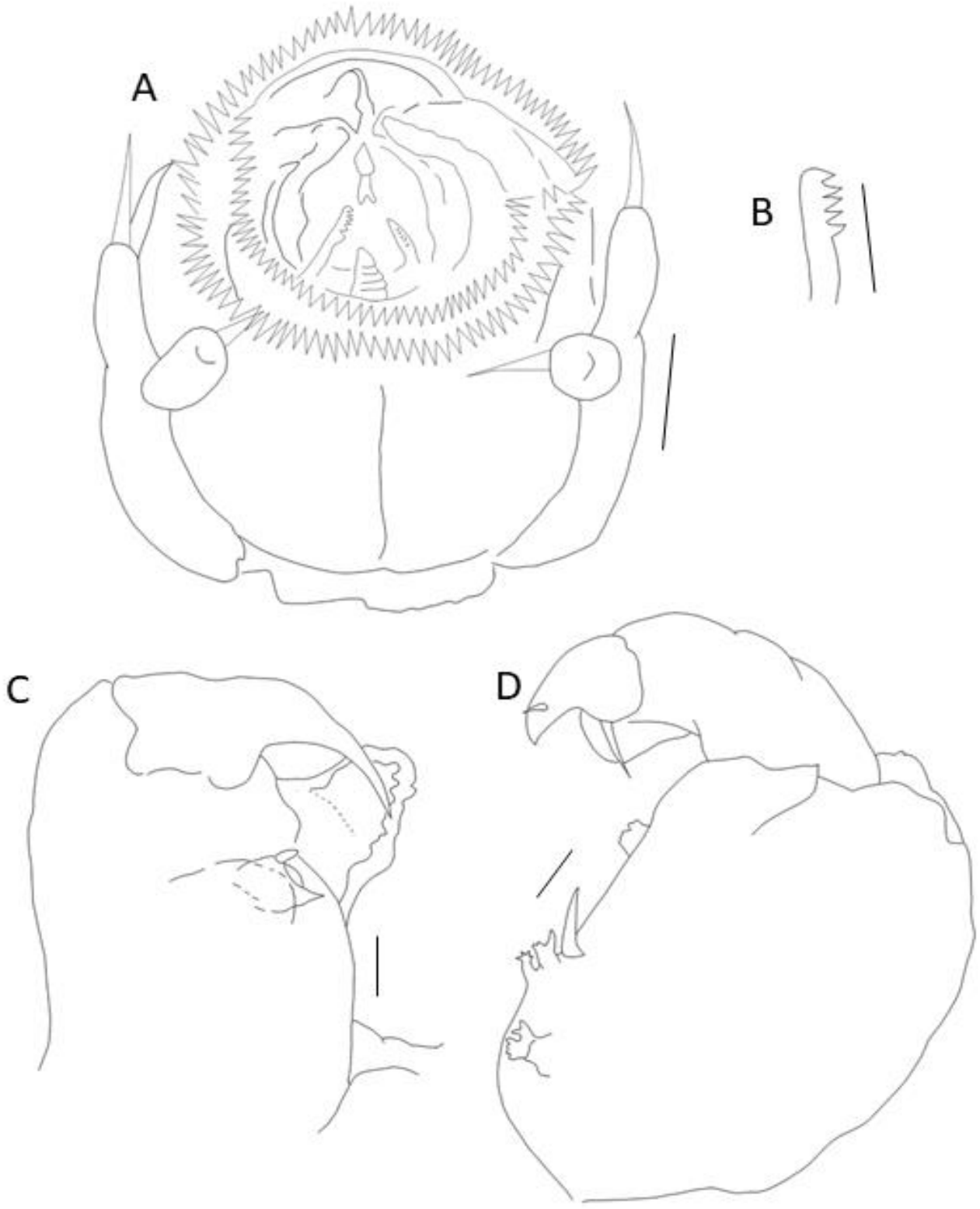

A
B
C
D

Figure 4.9: *Lophoura cf edwardsi* Kölliker, 1853 post-metamorphosis female. A. general habitus, trunk dorsal view, cephalothorax lateral view; B. general habitus, trunk ventral view, cephalothorax lateral view; C. general habitus, trunk lateral view, cephalothorax dorsal view. Scale bars: A – C = 1 mm.

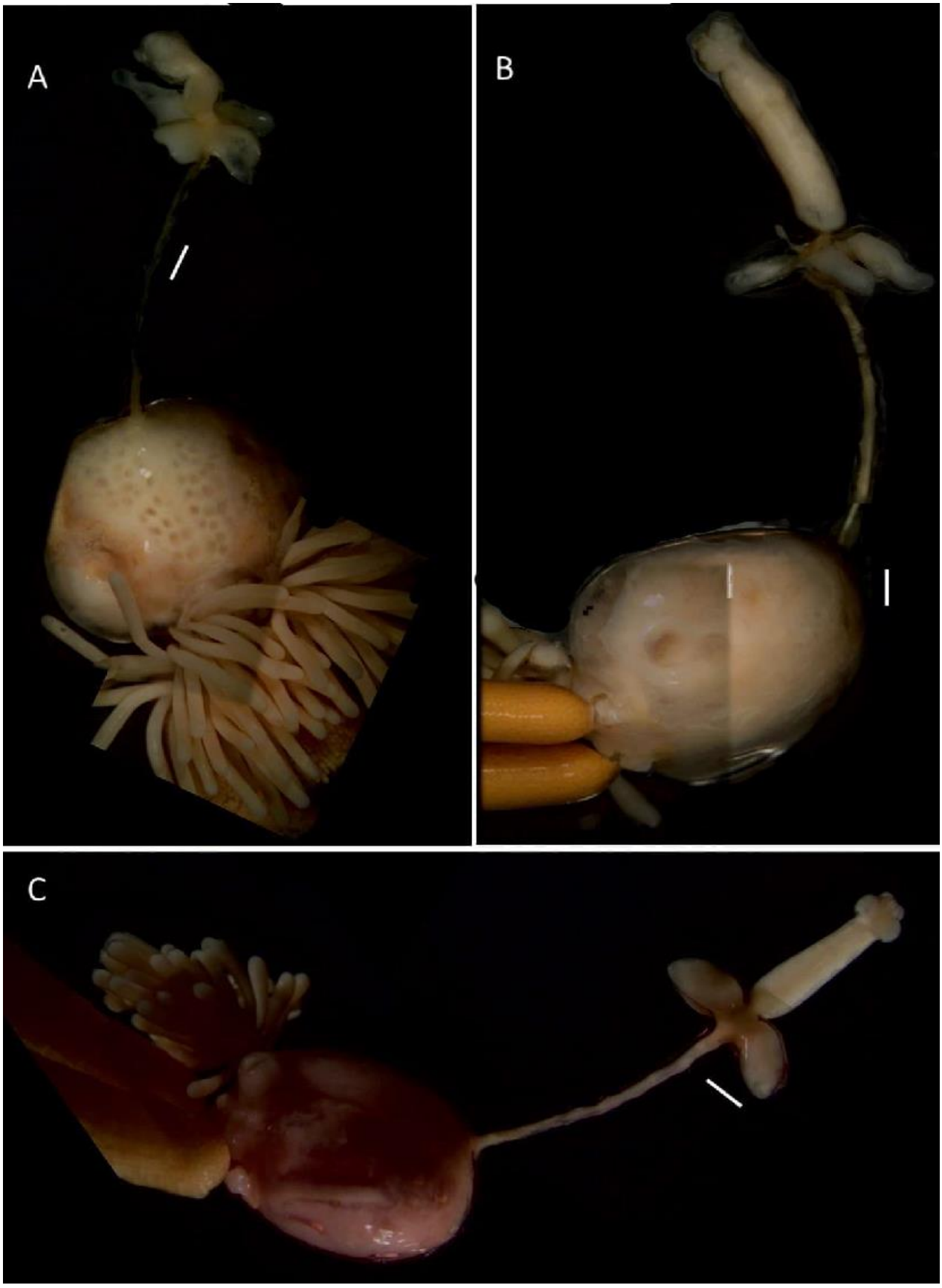

A
B
C

Figure 4.10: *Lophoura cf edwardsi* Kölliker, 1853 post-metamorphosis female. A. general habitus, trunk dorsal view, cephalothorax ventral view; B. cephalothorax, dorsolateral view; C. cephalothorax, anterior view; D, abdomen, dorsal view; E. antennule; F. antenna; G. maxilliped. Scale bars: A – D = 1 mm and E – G = 10 µm. (mp – maxillary process, ap – antennary process).

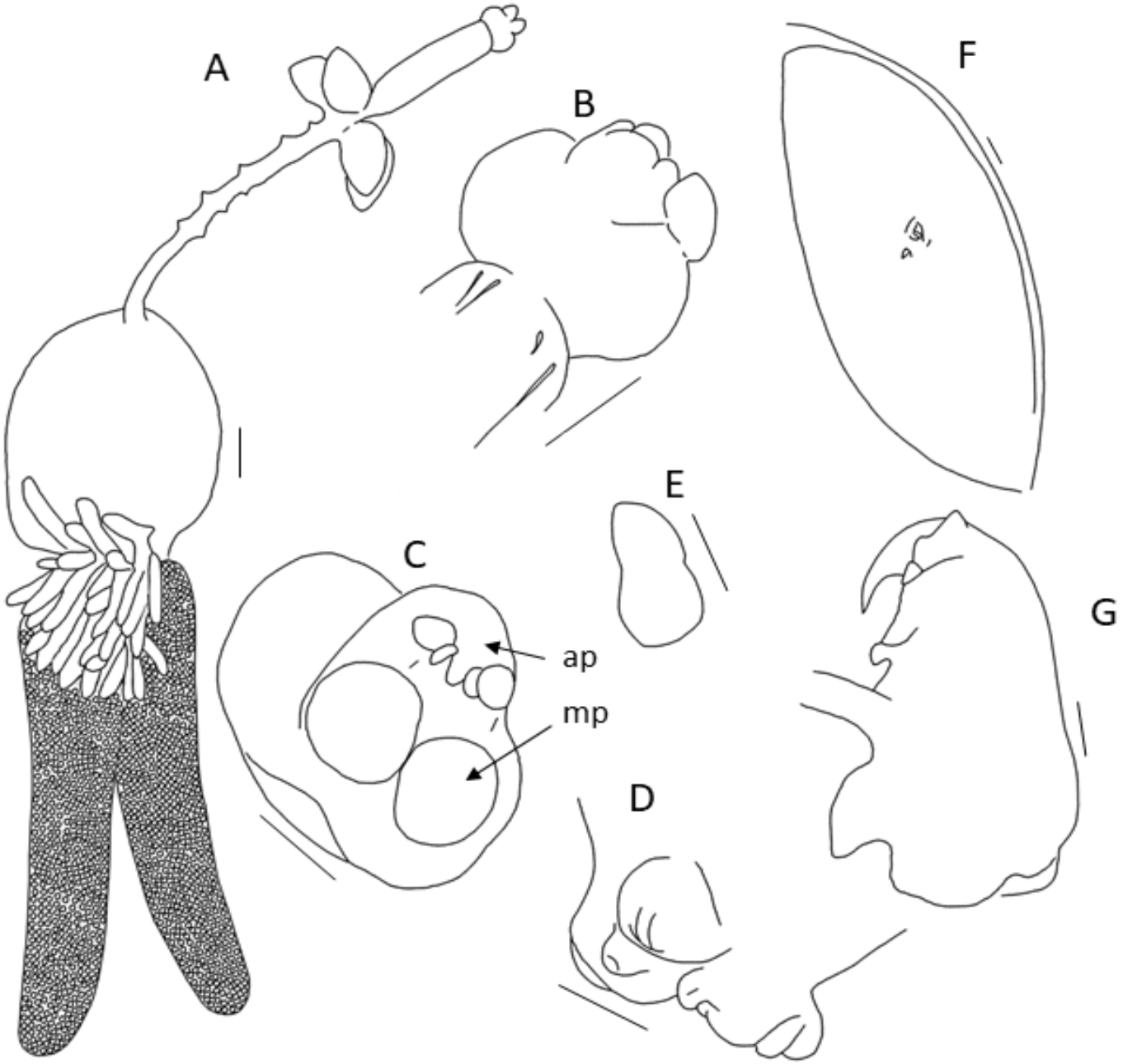

A
B
F
E
C
ap
mp
G
D

Figure 4.11: *Lophoura cf edwardsi* Kölliker, 1853 post-metamorphosis female. Scanning electron micrographs: A. cephalothorax anterior view; B. cephalothorax anterolateral view; C. antennule; D. antenna; E. cephalothorax, ventral view; F. maxilliped. Scale bars: A – B = 500 µm; C – D = 10 µm; E = 500 µm; F= 20 µm. (ap – antennary process, a1 – antennule, a2 – antenna, mp – maxillary process, mgp – maxillary gland pore, mxp – maxilliped, cg – circular groove).

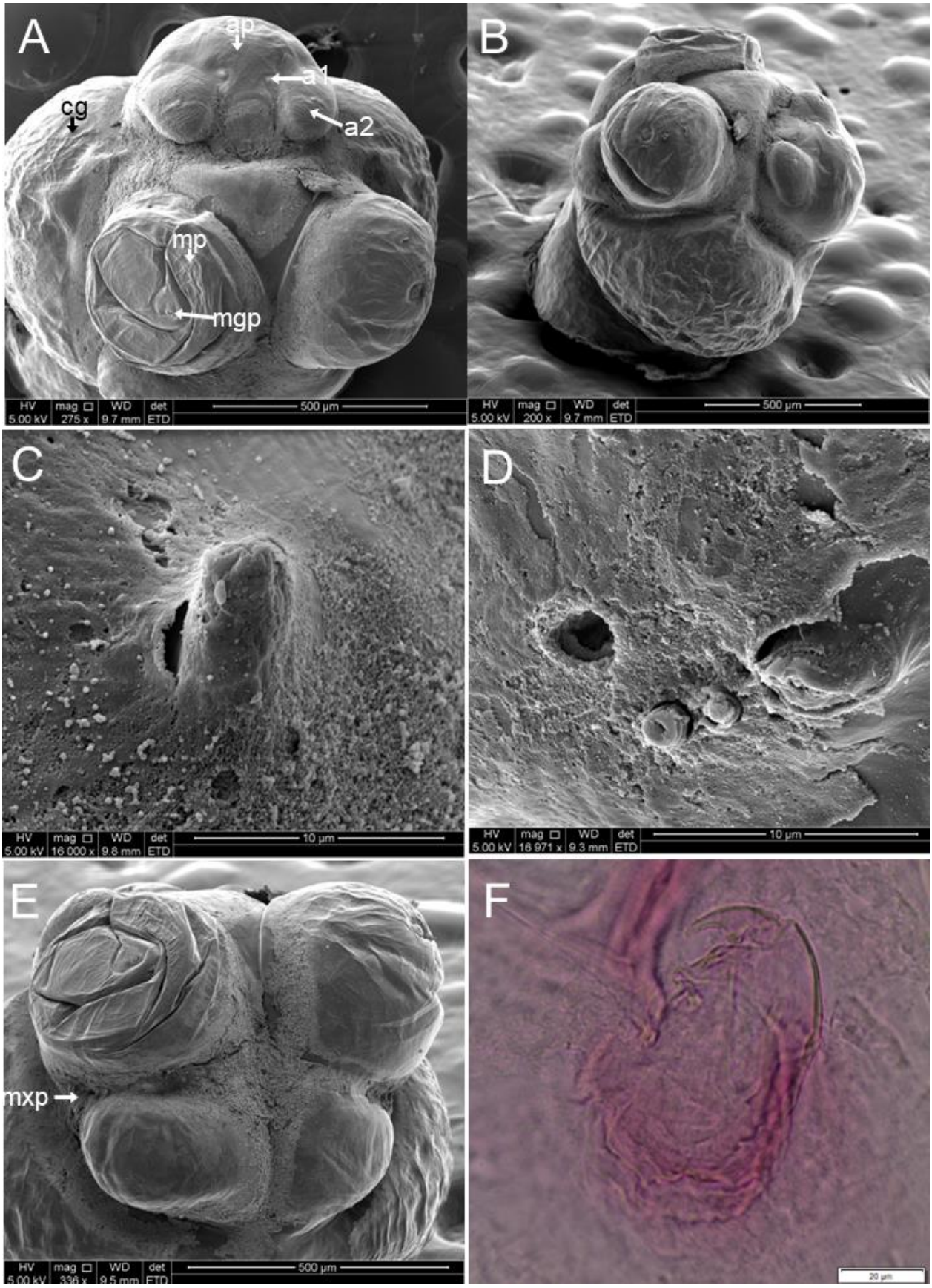

A
ap
a1
cg
a2
mp
mgp
HV 5.00 kV
mag 275 x
WD 9.7 mm
det ETD
500 µm
B
HV 5.00 kV
mag 200 x
WD 9.7 mm
det ETD
500 µm
C
HV 5.00 kV
mag 16 000 x
WD 9.8 mm
det ETD
10 µm
D
HV 5.00 kV
mag 16 971 x
WD 9.3 mm
det ETD
10 µm
E
mxp
HV 5.00 kV
mag 336 x
WD 9.5 mm
det ETD
500 µm
F
20 µm

Figure 4.12: *Lophoura caparti* (Nuñes-Ruivo, 1962) post-metamorphosis female. A. general habitus, trunk ventral view, cephalothorax dorsal view. B. holdfast organ; C. abdomen, dorsal view. Scale bars: A – C = 1 mm

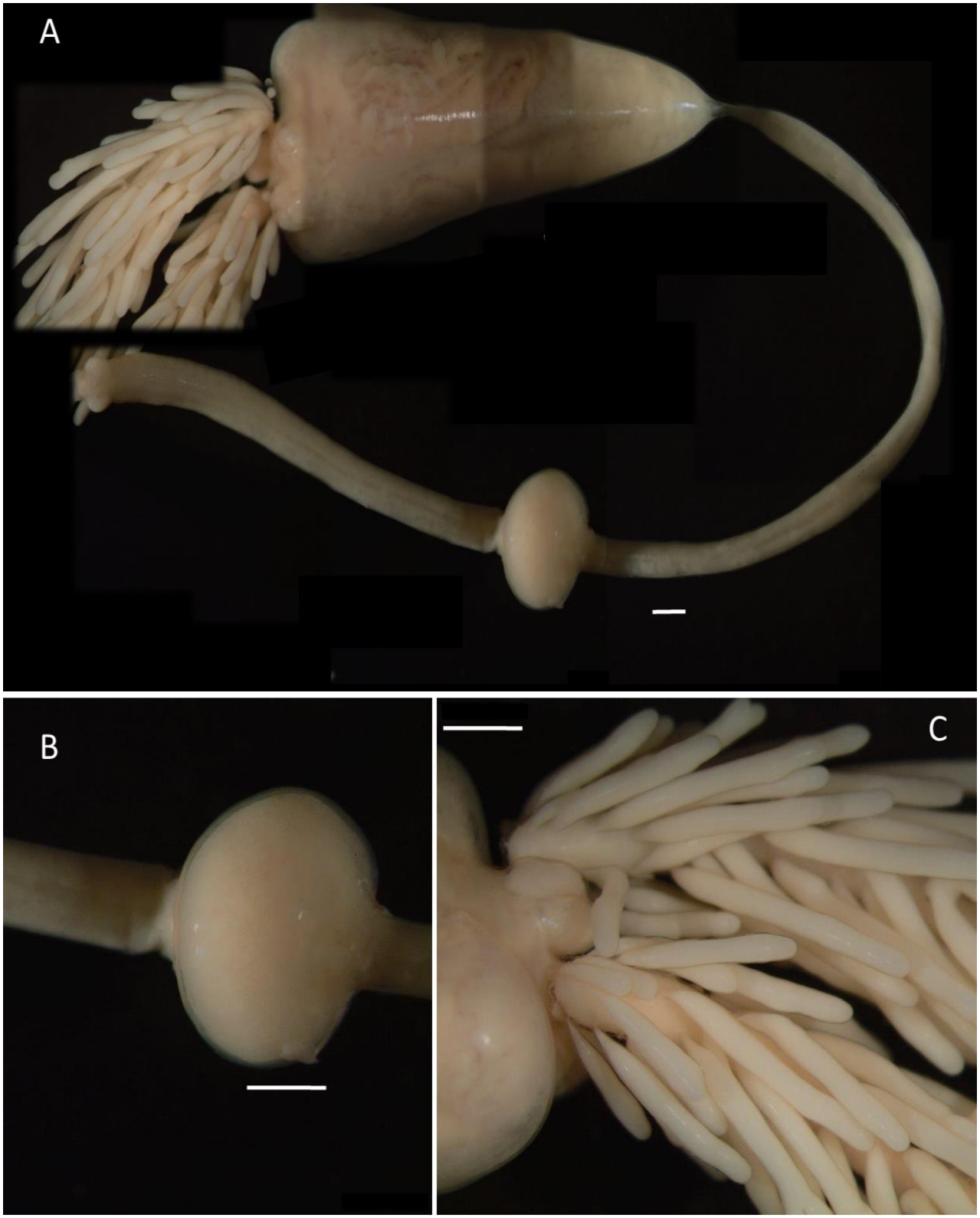

A
B
C

Figure 4.13: *Lophoura caparti* (Nuñes-Ruivo, 1962) post-metamorphosis female. A. general habitus, trunk dorsal view, cephalothorax ventral view; B. cephalothorax, ventral view; C. cephalothorax, dorsal view; D. abdomen; E. antennule; F. antenna; G. maxilliped. Scale bars: A – D = 1 mm and E – G = 10 µm. (mp – maxillary process, ap – antennary process).

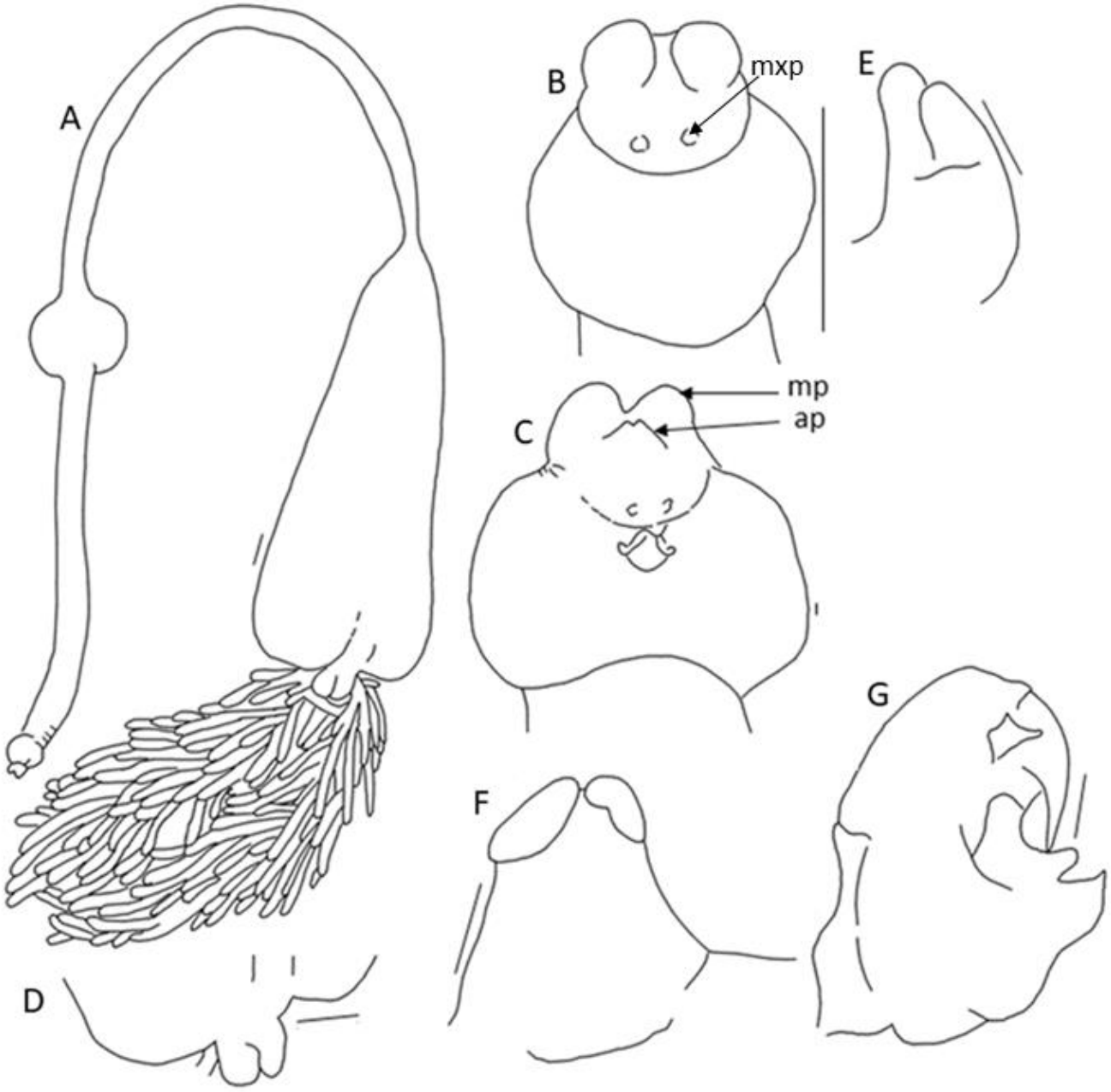

A
B
mxp
E
mp
ap
C
G
F
D

Figure 4.14: *Lophoura caparti* (Nuñes-Ruivo, 1962) post-metamorphosis female. A. cephalic region, anterior view; B. antennule; C. antenna; D. maxillule; E. maxilliped. Scale bars: B, C and E = 5 µm and D = 20 µm. (ap – antennary process, a1 – antennule, a2 – antenna, mp – maxillary process, mx1 – maxillule).

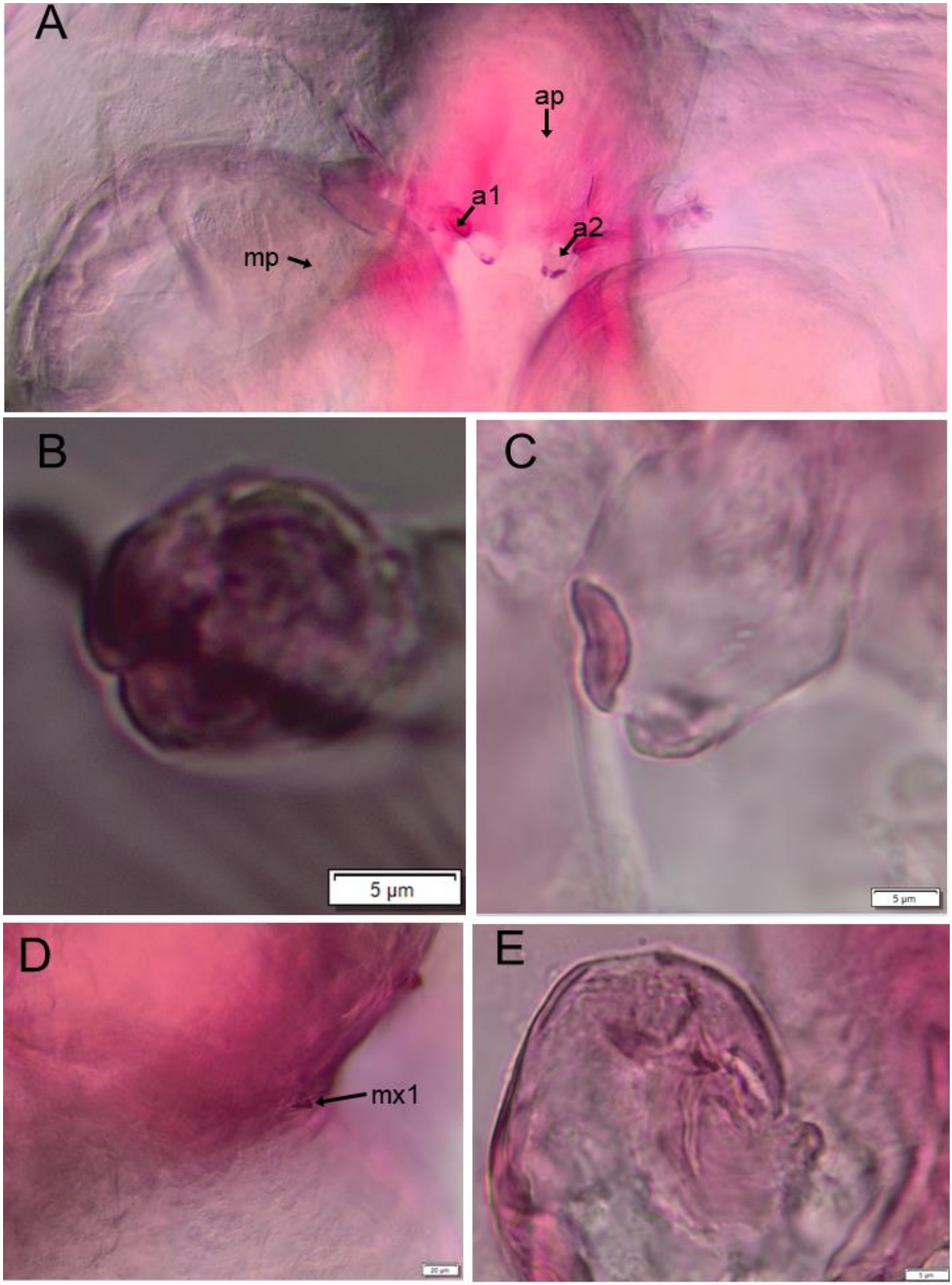

A
ap
a1
a2
mp
B
5 µm
C
5 µm
D
mx1
E

Figure 4.15: *Lophoura cornuta* (Wilson C.B., 1919) post-metamorphosis female. A. general habitus, trunk lateral view, cephalothorax lateral view; B. holdfast organ, lateral view; C. abdomen, lateral view. Scale bars: A – C = 1 mm.

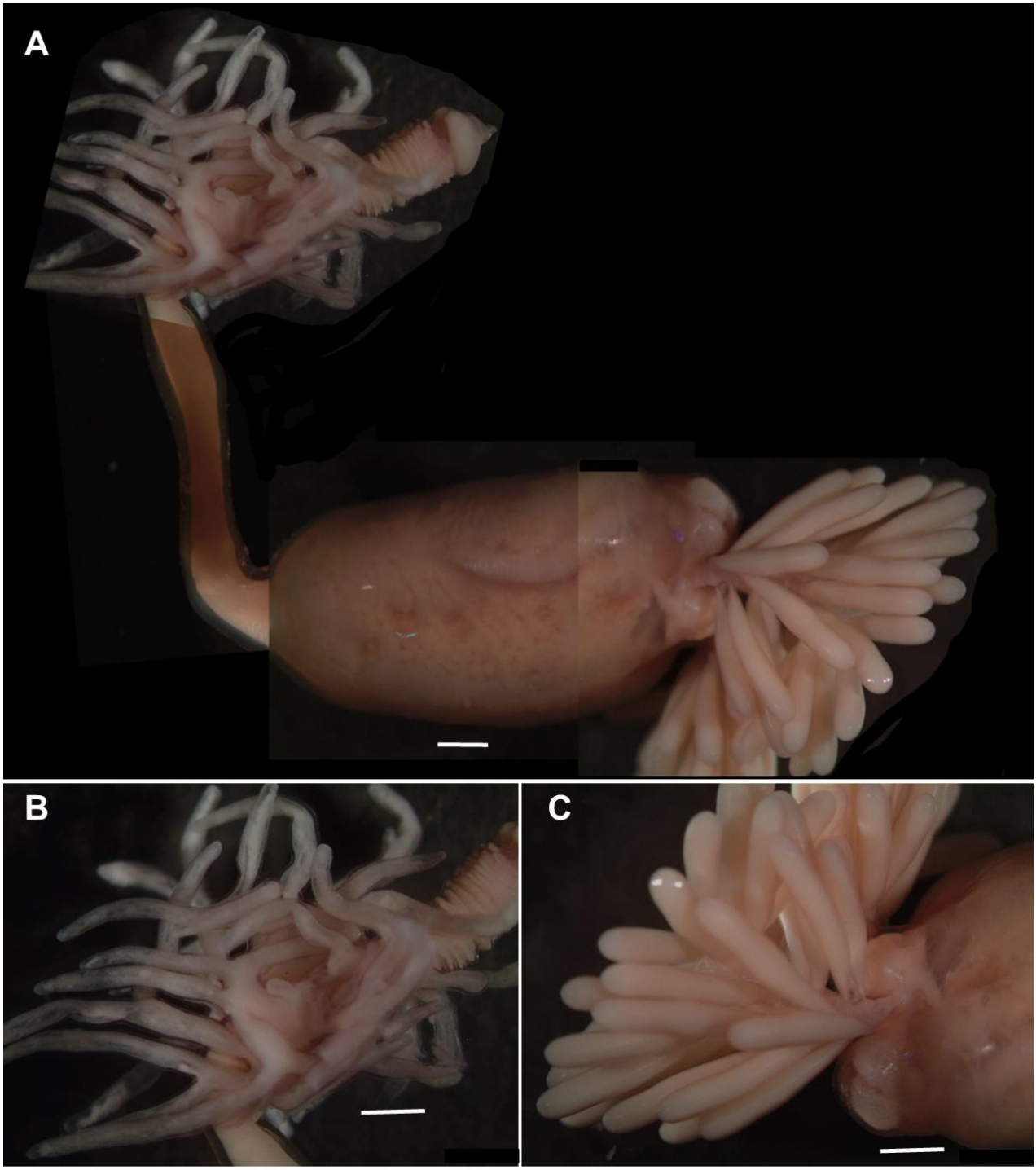

A
B
C

Figure 4.16: *Lophoura cornuta* (Wilson C.B., 1919) post-metamorphosis female. A. general habitus, trunk ventrolateral view, cephalothorax lateral view; B. general habitus, trunk ventrolateral view, cephalothorax lateral view; C. cephalothorax, lateral view; D. cephalothorax, anterior view; E. trunk, ventral view. Scale bars: A – E = 1 mm. (mp – maxillary process, ap – antennary process).

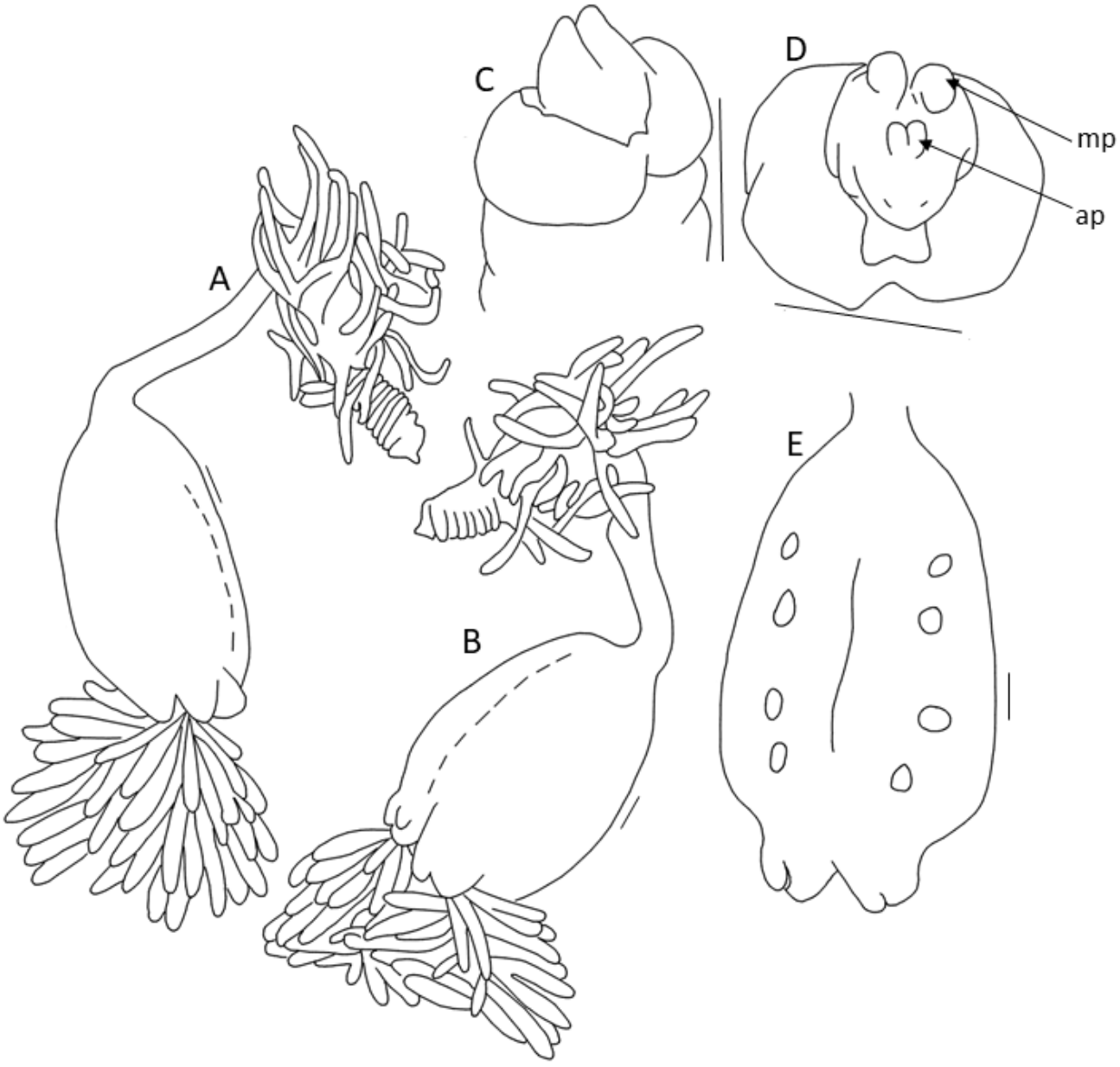

A
B
C
D
mp
ap
E

Figure 4.17: *Lophoura* sp. post-metamorphosis female. A. cephalothorax and holdfast organ, posterolateral view; B. cephalothorax and holdfast organ, anterolateral view; C. trunk, ventral view; D. trunk, ventral view; E. abdomen, dorsal view; F. posterior processes. Scale bars: A – F = 1 mm

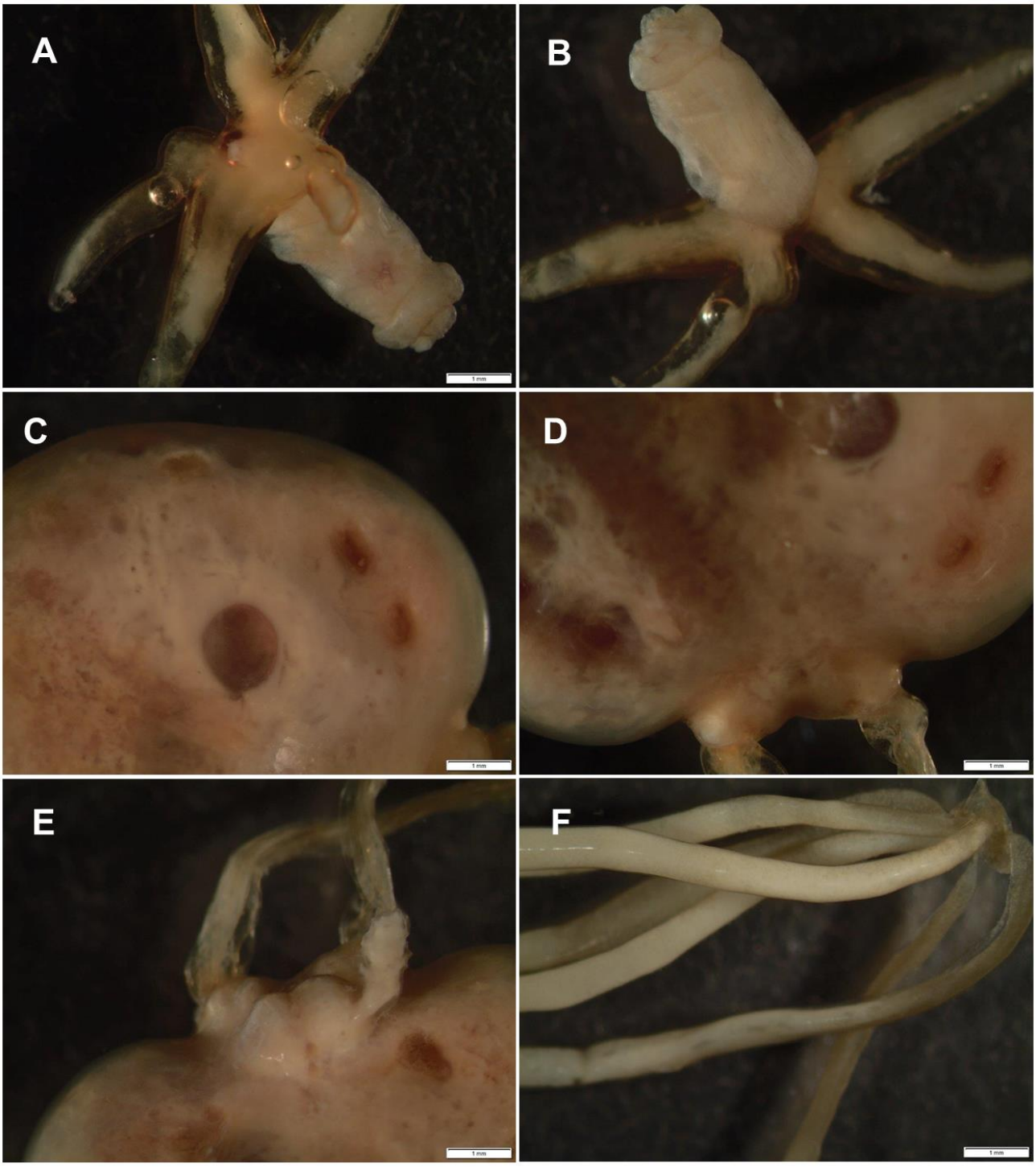

A
B
C
D
E
F

Figure 4.18: *Lophoura* sp. post-metamorphosis female. A. cephalothorax and holdfast organ, posterolateral view; B. cephalothorax, anterolateral view; C. cephalothorax, anterior view; D. trunk, ventral view; E. abdomen, dorsal view. Scale bars: A – E = 1 mm. (mp – maxillary process, ap – antennary process).

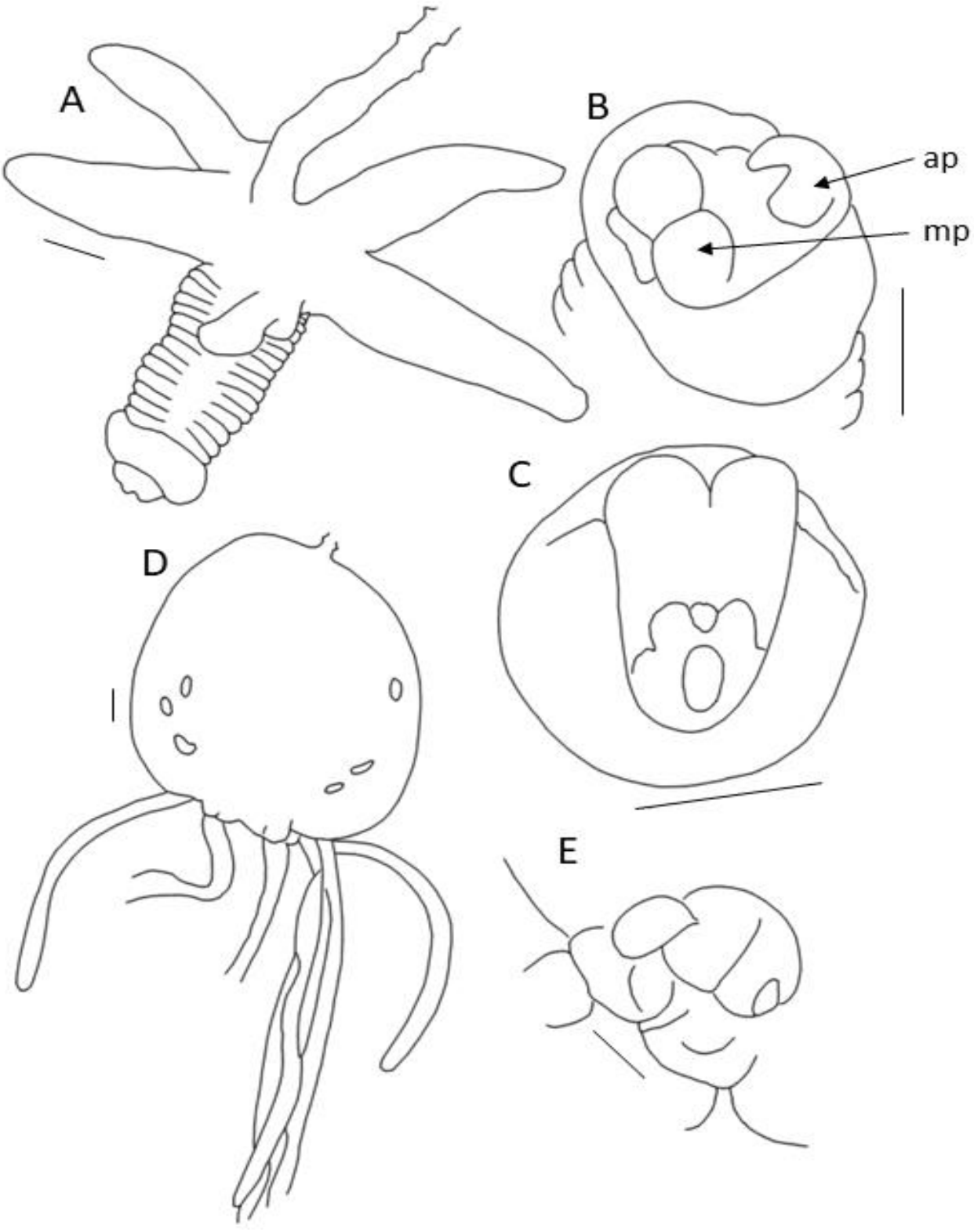
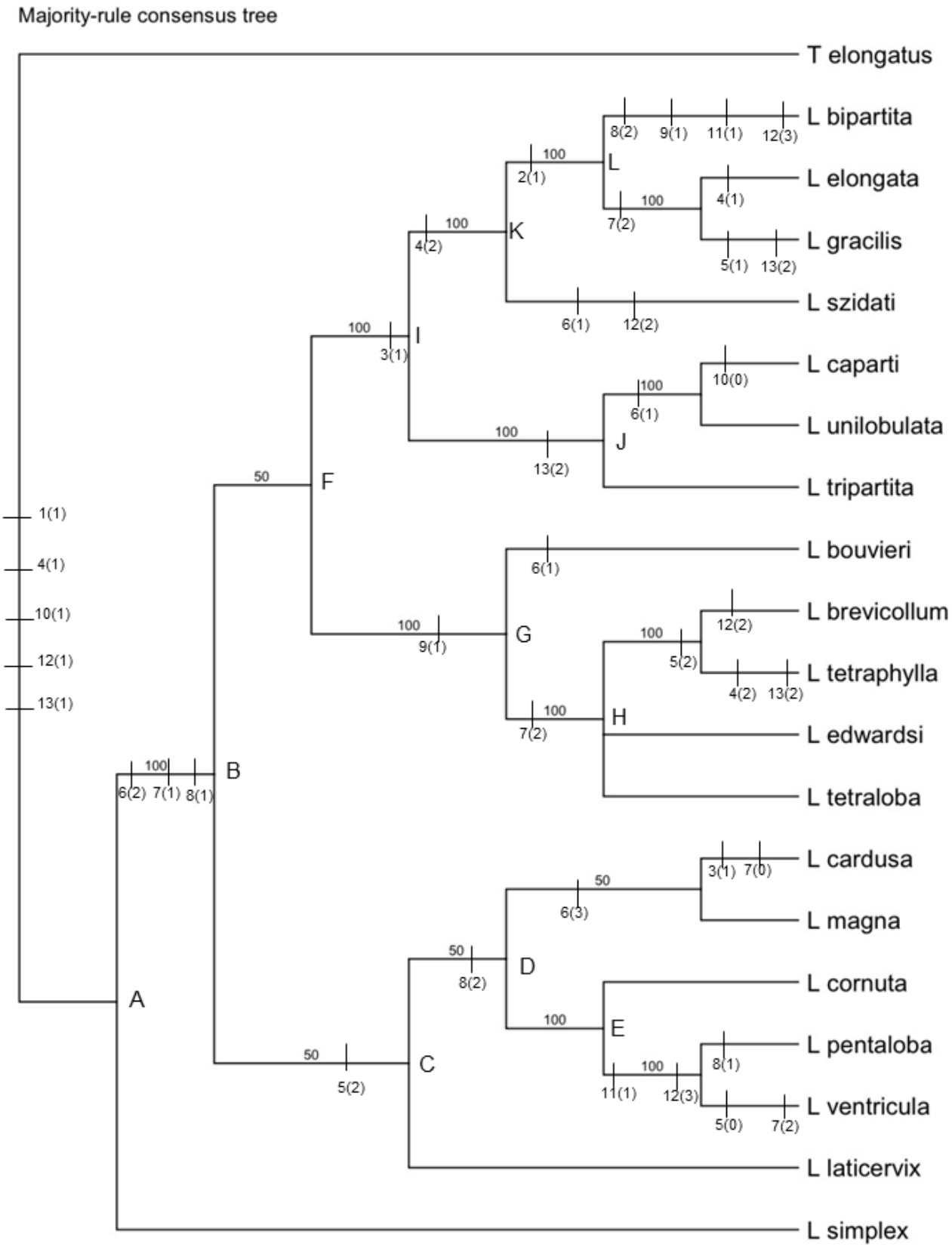

A
B
ap
mp
C
D
E

Figure 4.19**:** A 50% majority rule consensus tree of two most parsimonious trees (TL= 42, CI = 0.5238, HI = 0.4762, RI = 0.6078, RCI = 0.3184) estimating the phylogenetic relationships among *Lophoura* species (ingroup species), with *Tripaphylus elongatus* as an outgroup species. A – L represent nodes, while character transformations are marked on the branches.


Majority-rule consensus tree
T elongatus
L bipartita
L elongata
L gracilis
L szidati
L caparti
L unilobulata
L tripartita
L bouvieri
L brevicollum
L tetraphylla
L edwardsi
L tetraloba
L cardusa
L magna
L cornuta
L pentaloba
L ventricula
L laticervix
L simplex
A
B
C
D
E
F
G
H
I
J
K
L
1(1)
4(1)
10(1)
12(1)
13(1)
8(2) 9(1) 11(1) 12(3)
2(1)
4(1)
7(2)
4(2)
5(1) 13(2)
6(1) 12(2)
3(1)
10(0)
6(1)
13(2)
6(1)
12(2)
9(1)
5(2)
4(2) 13(2)
7(2)
6(2) 7(1) 8(1)
3(1) 7(0)
6(3)
8(2)
5(2)
11(1)
12(3)
8(1)
5(0) 7(2)
100
50

CHAPTER 5: Molecular systematics

5.1. Introduction

Recently there has been an increase in supplementing basic morphological studies with molecular data focusing on the 'DNA barcode' (the cytochrome oxidase I gene (COI)) with the primary goal of discrimination and identification of species. Additionally, the COI barcode is also used to estimate evolutionary processes, demographic history and aspects regarding the population biology of a species. However, the best approach is to combine morphological and molecular techniques in an integrative approach (Bucklin et al. 2021).

Sphyriidae females are highly transformed as a result of their mesoparasitic lifestyles, resulting in each species having a wide range of diverse characters (Kabata 1979) which makes species identification more complicated. Furthermore, some of the morphological variability is a result of the age of species, their site of attachment and possibly the water depth of the host's habitat. Thus, as recommended for marine copepods (Laakmann et al. 2020), integration of morphological and DNA-based taxonomy is preferable to avoid misidentifications. By supplementing morphological studies with molecular studies, conclusions can more easily be drawn as to whether it's a new species or cryptic species or just underdeveloped species (Castro-Romero et al. 2016).

Mitochondrial DNA, specifically the cytochrome c oxidase subunit I (COI) gene, mainly aids in resolving taxonomic affiliation of copepods at species level. It was previously used in relation to symbiotic Copepoda to determine phylogenetic relationships among siphonostomatoid families symbiotic on elasmobranchs (Dippenaar 2009); to distinguish between cryptic species in *Nessipus orientalis* (Dippenaar et al. 2010); to determine genetic diversity of *Nemesis* species (Mangena et al. 2014) and to discern polymorphism in Pennellidae (Castro-Romero et al. 2016). In this study, the use of DNA barcoding with COI was intended to identify and distinguish between species by intraspecific and interspecific variations based on sequence divergence (Meyer and

Paulay 2005). Currently the only COI data available on Genbank (https://www.ncbi.nlm.nih.gov) for members of Sphyriidae is that of *Tripaphylus elongatus* (accession nr: FJ447393.1).

5.2. Material and methods

Refer to Chapter 2.

5.3. Results

DNA extractions were done for all three collected species of *Sphyrion* and three species of collected *Lophoura* (Table 2.1 and Table 5.1). PCR was performed for DNA samples with desired concentrations, and PCR products were visualised on 1.5% agarose gel (Figs. 5.1. A – F).

Table 5.1: Representatives of Sphyriidae for which DNA extractions were done, the host species from which they were collected, DNA concentration, UV absorption at 260 nm (A260), UV absorption at 280 nm (A280), ratio 260/280 and ratio 260/230.

Species name	Host species name	DNA concentration (ng/µl)	A260	A280	260/280	260/230
1. *S. quadricornis*	*Coelorinchus simorhynchus*	113.5	2.270	1.094	2.08	0.77
2. *S. quadricornis*	*Coelorhinchus trunovi*	112.4	2.249	1.231	1.83	0.9
3. *S. lumpi*	*Coelorhinchus trunovi*	105.1	2.102	1.341	1.57	0.53
4. *S. lumpi*	*Allocytus verrucosus*	221.7	4.433	3,274	1.35	0.65
5. *S. lumpi*	*Coelorinchus simorhynchus*	187.5	3.749	2.231	1.68	0.53
6. *S. lumpi*	*Mesovagus antipodium*	86.8	1.737	1.057	1.64	0.57

7. *S. laevigatum*	*Genypterus capensis*	142.6	2.853	1.420	2.01	1.31
8. *S. laevigatum*	*Genypterus capensis*	114.1	2.283	1.681	1.36	0.34
9. *L. tetraloba*	*Coelorhinchus fasciatus*	158.8	3.176	2.329	1.36	0.68
10. *L. tetraloba*	*Lucigadus ori*	120.5	3.749	2.231	1.68	0.98
11. *L. cf edwardsi*	*Coelorhinchus fasciatus*	76.8	1.537	0.834	1.84	0.56
12. *L. caparti*	*Epigonus denticulatus*	107.7	2.153	1.026	2.10	1.89
13. *L. caparti*	*Epigonus denticulatus*	97.3	1.737	1.057	1.64	1.20

Even though the DNA concentrations seemed high enough for successful PCR, most DNA samples did not have the required 260/280 and 260/230 ratios of 1.8 and 2.0 respectively. Thus, even though there were some samples with apparent successful amplification of the COI gene (Figs. 5A – F) none was successfully sequenced. Apparently all the DNA samples were contaminated by phenol and carbohydrates since, all ratios of 260/230 were less than 2.0, which may have been caused by residual guanine which are common column-based kits (Wilfinger et al. 1997). However best laboratory protocols were practiced to avoid contamination.

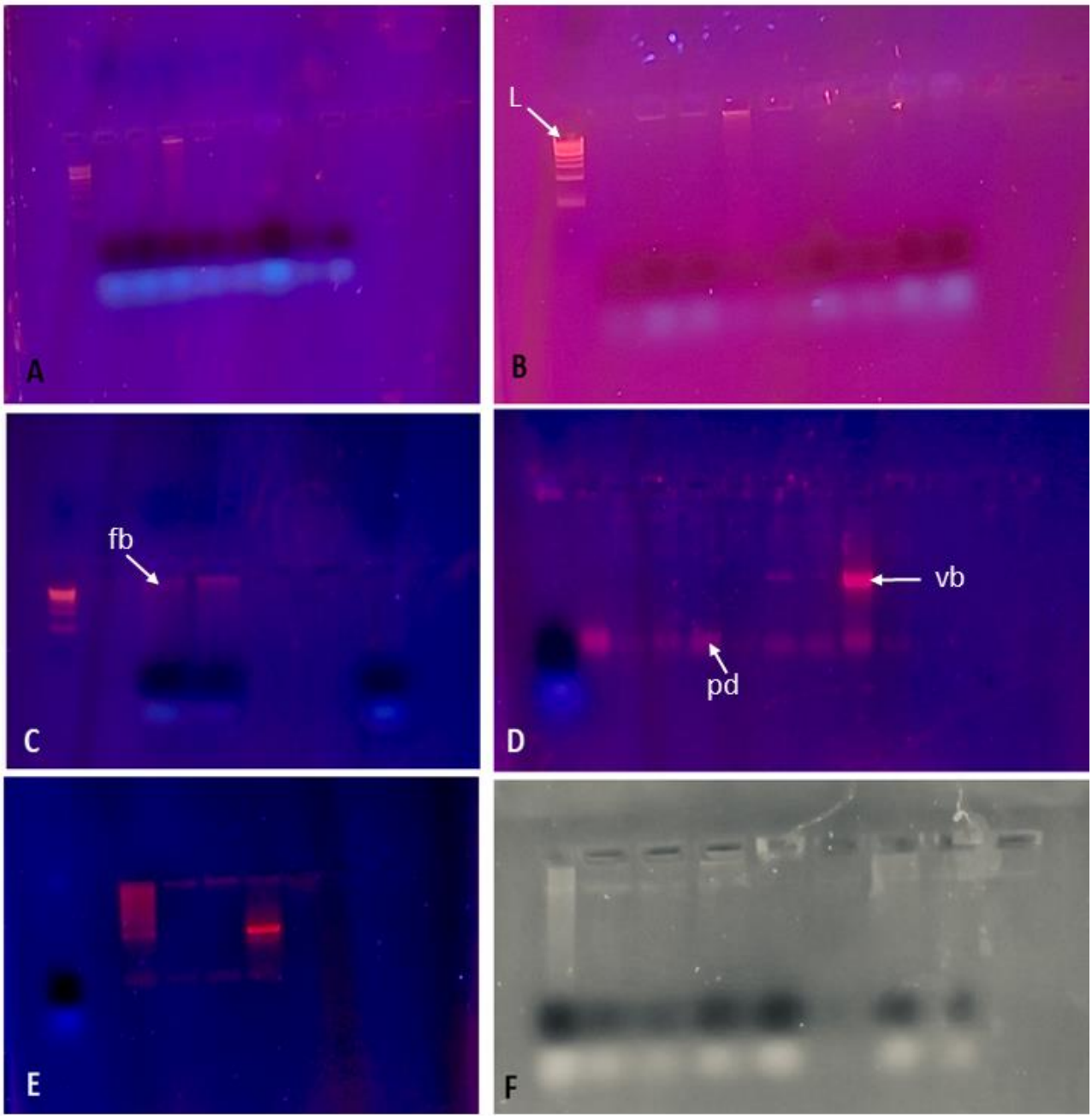
5.4. Discussion

Morphological examination of collected species of *Sphyrion* resulted in unexplainable variations amongst specimens e.g. *Sphyrion laevigatum* females (see Chapter 3, 3.3.1.1) from the same host species (*Genypterus capensis*) as well as *S. lumpi* females (see Chapter 3, 3.3.1.2) from different host species with different vertical distributions. Additionally, morphological differentiation between *Lophoura cf edwardsi* (see Chapter 4, 4.1.1.1) and *L. tetraloba* (see Chapter 4, 4.1.1.2) is problematic mostly due to incomplete descriptions of *L. edwardsi* (Kabata 1979) and lack of including this species in comparisons of species with a holdfast organ consisting of four main processes (Ho and Kim 1989; Gómez et al. 2010).

Thus, addition of molecular data may have assisted in clarifying the interspecific and intraspecific relationships of the species. However, the success of molecular identification is based on the quality of DNA and molecular markers. Different primer pairs (see Table 2.2) were used in an attempt to successfully amplify the COI gene. However, even apparently successful amplifications (see Figs. 5A – F) could not be sequenced. Reasons for failure may be low DNA quality, possibly degraded due to the collection methods used for firstly, the fish hosts and secondly, that of the parasites, but also by prolonged preservation (collected between 1991 and 2016) of specimens. Additionally, some specimens were stored in formaldehyde before being transferred to 70% ethanol.

It is recommended that sphyriid specimens should be dissected from their fish hosts as soon as the hosts are caught and immediately preserved in 70% EtOH for morphological examination and species identification and then soon be transferred to absolute EtOH to preserve the material for molecular analysis. Additionally, more sphyriids should be studied via integrated taxonomy to overcome any taxonomic confusion and reduce possible synonymies. Furthermore, sequence divergence between similar species with hosts of different vertical distribution can be used to support morphological findings of the effect of vertical distribution of fish on its ectoparasites (as discussed in Gómez et al. (2010)).

Figure 5.1: Examples of PCR products visualized with 1.5% agarose gel electrophoresis. A. PCR products of *Sphyrion* and *Lophoura* species (Ladder, *S*. *quadricornis*, *S*. *lumpi*, *S*. *laevigatum*, *L*. *tetraloba*, *L*. *edwardsi*, *L*. *caparti*, positive control and negative control respectively); B. PCR products of *Sphyrion* and *Lophoura* species (Ladder, *S*. *quadricornis*, *S*. *lumpi*, *S*. *laevigatum*, *L*. *tetraloba*, *L*. *edwardsi*, *L*. *edwardsi*, *L*. *caparti*, positive control and negative control respectively); C. PCR products of *Sphyrion* and *Lophoura* species (Ladder, *S*. *quadricornis*, *S*. *lumpi*, *L*. *edwardsi*, *L*. *caparti*, positive control and negative control respectively); D. PCR products of *Sphyrion* and *Lophoura* species (*S*. *quadricornis*, *S*. *lumpi*, *S*. *lumpi*, *S*. *laevigatum*, *L*. *tetraloba*, *L*. *edwardsi*, *L*. *caparti*, positive control and negative control respectively); E. PCR products of *Sphyrion* species (*S*. *lumpi*, *S*. *lumpi*, *S*. *laevigatum*, positive control and negative control respectively); F. PCR products of *Sphyrion* and *Lophoura* species (*S*. *quadricornis*, *S*. *lumpi*, *S*. *laevigatum*, *L*. *tetraloba*, *L*. *edwardsi*, *L*. *caparti*, positive control and negative control respectively). (L = ladder, fb = faint band, vb = visible band, pd = primer dimer).


A
L
B
fb
C
vb
pd
D
E
F

CHAPTER 6: General discussion and conclusions

South African marine waters have a very high biodiversity, which consist of highly studied and understudied groups (Schaeffner and Smit 2019), including symbiotic Copepoda on marine bony fish. This study aimed to report on selected representatives of Sphyriidae collected (between 1991 and 2016) from marine bony fish in coastal waters off southern Africa and to attempt to clarify any existing confusion regarding their taxonomy and systematics. This was done by providing morphological illustrations and descriptions/re-descriptions of members of Sphyriidae collected from bony fish off the South African coast.

Family Sphyriidae consists of two groups of genera, one which infect Osteichthyes and the other which infect Chondrichthyes (see Fig. 8 Gómez et al. (2010)). In this study collected species of two genera infecting Osteichthyes (*Lophoura* and *Sphyrion*), were studied. *Lophoura* and *Sphyrion* are closely related as estimated in previous cladistic analyses (Benz et al. (2006); Gómez et al. (2010)). However, their morphology differ, for instance (in post-metamorphosis females), *Lophoura* is characterised by possession of an elongated cephalothorax; a cylindrical neck with a holdfast near posterior end of cephalothorax; a trunk of different shapes and elevated perianal region; posterior processes with a porous peduncle bearing stalks of different shapes and lengths, and long, straight egg sacs with multi-seriate eggs (see Kabata (1979) and Chapter 4 in current study) whereas *Sphyrion* is characterised by possession of hammer-shaped (sphyra) cephalothorax; cylindrical neck; a round, flat trunk with a short abdomen; branched and grape-like posterior processes, and long, straight egg sacs with multi-seriate eggs (see Kabata (1979); Chapter 3 in current study). Even though the males of both genera resemble the males of Lernaeopodidae (Kabata 1979), the males of *Lophoura* is characterised by possession of a cephalothorax which is more than half of the total body length and an elongated trunk with clear/traces of segmentation with a pair of caudal rami posteriorly (see Wilson (1919); Nuñes-Ruivo (1962); Ho and Kim (1989) and Chapter 4 (4.3.1.1) in current study) whereas the males of *Sphyrion* is characterised by possession of a cephalothorax which is less than half of the total body length and an ovular trunk,

without segmentation, bearing small caudal rami on posterior margin (see Moran and Piasecki (1994); Dippenaar and Sebone (2022); see Chapter 3 in current study).

Lophoura species have been reported from four host orders whereas *Sphyrion* species have been reported from seven host orders, with Eupercaria and Gadiformes in common (Table 6.1). Some host species are infected by both *Sphyrion* and *Lophoura* species (Table 6.1) including *Antimora rostrata* (*S*. *lumpi* and *L*. *tetraphylla*); *Coelorinchus fasciatus* (*S*. *laevigatum*, *S*. *lumpi*, *S*. *quadricornis*, *L*. *cf edwardsi*, *L*. *laticervix* and *L*. *tetraloba*); *Coryphaenoides armatus* (*S*. *lumpi* and *L*. *pentaloba*); *Macrourus berglax* (*S*. *lumpi*, *S*. *quadricornis* and *L*. *bouvieri*); and *Macrourus holotrachys* (*S*. *lumpi* and *L*. *szidati*). Macrouridae seems to be the preferred host family of both *Sphyrion* (all three species, see 3.1) and *Lophoura* (twelve species, see 4.1) species. In contrast to *Sphyrion* species, which infect more than one host order, each of the *Lophoura* species have only been reported from one family, in one order (Table 6.1).

Table 6.1: Reported host species of *Lophoura* and *Sphyrion* species with geographic location (compiled from Wilson 1919, 1935; Nuñes Ruivo 1962; Hewitt 1964; Szidat 1971; Kensley and Grindley 1973; Kabata 1979; Ho 1985; Hogans and Dadswell 1985; Ho and Kim 1989; Boxshall 1989; Ho 1992; Moran & Piasecki 1994; Boxshall 2000; Castro-Romero and Gonzalez 2009; Gómez et al. 2010; Kazachenko 2017; Froese and Pauly 2022; Walter and Boxshall 2022; Dippenaar and Sebone 2022; and the current study).

Host order	Host species	Sphyriid species	Ocean reported from
Acropomatiformes	*Epigonus denticulatus* Dieuzeide, 1950	*L*. *caparti*	Atlantic Ocean, South Africa
	Epigonus telescopus (Risso, 1810)	*L*. *caparti*	Atlantic Ocean, Angola; Indian Ocean, South Africa
Anguiliformes	*Bassanago albescens* (Barnard, 1923)	*L*. *cornuta*	Atlantic Ocean, South Africa

	Histiobranchus bathybius (Günther, 1877)	*L. elongata*; *L. gracilis*; *L. simplex*	Atlantic Ocean (*L. gracilis* off New Jersey, USA; *L. simplex* off Challenger stn); Atlantic and Indian Ocean (*L. elongata* off Cape Point, South Africa)
	Synaphobranchus affinis Günther, 1877	*L. cornuta*	Atlantic Ocean, Japan
	Synaphobranchus brevidorsalis Günther, 1887	*L. cornuta*; *L. gracilis*	Atlantic Ocean (*L. gracilis* off New Jersey, USA) Pacific Ocean (*L. cornuta* off New Caledonia)
	Synaphobranchus kaupii Johnson, 1862	*L. gracilis*	Atlantic Ocean, New York Bight, USA
Aulopiformes	*Saurida undosquamis* (Richardson, 1848)	*S. quadricornis*	Indian Ocean, South Africa
Eupercaria	*Atractoscion aequidens* (Cuvier, 1830)	*S. laevigatum*	Indian Ocean, South Africa
	Calamus bajonado (Bloch & Schneider, 1801)	*L. tripartita*	Atlantic Ocean, Gulf of Mexico
	Pagellus bogaraveo (Brünnich, 1768)	*S. lumpi*	Unknown
Gadiformes	*Antimora rostrata* (Günther, 1878)	*L. tetraphylla*; *S. lumpi*	Atlantic Ocean (both New York Bight, USA; *L. tetraphylla* off Canada; *S. lumpi* off South Africa)
	Boreogadus saida (Lepechin, 1774)	*S. lumpi*	Arctic Ocean, Spitzbergen
	Coelorinchus braueri Barnard, 1925	*S. quadricornis*	South-east Atlantic Ocean
	Coelorinchus caelorinchus (Risso, 1810)	*L. edwardsi*	Atlantic Ocean, Portugal

	Coelorinchus fasciatus (Gunther, 1878)	*S. laevigatum*; *S. lumpi*; *S quadricornis*; *L. tetraloba*; *L. cf edwardsi*; *L. laticervix*	Indian Ocean (*S. laevigatum* off South Africa) Atlantic Ocean (*L. tetraloba*; *L. cf edwardsi* off South Africa); Pacific Ocean (*L. laticervix* off New Zealand)
	Coelorinchus innotabilis McCulloch, 1907	*S. quadricornis*	Pacific Ocean, New Zealand
	Coelorinchus marinii Hubbs, 1934	*S. lumpi*	Atlantic Ocean, Brazil
	Coelorinchus simorhynchus Iwamoto & Anderson, 1994	*S. lumpi*; *S. quadricornis*	Atlantic Ocean, South Africa
	Coelorinchus trunovi Iwamoto & Anderson, 1994	*S. lumpi*; *S. quadricornis*	Indian Ocean, South Africa
	Coryphaenoides armatus (Hector, 1875)	*S. lumpi*; *L. pentaloba*	Atlantic Ocean, New York Bight, USA (both)
	Coryphaenoides filifer (Gilbert, 1896)	*L. pentaloba*	Pacific Ocean, California
	Coryphaenoides nasutus Günther, 1877	*L. ventricula*	Pacific Ocean, Japan
	Coryphaenoides rupestris Gunnerus, 1765	*S. lumpi*	Northern Atlantic Ocean
	Coryphaenoides subserrulatus Makushok, 1976	*L. bipartita*	Pacific Ocean, Australia
	Gadus morhua Linnaeus, 1758	*S. lumpi*	Norwegian Sea
	Gaidropsarus ensis (Reinhardt, 1837)	*S. lumpi*	Atlantic Ocean, Canada
	Hymenocephalus striatissimus Jordan & Gilbert, 1904	*L. cardusa*	Pacific Ocean, Japan
	Kumba japonica (Matsubara, 1943)	*Lophoura* sp. (from Ho and Kim 1989)	Pacific Ocean, Japan
	Laemonema laureysi Poll, 1953	*S. lumpi*	Atlantic Ocean, Angola

	Lepidion ensiferus (Günther, 1887)	*L. magna*	Atlantic Ocean, Malvinas Islands
	Lucigadus ori (Smith, 1968)	*L. tetraloba*; *L. cf edwardsi*	Atlantic Ocean (*L. tetraloba*; *L. cf edwardsi* off South Africa)
	Macrourus berglax Lacepède, 1801	*S. quadricornis*; *S. lumpi*; *L. bouvieri*	Indian Ocean (*L. bouvieri* off Sudan) Atlantic Ocean (*S. lumpi* and *S. quadricornis* off Greenland)
	Macrourus holotrachys Günther, 1878	*S. lumpi*; *L. szidati*	Atlantic Ocean (*L. szidati* off Orkney Islands)
	Merluccius australis (Hutton, 1872)	*S. laevigatum*	Pacific Ocean, Chile
	Merluccius bilinearis (Mitchill, 1814)	*S. lumpi*	Atlantic Ocean
	Merluccius hubbsi Marini, 1933	*S. laevigatum*	Unknown
	Merluccius merluccius (Linnaeus, 1758)	*S. lumpi*	Atlantic Ocean
	Merluccius polli Cadenat, 1950	*S. laevigatum*	Unknown
	Mesovagus antipodium (Hubbs & Iwamoto, 1977)	*S. lumpi*	Atlantic Ocean, South Africa
	Molva dypterygia (Pennant, 1784)	*S. lumpi*	Arctic Ocean, East Greenland
	Nezumia bairdii (Goode & Bean, 1877)	*L. bouvieri*; *L. pentaloba*	Atlantic Ocean (*L. pentaloba* off New York Bight, USA; *L. bouvieri* off Martha's Vineyard, Block Island and New Jersey, USA)
	Nezumia condylura Jordan & Gilbert, 1904	*L. tetraloba*	Pacific Ocean, Japan

	Nezumia liolepis (Gilbert, 1890)	*L. brevicollum*	Pacific Ocean off Mexico and California
	Nezumia pulchella (Pequeño, 1971)	*L. unilobulata*	Pacific Ocean, Chile
	Nezumia stelgidolepis (Gilbert, 1890)	*L. unilobulata*	Pacific Ocean, Peru
	Nezumia umbracincta Iwamoto & Anderson, 1994	*Lophoura* sp.	Atlantic Ocean, South Africa
	Urophycis tenuis (Mitchill, 1814)	*S. lumpi*	Atlantic Ocean, Canada
	Ventrifossa nasuta (Smith, 1935)	*S. lumpi*	Indian Ocean, South Africa
Ophidiiformes	*Genypterus blacodes* (Forster, 1801)	*S. laevigatum*	Pacific Ocean off New Zealand and Chile; Atlantic Ocean off Falkland Islands
	Genypterus capensis (Smith, 1847)	*S. laevigatum*	Atlantic Ocean, South Africa; Indian Ocean, South Africa
Perciformes	*Anarhichas denticulatus* Krøyer, 1845	*S. lumpi*	Off Greenland
	Anarhichas lupus Linnaeus, 1758	*S. lumpi*	Atlantic Ocean (North Sea)
	Cyclopterus lumpus Linnaeus, 1758	*S. lumpi*	North Sea, Iceland
	Psychrolutes inermis (Vaillant, 1888)	*S. lumpi*	Atlantic Ocean, South Africa
	Psychrolutes macrocephalus (Gilchrist, 1904)	*S. lumpi*	Atlantic Ocean, South Africa
	Sebastes fasciatus Storer, 1854	*S. lumpi*	Atlantic Ocean, Canada
	Sebastes flammeus (Jordan & Starks, 1904)	*S. lumpi*	Pacific Ocean, Japan
	Sebastes mentella Travin, 1951	*S. lumpi*	Atlantic Ocean, Irminger sea; Mid-Atlantic ridge
	Sebastes norvegicus (Ascanius, 1772)	*S. lumpi*	Atlantic Ocean (off Cape Cod, Canada)

			North Sea, Iceland, Greenland, Newfoundland
	Sebastes viviparus Krøyer, 1845	*S. lumpi*	Unknown
Pleuronectiformes	*Glyptocephalus cynoglossus* (Linnaeus, 1758)	*S. lumpi*	Atlantic Ocean, Newfoundland
	Reinhardtius hippoglossoides (Walbaum, 1792)	*S. lumpi*	Atlantic Ocean (off Norway and Labrador, Canada)
	Solea solea (Linnaeus, 1758)	*S. lumpi*	Mediterranean Sea
Zeiformes	*Allocyttus verrucosus* (Gilchrist, 1906)	*S. lumpi*	Atlantic Ocean, South Africa

This study contributes to the knowledge of copepods symbiotic on marine Osteichthyes off South Africa and also adds to the knowledge of the marine siphonostomatoid biodiversity as well as their distribution. Additional information on the morphology of the selected Sphyriidae species is provided including full descriptions and illustrations of all valid *Sphyrion* species females (*S. laevigatum*, *S. lumpi* and *S. quadricornis*) and the males of *S. laevigatum* and *S. quadricornis* (Dippenaar and Sebone 2022) as well as that of five *Lophoura* species females (*L. caparti*, *L. cornuta*, *L. cf edwardsi*, *L. tetraloba* and *Lophoura* sp.) and the male of *L. tetraloba*. Furthermore, it adds to our knowledge of the hosts infected by these sphyriid species as well as information regarding the host specificity of the selected species. The knowledge of our world's symbiotic copepods can help the present and upcoming generations to better understand the world of copepods and their interactions with fish.

In future, the focus should be on resolving the confusion in the morphological description of *L. edwardsi* to the extent that it is clearly differentiated from *L. tetraloba* or their synonymy should be confirmed. Additionally, more specimens of *Nezumia umbracincta* should be examined for copepods since *Lophoura* sp. (Chapter 4 (4.3.1.4)) might be a new species specific to this host. Apparently, *N. umbracincta* is only distributed across southern African marine waters (Froese and Pauly 2022), hence *Lophoura* sp. (Chapter 4 (4.3.1.4)) might be endemic to southern Africa.

Molecular data should be used to attempt to estimate intraspecific and interspecific relationships between Sphyriidae species across each genus infecting marine Osteichthyes. Specific attention should be given to the intraspecific variations between *S*. *lumpi* individuals from different host species to determine if variations observed in the morphology (see Chapter 3 (3.3.1.2)) represent intraspecific variation or maybe another species. Additionally, molecular intraspecific variations may also provide possible explanations regarding the wide range of host orders infected by *S*. *lumpi* (Table 6.1). Similarly, intraspecific variations in the molecular data may also resolve the observed intraspecific morphological variations among *S*. *laevigatum* specimens collected from the same host species (see Chapter 3 (3.3.1.1)).

More marine deep-water fish should be examined for copepods to determine the diversity of sphyriid species infecting these fish and to increase knowledge about their biodiversity off South Africa. Additionally, more information regarding the habitat of the host species together with the morphological features of the infecting sphyriid is needed since it may provide valuable information regarding the relation between water depth and the variation in morphological features of the infecting sphyriids (Gómez et al. 2010).

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