Biodiversity and systematics of branchial cavity inhabiting fish parasitic isopods (Cymothoidae) from sub-Sahara Africa

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ABBREVIATIONS

CF – Condition factor; **COI** – Cytochrome c oxidase subunit I; **DELTA** – Descriptive Language for Taxonomy; **FHAI** – Fish Health Assessment Index; **FL** – Fork length; **GCF** – Gutted condition factor; **GSI** – Gonadosomatic index; **HSI** – Hepatosomatic index; **J** – Juvenile; **MEGA** – Molecular Evolutionary Genetics Analysis; **ML** – Maximum Likelihood; **MNHN** – National Museum of Natural History, Paris, France; **NCBI** – National Center for Biotechnology Information; **NWU** – North-West University, Potchefstroom Campus; **RV** – Research vessel; **SAM** – Iziko South African Museum, **SL** – Standard length; Cape Town; **SSI** – Splenosomatic index; **STDEVA** – Standard deviation; **TL** – total length; **W** – width.

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ABSTRACT

Isopods from the family Cymothoidae Leach, 1814 are well known ectoparasites of marine and freshwater fishes, most often attaching to the external surface, branchial chamber or buccal cavity of their hosts. Cymothoids have a cosmopolitan distribution but are mostly recorded in warmer, tropical regions from shallow, coastal waters, to the deep sea. The sub-Sahara African coastal region complies with these characteristics that favour the establishment of cymothoids as many species have already been recorded from this region. Within the Cymothoidae family, the branchial attaching genera *Norileca* Bruce, 1990; *Elthusa* Schioedte & Meinert, 1884 and *Mothocya* Costa, in Hope, 1851 have been recorded from the sub-Sahara African region.

Although it is expected that the biodiversity of these cymothoids would be much higher in this region than what is currently recorded, there are many challenges that obstruct the advancement of the taxonomy of these cymothoids. These challenges have hindered the research of cymothoids in many regions, leaving numerous species undiscovered or undescribed. This observation has led to the hypothesis that it is the lack of sampling and collection, rather than the lack of species, that accounts for the low number of branchial cavity attaching cymothoid species from the sub-Sahara African region. To test this hypothesis, unexamined specimens from sub-Sahara Africa were collected and examined, including museum material. These sample specimens were subjected to morphological analysis to provide full descriptions of each identified species, and to confirm the taxonomic placement of unidentified museum material. Molecular characterisation on fresh material was done by sequencing a fragment of the mitochondrial cytochrome oxidase I (COI) gene, providing genetic data and confirming morphological analysis. Diagnostic identification criteria have been provided, along with identification keys, to simplify and aid in the correct identification of species in future collections.

The genus *Norileca* contains three known species, of which only one species, *Norileca indica* (Milne Edwards, 1840), has been recoded from sub-Sahara Africa. *Norileca indica* is fully redescribed based on ovigerous females collected from Maputo Bay, Mozambique, from the branchial cavity of the fish host *Selar crumenophthalmus* Bloch, 1793. The first comprehensive description of a male specimen is also included and an identification key to the species of *Norileca* Bruce, 1990 is given. Furthermore, a fragment of the mitochondrial cytochrome oxidase I (COI) gene from *N. indica* was sequenced for the first time. This is the first molecular characterisation of a species of *Norileca*.

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Elthusa is considered to be among the most morphologically varied and species-rich genera of the family Cymothoidae, consisting of 31 described species. *Elthusa raynaudii* Schioedte and Meinert, 1884 is the only species that has been recorded from sub-Sahara Africa, and from southern Africa in particular. Three distinct species of *Elthusa* have been identified in the current study, *E. raynaudii* as well as two undescribed species. Specimens obtained from RV *Africana* and those collected from Alexander Bay during 1993 represent new species. In addition to the morphological analysis, each identified species was sequenced using a targeted part of the mitochondrial cytochrome oxidase I (COI) gene. These sequences were used to confirm the distinction between the species as well as to determine their phylogenetic placement and relationship to other genera from the family Cymothoidae.

The genus *Mothocya* contains 31 globally distributed species. Specimens resembling *Mothocya* were examined. This material originated from sub-Sahara African countries Nigeria and Kenya. From morphological analyses, three *Mothocya* species could be identified. These included one well-known and described species, *Mothocya renardi* (Bleeker, 1857) collected from Kenya. The remaining two species, both from Nigeria, resemble new and undescribed species. Full descriptions and diagnostic information were provided for these new species.

In addition to the description of four new branchial cavity attaching species, a case study was done on the effects that these ectoparasites might have on their hosts. For this case study, *Mothocya affinis* Hadfield, Bruce & Smit, 2015 and its host, the tropical halfbeak *Hyporamphus affinis* (Günther, 1866) were collected from Sodwana Bay, South Africa. By considering the relatively large size and attachment techniques of these parasitic cymothoids to their hosts, it was expected that they would induce some measure of negative impact on the fish host's health. This led to the second hypothesis, that branchial cavity inhabiting cymothoids would have a quantifiable or noticeable negative impact on the health of its host. In order to test this second hypothesis, the case study was executed to determine the visible effects and change in health condition of infected hosts compared to uninfected fish. A fish health assessment yielded no significant difference in the condition and health of infected hosts, compared to uninfected ones. Although condition indices provided no substantial evidence of internal health effects, the physical effects of these branchial attaching cymothoids were evident, especially at the site of attachment.

Thus, both hypotheses were confirmed. An increase in sampling and collection of cymothoids across sub-Sahara Africa yielded four new species from two branchial cavity inhabiting cymothoid genera. In addition, the genetic data of all species encountered was obtained. Frayed and discoloured gills, shortened opercula, and a physical depression created by the attachment of the gill parasite were noticed. This provides evidence that branchial cavity attaching cymothoid genera can have a negative effect on the general health of their fish hosts.

Keywords

Cymothoid, molecular characterisation, morphological analysis, *Norileca*, *Elthusa, Mothocya*, taxonomy, fish health assessment.

CHAPTER 1: INTRODUCTION

The term “parasite” often resonates to people as something that cause diseases and even death as general perception is usually based on well known, medical and veterinarian important parasites and how they are portrayed (Bush et al., 2001). In fact, most plant and animal species are most likely to be infected with one or more parasite during their lifetime. According to the Oxford Dictionary of Biology, parasitism can be defined as “an association in which one organism (the parasite), lives in, or on the body of another (the host), from which it obtains its nutrients”. This symbiotic relationship can be defined in many ways, depending on the research interest and context in which it is used e.g. ecological, physiological, medical and/or economical. Parasitism is considered a very successful lifestyle; often going unnoticed (Rohde, 2005).

Parasites are usually most abundant in tropical and subtropical terrestrial regions, mainly because of the high species diversity and favourable environmental transmission conditions (Bush et al., 2001). This is no different in aquatic ecosystems, where fishes can act as final or intermediate hosts of adult and juvenile forms of parasites. There are numerous applications of parasites to research. They can be used as indicators of the overall health of an ecosystem and how they contribute to shaping ecosystem structures and ecology (Landsberg et al., 1998; Hudson et al., 2006); or as biological tags for the study of commercially important fish species (MacKenzie et al., 2008; Lester and MacKenzie, 2009). This includes the use of parasites in geographical studies, to determine the distribution of infected hosts via migration (Williams et al., 1992; Rohde, 2002). Parasites can be used for the monitoring of pollution, as they can be indicative of pollution levels that the host is exposed to, or how pollution affects the transmission of parasites between hosts (Williams and MacKenzie, 2003; Sures, 2004). Biodiversity studies frequently include parasites as they can be responsible for the incline or decline of host biodiversity, ultimately altering ecosystem health and community structure (Marcogliese, 2003; Hudson et al., 2006).

The grouping of parasites is based on the position that they occupy on the host (ecto- or endoparasitic); the size of the parasite (macro- or microparasitic); as well as the duration of infection and dependence on its host (permanent, obligate, facultative) (see Rohde, 2005). Ectoparasites occupy the exterior of the host’s body surface (Bush et al., 2001), such as ticks and fleas of mammals. Similarly, in marine systems, these ectoparasites can be seen covering the external surfaces of the host and are abundant and diverse. Many of these marine ectoparasites belong to the subphylum Crustacea Brünnich, 1772.

1.1 Subphylum Crustacea Brünnich, 1772

Crustaceans are the most diverse and abundant of the metazoan groups (Lester, 2005) and are thought to have a predominant marine origin (Moore, 2006). The approximate 66 914 known crustacean species (Zhang, 2011) are found in freshwater, terrestrial and marine environments. Countless marine inhabitants are essential role-players in food chains and in the preservation of a balanced ecosystem (Barnes, 1987). Although crustaceans are mostly free-living, many are associated symbionts of other animal species. Crustacean diversity and abundance have resulted in a complex hierarchy of classification to group these taxa (Barnes, 1987).

Crustaceans are characterised by features that are more commonly associated with aquatic, rather than terrestrial forms, such as the hardened cuticle that results from calcium salts and tanned proteins (Moore, 2006). Additional distinctive features include five pairs of head appendages (two pairs of antennae and maxillae, and one pair of mandibles). The class Malacostraca Latreille, 1802, is characterised by an anterior non-segmented rostrum and a non-segmented telson with uropods at the posterior end, creating a tail fan (Hickman et al., 2008).

1.2 Order Isopoda Latreille, 1817

As one of the largest orders within the crustacean subphylum, the isopods form part of the superorder Peracarida and are considered to be the most morphologically diverse and species rich order of crustaceans, ranging in size from 1 mm up to 40 cm (Bruce, 2001; Hadfield, 2012; Kazmi and Yousuf, 2013). They are distributed worldwide in fresh, marine and terrestrial habitats, often recognised by locals as pill bugs, wood lice, slaters or snow bugs (Bruce, 2001; Ghani, 2003). Isopods can easily be identified by their dorso-ventrally flattened body without a carapace (Kensley and Schotte, 1989). Some species have the ability to adapt their body colour to the background of their environment with the aid of chromatophores, making colour an unreliable feature for species identification (Barnes, 1987). Brandt and Poore (2003) further characterised isopods by the cephalon being attached to the anterior margin of pereonite 1, with forwardly projecting coxae 1. The movability and distinction between cephalon and pereonite may vary between taxa. In most cases, the pereonite 1 and coxae 1 are fused together, not creating a distinct margin between the segments as with the rest of the pereonites and coxae. The pleon consist of six pleonites, from which pleonite 6 is fused with the telson to create the pleotelson (Brandt and Poore, 2003).

Marine isopods have been found in almost all ecological niches ranging from hadal and abyssal depths, to the sea shore (Kensley, 1978). They form a dominant part of the marine bottom-dwelling fauna biodiversity (Bruce, 2001). The importance of marine isopods lies within their ecological role and potential anthropogenic uses. They exhibit considerable variations in terms of their ecological roles specifically regarding their feeding mode (Sidabalok, 2015). The free living isopods are predominantly detritivores, feeding on and breaking down dead plant or animal matter (Lester, 2005). Browsers, carnivores and scavengers create potential anthropocentric purposes for these isopods as shark carcass cleaners (Poore and Bruce, 2012) and possible bio-indicators of water quality as well as marine pollution (Lee, 1977). They can also be used as health and diversity bio-indicators of coral reefs (Jameson et al., 1998), due to them being susceptible to environmental change, easy to collect, slow moving, non-migrating and not exploited for human use (Sidabalok, 2015).

Parasitic or fish associated isopods can be used as model parasites for experimental and observational research objectives due to their large size, making them easy to observe and handle; their survivability; ease of manipulation and range of association and ubiquity (Bunkley-Williams and Williams, 1998). These characteristics are not always available in other types of parasites. These parasitic isopods have adapted to survive on both fish and crustacean hosts, mainly found in warm, tropical marine waters (Lester, 2005).

1.3 Parasitic Isopoda

From the 95 known and accepted Isopoda families, only eight are parasitic, namely Bopyridae, Corallanidae, Cryptoniscidae, Cymothoidae, Dajidae, Entoniscidae, Gnathiidae and Tridentellidae (Lester, 2005; Smit et al., 2014). The three major groups of parasitic isopods are the epicaridians that are parasites of crustaceans as juveniles and adults; the gnathiids that are parasites of fish only during their larval stages (adults are free-living and non-feeding); and the cymothoids, which are parasites of fish as juveniles as well as adults (Lester, 2005; Ravichandran et al., 2009; Hadfield, 2012). These extremely diverse organisms vary greatly in terms of body shape and size.

In general it is thought that parasitic isopods feed on host blood or haemolymph by using their complex mouthparts that include maxillipeds and mandibles that pierce and slit open the flesh of the host tissue (Lester, 2005). These parasites are considered to be intermittent feeders as they store their food in the hind gut and slowly digest the contents as it moves to the midgut (Lester, 2005; Ravichandran et al., 2009).

Brusca (1975) is of the opinion that man must have been aware of parasitic isopods since taking up fishing in the ocean. This explains why the first fish parasitic isopods where already described hundreds of years ago. Nevertheless, parasitic isopods have, until recently, seldom been studied in the sub-Sahara African region, causing limited data availability on their biodiversity, distribution, hosts and ecology (Hadfield et al., 2010; Hadfield, 2012). The lack of information regarding parasitic isopods in Africa and other parts of the world cannot be attributed to a low diversity of these isopods, but rather the lack of researchers interested in these studies (Trilles, 1994). It is essential to gather knowledge and information on parasitic isopods in order to overcome the challenge of determining the effects that these isopods might have on fish hosts and host populations (Paperna and Overstreet, 1981; Hadfield, 2012). Cymothoid isopods are representatives of these parasitic isopods of fish and other crustacean species (Verma, 2005).

1.4 Cymothoidae Leach, 1814

1.4.1 Introduction to the Cymothoidae

The isopod family, Cymothoidae, contains some of the largest living isopods (Brusca, 1981) and represents obligate ectoparasites of various marine, fresh and brackish water fish species (Kensley and Schotte, 1989; Smit et al., 2014). Following the Sphaeromatidae family, the Cymothoidae are the most abundant in described genera and species. Schioedte and Meinert (1884) were among the first authors to dedicate an entire publication on the description of cymothoids in particular. As a result, the Cymothoidae taxonomic history dates far back. The rather large size of these parasites (>6 mm) made it possible for taxonomists to study them in the early years, as they are easy to notice, collect and handle (Smit et al., 2014). To date, there are 43 cymothoid genera recognised and accepted (Smit et al., 2014; Hadfield et al., 2017b), and with the increase in research on cymothoids recently, this number is bound to rise in the future.

Many taxonomists are of the opinion that cymothoids are one of the most difficult isopod taxa to work with (Brusca, 1981; Hadfield et al., 2010; Trilles and Randall, 2011; Smit et al., 2014). They closely resemble the family Aegidae, which are facultative/temporary ectoparasites of fish (Brusca, 1975), but are distinguished from the latter by having smaller eyes, pleopods without setae and often an asymmetrical body shape. The pereopods of the cymothoids are all prehensile with a strongly hooked dactylus longer than propodus (Kensley, 1978; Kensley, 1989; Kensley and Schotte, 1989), whereas only the first three

pereopods of Aegidae are prehensile and the rest are ambulatory, meaning they are adapted as walking appendages (Kensley, 1978; Lester, 2005).

Cymothoid parasites can further be distinguished from other isopod families by the eyes that are laterally positioned (when present); a pleon with free pleonites; reduced antennae and antennulae (Kensley and Schotte, 1989), with peduncle and flagellum not easily distinguishable (Kensley and Schotte, 1989; Bruce et al., 2002); mandibular palp present with three articulated segments; maxilla with a lateral lobe and smaller medial lobe, each with a minimum of two robust setae (Brandt and Poore, 2003); prehensile pereopods; lamellar pleopods lacking marginal setae (Kensley and Schotte, 1989), some with fleshy folds; and appendages are mostly or completely lacking any kind of setae, with the exception of the mouthparts (Bruce et al., 2002).

Some cymothoids present a twisted body shape, ranging in degree from weakly twisted to strongly twisted (Bruce et al., 2002). Brusca (1975) mentions that the twisted body can most likely be attributed to the physiological stress on the parasite's body due to the available growth space and attachment position on the host. Many adults are recorded with having distorted or curved bodies due to their habit of attaching and fitting into snug spaces on the fish host. The growth of external attaching cymothoids is not limited by space and thus they are frequently symmetrical (Kensley, 1978).

Cymothoids are found attached to the host skin surface (exterior body); inside the buccal cavity; inside the branchial cavity; or in the muscle tissue of the host (Ghani, 2003; Hata et al., 2017). This position of attachment is usually genus or species specific (Kensley and Schotte, 1989; Smit et al., 2014) and host specificity can range from highly specific to general (Bennett, 1993; Ghani, 2003; Smit et al., 2014). While attached, cymothoids feed mainly on host blood, and additionally on host mucus as well as epithelial and subcutaneous tissues (Bunkley-Williams and Williams, 1998).

Since cymothoids are obligate parasites, they do not change their host or leave their host during their life cycle. The presence of these parasites on elasmobranchs and other invertebrates may indicate the occurrence of trawl transfer between hosts (Bunkley-Williams and Williams, 1998). This may happen when the cymothoid detaches from its host and accidentally attaches to another organism (Ateş et al., 2006; Woo, 2006). Occasional transfers have also been reported, when swimming juveniles swimming males are in search of hosts or females (Ateş et al., 2006). Brusca (1975) noted how cymothoids abandon their host as soon as the host is stressed or dying, by detaching and moving away.

1.4.2 Life cycle and development

Over a century ago, it was discovered that cymothoids are protandrous hermaphrodites (Mayer, 1879; Richardson, 1904; Trilles, 1994). All juvenile broods are males (Woo, 2006) that develop into mature females when it is the first to attach to its host (Kensley, 1978). Richardson (1904) mentions that these males possess the reproductive organs of both sexes until they develop into a female through moulting, losing male reproductive organs and developing oostegites. Other males that later attach to the same fish will remain male (Lester, 2005). This phenomenon was apparently first discovered with the genera *Cymothoa, Nerocila, Anilocra and Icthyoxenous* (Montgomery, 1895; Richardson, 1904)

This sexual inversion is regulated by androgenic and neurohormonal processes (Trilles et al., 2011) and can be influenced by the presence of a female which secretes sexual pheromones to prevent the male from turning into a female. This male will only be able to develop into a female with the death of the first female. The lifespan of these adult females are expected to vary according to the lifespan of the fish host (Woo, 2006).

Gravid females will release their eggs into the brood pouch, formed by the oostegites. When the eggs hatch, they undergo moulting until they reach the “pullus 2” stage. These “pullus 2” larvae are released as free-swimming manca larvae, becoming parasitic within a day or two when they need to infest a host fish in order to feed and survive.

The developmental changes of cymothoid isopods can make them a complicated group of species to identify based on morphology (Joca et al., 2015). The identification of cymothoid isopods is further complicated by changes in morphology during development. Beyond a change in sex, these ontogenetic changes include an increase in pleotelson width and decreases in gonopod length, eye perimeter, uropod perimeter and first antenna length (Cook and Munguia, 2015). As a result, species identifications are generally limited to ovigerous females (Bunkley-Williams and Williams, 2003).

1.4.3 Taxonomy and systematics

Representatives of the family Cymothoidae are considered to be a highly derived, monophyletic lineage of isopods (Thangaraj et al., 2014; Martin, 2015), consisting of 369 known and accepted species (Hadfield et al., 2017b). Brusca (1981) initially suggested distinct lineages between the externally attaching cymothoid isopods and the internally attaching cymothoids. Bruce (1990) mentions that this interpretation of isopod lineages was based largely on morphological characteristics that exclude appendages such as pleopods

and mouthparts. With consideration to the more recently available descriptive data, the simple lineage patterns suggested by Brusca (1981) were rejected (Bruce, 1990). Ketmaier et al. (2008) and Thangaraj et al. (2014) supported the proposal of three distinct evolutionary Cymothoidae lineages: Anilocrinae, Livonecinae and Cymothoinae, based on the construction of phylogenetic trees using molecular techniques. In contrast, Jones et al. (2008) and Hata et al. (2017), also using molecular methods, suggested that the branchial attaching cymothoids were found to be the most likely ancestral attachment mode of cymothoids, from which the buccal and external attaching modes have evolved. Based on morphological characteristics, Hadfield (2012) suggested that externally attaching genera were more derived than the buccal and branchial attaching genera. Recent studies now also provide evidence of attachment site flexibility within a genus (Thangaraj et al., 2014).

1.4.4 Taxonomical challenges

In most cases, species from the family Cymothoidae were originally described with a typological approach, without the technology of modern day molecular characterisation. Generally there was only a single specimen available at the time of the original description thus polymorphism could not be noted. Brusca (1981) advised to examine a large array of collected specimens, if possible, to enable accurate descriptions, and to recognise variability and polymorphism within characteristics.

Polymorphism is a prominent characteristic in isopods that is especially evident between ovigerous females and males (Naylor, 1972; Smit et al., 2014), as the males are normally narrower and smaller in length and width ratios than the female specimens (Lester, 2005; Woo, 2006). The main differences between male and female specimens, other than the noticeable difference in size, include the presence of appendix masculina on the second pleopod and the presence of penes in male specimens, as well as developed oostegites in female specimens. Male specimens tend to be morphologically similar (Brusca, 1975) and cymothoids are typically unidentifiable during their egg or juvenile stage (Criscione et al., 2005). Male descriptions and identifications may aid in the collection of ecological data such as host and locality records and may even provide unknown information regarding sex change in a species.

The absence of high power magnification microscopy limited early taxonomists by only being able to describe and illustrate features and characteristics that were visible with the naked eye or with basic microscopy. Consequently, the description and illustration of smaller body

parts, such as the pleopods and mouthparts, could not be done with the same detail and accuracy as those of recent descriptions.

Many older species names may possibly be relegated and specimens should be subjected to re-identification. Morphological re-descriptions are essential for the revision of older, published species. Many type specimens from original or early descriptions have been lost or were poorly preserved, making them very fragile. In some cases, a type specimen has not been assigned or fully described (Bruce, 1990; Hadfield et al., 2016b). In other cases, where type material has been assigned and is available, many of them lack critical information about where it has been stored, the hosts and localities from which it had been collected, as well as the date from which it was collected (Brusca, 1975). Revisions of many early described genera and species are done today, aiming to eliminate future uncertainties and confusion that may exist due to insufficient descriptions and available information (Hadfield et al., 2016b).

Another challenge of cymothoid taxonomy lies in the morphological variability of members of this family. From previous taxonomic studies it became clear that intra-specific variations were often confused with inter-specific variations, leading to multiple misidentifications of genera and species. Lester (2005) provided evidence of the inter-specific variations that occur within mature females from different isopod species. Highly intra-specific morphological features were sometimes used as diagnostic characteristics in separating species, which actually belonged to the same species (Brusca, 1981). Consequently, many new species have been erroneously named on the basis of these differences and were later synonymised with known species (Smit et al., 2014). Along with the discovery and identification of cymothoids, numerous genera and species legitimacy and validity are currently being questioned and evaluated (Smit et al., 2014). Even today, it is still challenging to distinguish between valid names and to determine which names need to be placed in synonymy. Some synonymised species require redescriptions and revisions to make clear distinctions between them (Hadfield et al., 2016b).

The morphological variability observed in the cymothoids can be attributed to the parasitic lifestyle of cymothoids, polymorphism and sister species (Kensley and Schotte, 1989; Smit et al., 2014). The differentiation between intra-specific and inter-specific variations was often confused or overlooked in previous taxonomic studies (Ghani, 2003). Many of the defining characteristics used to describe and identify the cymothoids are variable and can often be damaged or disfigured as a result of unusual growth or damage from predators. When these imperfections are not taken into account, it contributes to misidentifications and faulty data on the species (Hadfield et al., 2010; Smit et al., 2014).

Due to the variations in attachment site, the cymothoids developed different specialised morphological adaptations based on their feeding strategy, direct environment and available space. These adaptations cause difficulties in choosing morphological character sets for the identification and phylogenetic reconstruction of species (Ketmaier et al., 2008; Thangaraj et al., 2014). These difficulties, including the fact that some taxonomic errors were made by excluding type species, have caused cymothoid taxonomy to become confusing over the years (Hadfield, 2012; Smit et al., 2014).

1.4.5 Effects on host

Pawluk et al. (2015) stated that evolutionary diversification is driven, in most cases, primarily by the association between parasites and their hosts. This association is essential in the understanding of ecological stability and the possible implications that they might have on fishing industries and aquaculture. Fish parasites may have several biological influences on the host such as alterations in its behaviour, health deterioration, and changes in physical distribution (Rohde, 2005). These effects may alter the total fish population, the overall fitness, and population demographics of individuals. The pathogenicity of cymothoids depends on the position of attachment to its host, its feeding strategy, the manner in which they attach as well as their size relative to the host (Östlund-Nilsson et al., 2005).

A study done by Östlund-Nilsson et al. (2005) provided results of reduced swimming speed and endurance of hosts infected with the externally attaching *Anilocra apogonae* Bruce, 1987, at elevated water speeds. This might be due to the resistance and drag that the parasite inflicts, especially observed with the externally attaching genera. These observations confirm that cymothoid parasites may have a negative impact on fish, regardless of their body condition index.

Adult cymothoids, such as the buccal attaching *Ceratothoa oestroides* (Risso, 1816) have proven to hinder or constrain the normal growth pattern and reproduction of fish hosts (Horton and Okamura, 2001; Ravichandran et al., 2009). This might be due to the loss of vital nutrition through the feeding of the cymothoid parasite. The buccal attaching cymothoid genera are known to inhibit the natural growth pattern and weight of fish hosts by hindering the amount of food ingested (Rameshkumar et al., 2013b). In addition, the buccal attaching genera, with *Ceratothoa* Dana, 1852 as an example, constrain the development of the host's oral structures, and can even completely replace the host's tongue in some cases (Lester, 2005). Parker and Booth (2013) reported considerable damage and deformation of the

mouth structures of the largespot pompano, *Trachinotus botla* (Shaw, 1803), infected by *Cymothoa borbonica* Schioedte & Meinert, 1884.

Romestand and Trilles (1977a) reported a 50% reduction in the total length and weight of the host tongue after a buccal cymothoid, *Ceratothoa oestroides* (Risso, 1816), infection. Östlund-Nilsson et al. (2005) found that, when provided with less than a normal food quota, fish hosts infected with *Anilocra apogonae* Bruce, 1987, were subjected to greater weight loss than uninfected fish, and were thus typically smaller in size. Colorni et al. (1997) and Brusca and Gillian (1983) presented contrary results, where the growth, feeding or respiration ability of infected fish hosts were not constrained when compared to uninfected hosts of the same size.

Permanently attached cymothoid parasites drain the nutrition from the host, affecting reproductive performance and/ or growth rate (Lester, 2005). Fogelman et al. (2009) provided evidence of parasitic castration and found that infected male fish hosts had smaller gonads in comparison to uninfected males, and that infected female hosts had considerably less and smaller eggs than uninfected females.

In a study by Adlard and Lester (1994) on the effects of the externally attaching cymothoid *Anilocra pomacentri* Bruce, 1987 on fish populations, a decline in growth and reproduction were confirmed. Infected female hosts were only able to produce approximately 12% of the amount of eggs as an uninfected female from the same size. They added that these effects may alter the survivability of infected fish hosts, as the mortality rate of infected juvenile hosts were 22% higher than that of uninfected juveniles.

Changes in the behaviour of hosts include evasive reactions from the host in response to the attachment of an externally attaching cymothoid, such as *Nerocila acuminata* Schioedte & Meinert, 1881. In some cases, where the cymothoid attaches to sensitive areas (near the eyes or anal opening), the host will display aggressive behaviour by swimming rapidly, wiggling the body or rubbing against objects in an attempt to detach the cymothoid (Segal, 1987).

Damage to host tissue can be caused by erosion of the tissue and epidermis due to constant pressing against the cymothoid parasite, or by means of deformation as the host tissue grows around the body of the parasite (Romestand and Trilles, 1977a; Bunkley-Williams and Williams, 1998; Carrassón and Cribb, 2014). Tissue damage can also be caused by crypting (a necrotic eroding reaction of host tissues pressed against the parasite) or deformation (host growing around the parasite). Rand (1986) noted the development of lesions at the site of attachment on the host as a result of externally attaching *Nerocila acuminata* Schioedte &

Meinert, 1881. The tissue at the attachment site had started to erode, compress and become inflamed and haemorrhagic ulcers had developed. Lester (2005) reported on the tissue damage of the host penetrating cymothoids such as species from the genus *Ourozeuktes* Milne Edwards, 1840. The development of pressure atrophy from adjacent host tissue and muscles, were evident, as well as the pouch-like depression created by the cymothoid inside the skin of its host. Evidence of poor condition of hosts has been recorded by Kroger and Guthrie (1972) where hosts infected with *Olencira praegustator* (Latrobe, 1802), displayed haemorrhaging of various tissue such as the eyes, fins and snouts. Bodily scars and cloudy lenses were also visible.

Furthermore, certain cymothoids, including *Anilocra nemipteri* Bruce, 1987, may alter the metabolic rate of their hosts by increasing the amount of oxygen consumed (Binning et al., 2013), reducing aerobic capacity as well as maximum swimming speeds of their hosts. As a result of an elevated metabolism, infected fish will need to increase their foraging time to obtain their metabolic requirements. Increased foraging time may lead to a higher predation risk, especially if the fish's swimming capabilities are impaired or reduced by the presence of *Anilocra apogonae* Bruce, 1987 (Östlund-Nilsson et al., 2005). These results were repeated in a study done by Binning et al. (2013), providing evidence of an elevated standard metabolic rate, as well as reduced aerobic capacity and maximum swimming speeds of infected fish in comparison to uninfected fish. These publications confirmed the drag effect that ectoparasites, such as the cymothoids, have on their hosts. This effect has a noteworthy influence on the swimming speed and capacity of infected fish hosts, especially at high water speeds. Although all of these effects are not lethal to the infected fish, it has the potential to alter and reduce the overall fitness and population demographics of individuals.

Nutrient deprivation together with the hook-like attachment of the cymothoids may cause the fish to become more vulnerable to fatal diseases and pathogens, bacterial growths and ulceration. Previous studies have provided evidence of such dense bacterial growths and fungal strains at the infected site of infected fish host (Rameshkumar et al., 2013b). The bacterial count and richness of bacterial growths at the lesions of infected fish may have an overall influence on the total fish population. With investigation and analyses of the bacterial load and infectious agents on the infected fish hosts, cymothoid infestation can possibly be applied as a marine ecosystem health indicator for changing environments (Rameshkumar et al., 2013b).

Horton and Okamura (2003) reported on the haematological effects of a blood feeding buccal cavity cymothoid parasite, *Ceratothoa oestroides* (Risso, 1816). The results included significantly lower haematocrit, haemoglobin and erythrocyte values of infected fish hosts

compared to uninfected hosts. The leucocyte count has also significantly increased, suggesting that the host's immune response is activated by the presence of the cymothoid parasite. These effects may ultimately cause anaemia as well. Adlard and Lester (1995) and Romestand and Trilles (1977b) both confirmed the decrease in the total amount of erythrocytes of hosts infected with *Anilocra physodes* (Linnaeus, 1758), *Ceratothoa oestroides* and *Emetha audouini* (Milne Edwards, 1840). Romestand and Trilles (1977b) further mentioned that the spleen of infected hosts tend to undergo hypertrophy and hypervascularisation.

1.5 Branchial cavity inhabiting Cymothoidae

Branchial cavity inhabiting cymothoids are typically found in pairs, consisting of a female and a male, attached in opposite gill cavities of a host (Aneesh et al., 2016b). In a study done by Ravichandran et al. (2011), the abundance of cymothoids per gill cavity of most infected fish was only one, while some were infected with two cymothoids per gill cavity. All ovigerous females, with the exception of *Ryukyua circularis* (Pillai, 1954), have a strongly twisted body shape. The twisting of the body, either to the left or to the right, can be attributed to the positioning and attachment of the cymothoid to either the left or the right gill chamber (Aneesh et al., 2016b). To ensure better attachment and use of limited available space, these cymothoids usually have dorsally flattened bodies (Colorni et al., 1997; Jithin et al., 2016).

Two different attachment positions have been recorded for the branchial cavity inhabiting cymothoids. Some cling to the inner surface of the operculum, while others attach to the inside of the gill cavity floor, both with the cephalon to the anterior end of the host (Jithin et al., 2016). The degree of damage inflicted by a branchial cymothoid is related to the relative size of the parasite to the host as well as the duration of its attachment (Romestand and Trilles, 1977a).

These cymothoids mainly feed on the gill filaments (Kroger and Guthrie, 1972), possibly causing blood loss, a decrease in respiratory efficiency, and a reduction in growth rate (Lester, 2005; Östlund-Nilsson et al., 2005). Brusca (1975) debated that this gill damage may rather be due to mechanical erosion and not feeding behaviour from the parasite. Stephenson (1976) agrees that the erosion of gill lamellae is most likely due to the presence of a cymothoid, and the space restriction it creates within the gill cavity, and not as a result of the cymothoid feeding.

Branchial cavity attaching cymothoids cause severe damage to the gill arches of the fish host, especially those on which the brood pouch of the female cymothoid is resting (Ravichandran et al., 2011). A well developed and large brood pouch often causes a pit-like depression within the gill cavity of the host due to the space restriction and growth of the brood pouch (Aneesh et al., 2016b). This phenomenon is especially noticeable where ovigerous females (such as those from the genus *Mothocya* Costa, in Hope, 1851) are present, suggesting that ovigerous females have a greater negative impact on the host.

In some cases where only one of the gill chambers have an attached cymothoid, the fish host may experience an imbalance in posture, which it needs to compensate for by increasing the rate at which the pectoral fin moves. This is especially evident branchial attaching cymothoids, such as the *Norileca* Bruce, 1990, where only one of the gill chambers is usually infested (Brusca, 1975).

The increase of bacterial growth in the branchial respiratory region of an infected fish host has proven to affect respiration of the host by battering and fusion of the lamellae (Rameshkumar et al., 2013b). This reduces the respiration (Trilles, 1994) and nitrogenous waste excretion of the host (Ravichandran et al., 2011). Bacteria and microbes present in the branchial region of a cymothoid infested host are possibly due to lesions and contamination with respiratory water. This may result in a reduction of respiration and nitrogenous waste excretion (Trilles, 1994; Ravichandran et al., 2009).

Other detrimental effects of branchial attaching cymothoids include anaemia (Lester, 2005; Ravichandran et al., 2009), underdeveloped gills as well as pericardial and heart compression as a direct result of the presence of the cymothoids (Trilles, 1994).

1.6 Sub-Sahara African Cymothoids

The sub-Saharan Africa region includes all African countries with a geographical distribution south of the Sahara, thus excluding Western Sahara, Morocco, Algeria, Tunisia, Libya, and Egypt. Figure 1.1 illustrates the included African countries. From the sub-Sahara African region, 13 cymothoid genera have been recorded. Table 1.1 summarises the cymothoid species that has been recorded from the sub-Sahara African region.

Figure 1.1: Sub-Sahara African countries included in the study region (Shingler, 2016).

Table 1.1: Cymothoidae Leach, 1814 species that has been recorded from the sub-Sahara African region.

Sub-Sahara African Cymothoids	Country	Most recent location reference
***Anilocra* Leach, 1818**		
A. acuminata Haller, 1880	Réunion Island	Haller (1880)
A. capensis Leach, 1818	South Africa	Kensley (1978)
A. coxalis Schioedte & Meinert, 1881	Zanzibar	Schioedte and Meinert (1881)
A. guinensis Bovallius, 1887	Guinea	Bovallius (1887)
***Ceratothoa* Dana, 1852**		
C. africanae Hadfield, Bruce & Smit, 2014	South Africa	Hadfield et al. (2014a)
C. carinata (Bianconi, 1869)	Mozambique	Schioedte and Meinert (1883)
C. collaris Schioedte & Meinert, 1883	Algeria	Ramdane and Trilles (2008)
	Mauritania	Trilles (1977)
	Senegal	Trilles (1979a)
C. famosa Hadfield, Bruce & Smit, 2014	South Africa	Hadfield et al. (2014a)
C. guttata (Richardson, 1910)	Madagascar	Bruce and Bowman (1989)
C. retusa (Schioedte & Meinert, 1883)	Mozambique	Bruce and Bowman (1989)
	South Africa	Hadfield et al. (2014b)
***Cinusa* Schioedte & Meinert, 1884**		
C. tetrodontis Schioedte & Meinert, 1884	South Africa	Hadfield et al. (2010)
***Cymothoa* Fabricius, 1787**		
C. borbonica Schioedte & Meinert, 1884	Djibouti	Trilles (1975)
	Madagascar	Trilles (1979a)
	Mozambique	Barnard (1926)
	Réunion Island	Trilles (1975)
	Somalia	Trilles (1975)
	South Africa	Barnard (1940)
C. epimerica Avdeev, 1979	Seychelles	Trilles (2008)
	Mauritius	Leach (1818)
C. eremita (Brunnich, 1783)	Mozambique	Unpublished
	Seychelles	Milne Edwards (1840)
	Zanzibar	Stebbing (1910)
C. hermani Hadfield, Bruce & Smit, 2011	Zanzibar	Hadfield et al. (2011)
C. plebeia Schioedte & Meinert, 1884	Angola	Brian and Dartevelle (1949)
	Benin	Trilles (1975)
	Cameroon	Trilles (1975)
	Cape Verde	Van Name, 1920
	Côte d'Ivoire	Trilles (1975)
	Democratic Republic of the Congo	Brian and Dartevelle (1949)

Table 1.1: Continued.

Sub-Sahara African Cymothoids	Country	Most recent location reference
	Ghana	Trilles (1975)
	Senegal	Rokicki (1984)
C. selari Avdeev, 1978	Madagascar	Hadfield et al. (2013)
C. sodwana Hadfield, Bruce & Smit, 2013	South Africa	Hadfield et al. (2013)
***Elthusa* Schioedte & Meinert, 1884**		
E. raynaudii (Milne Edwards, 1840)	South Africa	Kensley (1978)
***Glossobius* Schioedte & Meinert, 1883**		
G. hemiramphi Williams and Williams, 1985	Angola	Bruce and Bowman (1989)
	Ghana	Bruce and Bowman (1989)
	Guinea	Bruce and Bowman (1989)
	Liberia	Bruce and Bowman (1989)
	Senegal	Bruce and Bowman (1989)
	Sierra Leone	Bruce and Bowman (1989)
***Ichthyoxenus* Herklots, 1870**		
I. africana (Lincoln, 1972)	Tanzania	Lincoln (1972)
I. micronyx (Miers, 1880)	Mauritius	Miers (1880)
I. tanganyikae (Fryer, 1965)	Tanzania	Fryer (1965)
***Mothocya* Hope, 1851**		
M. affinis Hadfield, Bruce and Smit, 2015	South Africa	Hadfield et al. (2015)
M. arrosor Bruce, 1986	Kenya	Bruce (1986)
M. collettei Bruce, 1986	Kenya	Bruce (1986)
M. longicopa Bruce, 1986	Guinea	Bruce (1986)
M. plagulophora (Haller, 1880)	Comoros Islands	Bruce (1986)
	Kenya	Bruce (1986)
	Madagascar	Monod, 1971
	Mauritius	Bruce (1986)
	Mozambique	Hadfield et al. (2015)
	Somalia	Bruce (1986)
	Zanzibar	Bruce (1986)
M. renardi (Bleeker, 1857)	Kenya	Bruce (1986)
	Madagascar	Trilles (1976)
	Mozambique	Bruce (1986)
	South Africa	Hadfield et al. (2015)
M. trillesi (Rokicki, 1986)	Senegal	Rokicki (1986)
***Nerocila* Leach, 1818**		
N. armata Dana, 1853	Cameroon	Monod (1927)
	Democratic Republic of the Congo	Brian and Dartevelle (1949)
N. livida Budde-Lund, 1908	Zanzibar	Budde-Lund (1908)
N. orbignyi (Guérin-Méneville, 1832)	South Africa	Bruce (1987)

Table 1.1: Continued.

Sub-Sahara African Cymothoids	Country	Most recent location reference
N. phaiopleura Bleeker, 1857	South Africa	Barnard (1925)
N. serra Schioedte & Meinert, 1881	Madagascar	Bruce and Harrison-Nelson (1988)
	South Africa	Bruce and Harrison-Nelson (1988)
N. trichiura (Miers, 1877)	Mauritius	Miers (1877)
	Senegal	Bruce and Harrison-Nelson (1988)
***Norileca* Bruce, 1990**		
N. indica (Milne Edwards, 1840)	Madagascar	Rokicki (1982)
	Mayotte Island	Trilles (1976)
	Mozambique	Rokicki (1982)
***Pleopodias* Richardson, 1910**		
P. neilbrucei Hadfield & Smit, 2017	South Africa	Hadfield and Smit (2017)
P. vigilans Richardson, 1911	Sudan	Richardson (1911)
***Rhiothra* Schioedte & Meinert, 1884**		
R. callipia Schioedte & Meinert, 1884	Mauritius	Schioedte and Meinert (1884)

The branchial cavity inhabiting genera among the above mentioned genera are *Elthusa* Schioedte & Meinert, 1884; *Mothocya* Hope, 1851; *Livoneca* Leach, 1818 and *Norileca* Bruce, 1990. For the purposes of this study, the focus will be on the genera of *Elthusa, Mothocya* and *Norileca*.

Species of *Norileca* are commonly recorded from pelagic fishes and mainly distributed in the Indo-West pacific regions and Mozambique area (Bruce et al., 2002). There are three known species: *Norileca borealis* Javed & Yasmeen, 1999, *N. triangulata* Richardson, 1910 and *N. indica* (Milne Edwards, 1840). Species from the genus *Elthusa* Schioedte and Meinert, 1884 have a cosmopolitan distribution in all oceans (Bruce et al., 2002). *Elthusa* is considered to be among the most morphological varied and species-rich genera with currently 32 known species. *Elthusa raynaudii* is the only species that has been described from sub-Saharan Africa. The genus *Mothocya* has a similar cosmopolitan distribution to *Elthusa* (Bruce et al., 2002), and are parasitic on atheriniform and beloniform fishes (Bruce, 1986; Hadfield et al., 2015; Aneesh et al., 2016a).

Many *Mothocya* and *Elthusa* species have been misidentified, and placed with the genus *Irona* Schioedte and Meinert, 1884. *Irona* has since been synonymised with *Mothocya* (Bruce, 1986; Hadfield et al., 2014c).

Gibbons (1999) explained that although southern African marine faunal biodiversity represents high richness and diversity, incomplete studies and evaluations as well as inaccurate identifications and descriptions prevent global comparisons of species richness

and endemism. Despite many taxonomical challenges, many more cymothoid species are yet to be described. The source of these species would most probably be from isopod families that have rarely been studied in the past; the discovery of species within new regions and habitats; the collection of isopods from habitats that are not easily accessible to collect samples from; as well as the discovery of cryptic species with the aid of molecular characterisation and analysis (Poore and Bruce, 2012). Poore and Bruce (2012) conclude that the high species diversity of certain families can be ascribed to increasing taxonomic studies regarding these families, including describing species and systematics.

1.7 Hypotheses, aims and objectives

Members of the family Cymothoidae Leach, 1814, are cosmopolitan but most abundant in warmer/temperate to subtropical waters (Brusca, 1975; Kensley and Schotte, 1989; Hadfield, 2012). The focus of this dissertation was with the marine species from the family Cymothoidae. Tropical regions contain the greatest diversity of cymothoid isopods (Smit et al., 2014). Cymothoids have been reported from deep waters in some cases, but they are mainly found in shallow, coastal waters (Ateş et al., 2006). Since most of the sub-Sahara African coastline is characterised by warm, temperate oceans, it would be expected to have a higher diversity of branchial cavity inhabiting cymothoids than that which is currently known. These expectations led to the hypothesis that it is the lack of sampling and collection, rather than the lack of species that accounts for the low number of *Elthusa, Norileca* and *Mothocya* species from the sub-Sahara African region. To test this hypothesis, the aims of this study were:

- To collect material resembling *Elthusa, Norileca* and *Mothocya* branchial cavity inhabiting Cymothoidae, from the sub-Sahara African region;
- to determine the biodiversity and systematics of these genera from this region by means of morphological analysis and identification, as well as confirmation thereof, with molecular characterisation;
- to provide full descriptions of each identified species (male and female material), where possible;
- to provide COI gene sequences from the identified species where possible, for future reference and contribute to the available Cymothoidae sequence data on GenBank;

- to provide diagnostics and identification criteria for the correct placement of species for future studies and collections.

In order to achieve the aims of this hypothesis, the objectives were:

- to collect preserved, donated material of *Elthusa, Norileca* and *Mothocya* specimens from national and international colleges; national and international museums; personally collected fresh material from permitted sites; and material previously collected by of members of the *NWU – Water Research Group*;
- to provide detailed morphological descriptions of examined and identified material as well as digital illustrations of described species;
- to determine diagnostic characteristics and species identification keys where applicable.
- to do molecular analysis by generating COI gene sequences, where possible;
- to provide an appropriate phylogenetic tree to confirm the identity and genetic placement of identified material.

Furthermore, by considering the relatively large size and attachment strategy of these parasitic cymothoids to their hosts, it is expected that they would have a negative impact on the host's health. This led to the second hypothesis, that branchial cavity inhabiting cymothoids would have a quantifiable negative impact on the health of its host. To test this hypothesis, the aim of this section of the study was:

- to provide a case study of the impact of branchial cavity inhabiting cymothoids on their fish hosts, using a southern African species as an example.

In order to achieve this aim, the objectives were:

- to collect fresh material of branchial cavity inhabiting cymothoids with their accompanying fish host, from a southern African area;
- and to conduct an in-field Fish Health Assessment (FHA) on the collected fish hosts (infected and uninfected) by considering the appropriate Fish Health Assessment Indices (FHAI) and condition factors.

1.8 Layout of dissertation

The first chapter provided a literature review in order to introduce parasites in general, crustacean parasites and then specifically the parasitic isopods. The family Cymothoidae is discussed, including diagnostic characteristics, life cycle and development, their taxonomy and challenges that hinder the taxonomy, as well as molecular research done on cymothoids and their reported effects on hosts. The branchial cavity inhabiting cymothoids are discussed, concluding with the sub-Sahara African genera and the three focus genera of this study, namely *Norileca, Elthusa* and *Mothocya* (Chapter 1).

It is followed by a broad outline of the materials and methods used to achieve the aims of this project and to test the hypotheses (Chapter 2). The first genus is discussed with a redescription and molecular characterisation of *Norileca indica*, with a key to the genus (Chapter 3). Species from the second genus, *Elthusa* are described with accompanying molecular characterisation, a key to sub-Sahara species, and a summary of the genus (Chapter 4). A review of the third genus, *Mothocya* is provided, along with a case study of its effect on the host (Chapter 5). The chapters conclude an overall discussion and final remarks, as well as recommendations for future studies (Chapter 6). A full bibliography and additional appendices are provided at the end of the dissertation.

CHAPTER 2: GENERAL METHODOLOGY

2.1 Material collection and sites

2.1.1 Preserved material

These consisted of donations from national and international colleagues. Material from the genus *Norileca* was collected by local fishermen via line-fishing at Maputo Bay, Mozambique (26°00′17″S 32°54′52″E) during 2013, and donated by Dr Wynand Vlok. The natural harbour of Maputo Bay (previously known as Delagoa Bay) stretches over 90 km in length and 32 km in width (with a total surface area of approximately 1875 km^2) along the Indian Ocean. It lies within a changeover between tropical and temperate climates. The depth of the bay ranges between 1–20m and is influenced by its connections with five discharging rivers. Maputo Bay is characterised by the rich biodiversity that it encompasses (Canhanga and Dias, 2005). This environment creates the perfect opportunity for local fishing via gill-nets, seining and line-fishing as well as commercial prawn trawling (Guissamulo and Cockcroft, 2004) (see Figures 2.1 and 2.3c–d).

Material from the genus *Elthusa* came from different localities. Prof Nico J. Smit collected and donated material from the intertidal zone of Alexander Bay, South Africa (28°35′S; 16°29′E) during 1993 (Figures 2.1 and 2.3e). This South African town is where the Orange River enters into the Atlantic Ocean. Other *Elthusa* material, also donated by Prof. Nico J. Smit, was collected from the deck and sorting table of the *Africana* Research Vessel (RV) during their 2003 expedition along the south coast of South Africa (Figures 2.1 and 2.2e–f). The *Africana* RV was commissioned in Cape Town during 1982 by the South African Department of Agriculture, Forestry and Fisheries. Its main purposes are research and monitoring of South African fisheries (SADCO, 2017). Dr Kerry A. Malherbe collected and donated material from the *Dr Fridtjof Nansen* Research Vessel during its 2010 expedition along the western coast of South Africa (Figures 2.1 and 2.2 c–d). The *Dr Fridtjof Nansen* RV was built in 1993 and is operated by the Norwegian Institute of Marine Research. To this day, its aim is to improve the fisheries management of developing countries. In both RV collections, masses of fishes were caught and collected with trawl nets. These fishes were then sorted and isopods were removed from the fish (where possible) or the sorting table, placed into containers with 70% ethanol, labelled and transported back to the laboratory for storage (see Figures 2.1 and 2.2c–f).

Specimens of the genus *Mothocya* were collected at Lamu archipelago in Kenya (2°06'7.20" S 41°01'8.40" E) during 2009 by David Modry and donated by Roman Kuchta. The Swahili cultured Lamu archipelago can be described as a collection of low-lying Indian Ocean

islands, on the northern coast of Kenya (Figures 2.1 and 2.3b). These islands form a series of creeks, channels and mangrove forests. The marine habitats of Lamu archipelago include seagrass beds as well as coral reefs, with sandy beaches and coastal forests.

Other material, originating in Nigeria, was collected from Andoni Creek of the Niger Delta (4°28' to 4°45'N; 7°45'E) during 2015 by CB Powell and Babatunde Olaosebikan; and from Birakiki, seaward end of Hughes Channel, Bonny Town (4°34'10''N; 7°08'E), during 1985, also collected and donated by Babatunde Olaosebikan. The Niger Delta connects with the Atlantic Ocean at the southern region of Nigeria and has a surface area of about 112 110 km^2 (Figures 2.1 and 2.3a). It is characterised by sandy island beaches and widespread mangrove swamps.

Preserved material was also borrowed from various museum deposits. Three additional *Elthusa* material specimens were borrowed from the Iziko South African Museum in Cape Town (SAM) (deposits numbers: A19448–150–052–3129, A19458–150–061–3044 and A11001) and from the National Museum of Natural History in Paris, France (MNHN) (deposit number: MNHN–Is692 IU–2016–9885) (see Figure 2.2a–b).

2.1.2 Fresh material

A known and described species, *M. affinis* Hadfield, Bruce and Smit, 2015, was collected from the Sodwana Bay National Park (27°32′S; 32°41′E) (see Figure 2.1 and 2.3f), located on the east coast of South Africa. It has been classified as one of the most popular diving sites worldwide due to its rich marine biodiversity and coral reefs. In this case, fresh material was essential for the case study on the effects of branchial cavity inhabiting cymothoids on fish hosts. Here, fish hosts were caught and collected by line-fishing with the assistance of local fishermen and members of the North-West University (NWU) – Water Research Group (WRG) (see Figure 2.5a).

Figure 2.1: Cymothoid material collection sites. These include donated material and those from respective research vessels. A key is provided.

Figure 2.2: **a** Iziko South African Museum, Cape Town (Iziko Museums of South Africa, 2017), photo by Carina. **b** National Museum of Natural History in Paris, France (French Pamphlet Collections at the Newberry Library, 2012). **c** On board the *Dr Fridtjof Nansen* RV, sorting through a traw, photo by KA Hadfield Malherbe. **d** *Dr Fridtjof Nansen* RV, photo by KA Hadfield Malherbe. **e–f** On board the *Africana* RV, sorting through a trawl, photos by NJ Smit.

Figure 2.3: **a** Villagers of the Niger Delta rely on fishing for survival and economic growth (Ross, 2013). **b** Lamu archipelago, Kenya (The 50 Treasures of Kenya, 2013), photo by H Fiebig. **c** Maputo Bay, Mozambique. Local fishermen and villagers delivering the catch of the day. **d** The mangrove-lined, sandy beaches of Maputo Bay at low tide. **e** Alexander Bay, South Africa, photo by Nico J. Smit. **f** Sodwana Bay National Park beach.

2.2 Host condition

The host condition was only determined for the case study to determine the effect of the branchial cavity attaching *Mothocya affinis* Hadfield, Bruce and Smit, 2015, that was collected as fresh material from the Sodwana Bay National Park. The identification of fish hosts was done with the aid of Smith and Heemstra (1986). At the field lab, each fish was weighed (in grams) using a R200D Sartorius research scale (Zeiss, West Germany), and measured (in mm) using a standard 30cm ruler (Figure. 2.5c) or tape measure in the case of longer fish. Each fish was measured in terms of its standard length (SL) (the total length from the tip of the nose to the end of the tail), fork length (FL) (the total length from the tip of the nose to the fork of the tail fin) and the total length (TL) (the total length from the tip of the nose to the end of the tail fin) (see Figure 2.4).

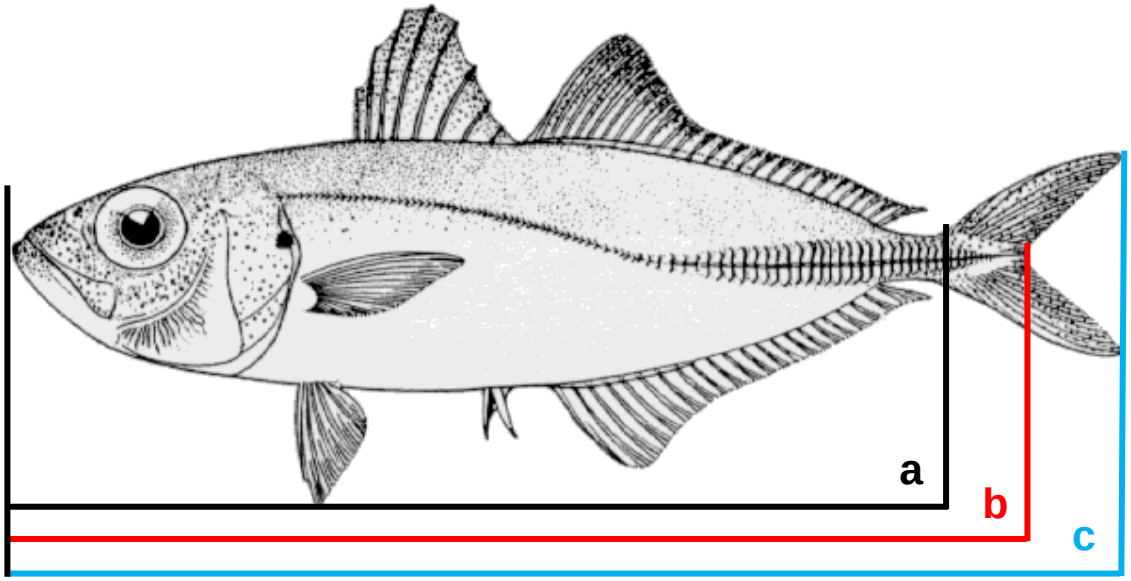

Figure 2.4: *Selar crumenophthalmus* (Bloch, 1793) body measurements. Illustration from Johnson (1978). **a** Standard length. **b** Fork length. **c** Total length.

All hosts were humanely killed following approved procedures. External examination of various features and structures of each host was done to record any damages and defects. During this step, any and all branchial attaching cymothoid isopods were removed and collected in bottles containing 70% ethanol. Dissection was done to examine internal organs (Figure 2.5d). The liver, spleen and gonads were removed and weighed to determine the hepatosomatic index (HSI), splenosomatic index (SSI) as well as the gonadosomatic index (GSI) (see McHugh et al., 2015). The following formula was used for index calculation:

Weight of the organ (in grams) / Total body weight of the fish x 100

The weight measurements of the fish were used in the calculation of the condition factor (CF) and gutted condition factor (GCF), using the total length of the fish as the statistical standard. The following formula was used for CF and GCF calculation (Barnham and Baxter, 2003; Nash et al., 2006):

K (Fulton's condition factor) = W (total weight / gutted weight) (g)/L (total length) ^{3}

A rapid fish health assessment was done following the variables from Goede and Barton (1990) and Adams et al. (1993). The values assigned to each variable were slightly modified according to this study. A value of zero was assigned for variables that displayed a normal / healthy condition. As the condition of a variable decreased / worsened, the assigned value increased. Moderate conditions were assigned 10, whereas moderate-severe conditions were assigned 20. Where a variable was considered to be abnormal / severe, a value of 30 was assigned.

2.3 Morphological analysis

The measurements of each cymothoid were recorded in terms of the total body length and total width. Total body length was taken from the longest region of the body, reaching from the anterior point of the cephalon to the most posterior point of the pleotelson. The width measurement was taken from one lateral margin to the other, through the widest region of the body. Notation was made of any and all deformities, damage and contorting that may influence the body measurements. Isopods were subjected to morphological identification by individually illustrating all body parts and appendages with the aid of a Nikon SMZ1500 Stereo Microscope as well as a Nikon Eclipse80i Compound Microscope, both equipped with drawing tubes (Figure 2.6a–b). This procedure involves the careful dissection of pereopods, pleopods and mouthparts of selected specimens with the aid of dissection needles, forceps and scalpels. The position of specimens and dissected parts were manipulated to obtain a direct and complete view so as to eliminate errors in illustrated ratios of segments.

Figure 2.5: **a** Local fisherman assisting in the collection of hosts from Sodwana Bay, South Africa. **b** Aerated bucket containing collected fish hosts for transport to the field lab. **c** Taking host measurements with a standard ruler. **d** Dissected internal organs of a fish host for the determination of condition indices. **e** Processing hosts for fish health assessment.

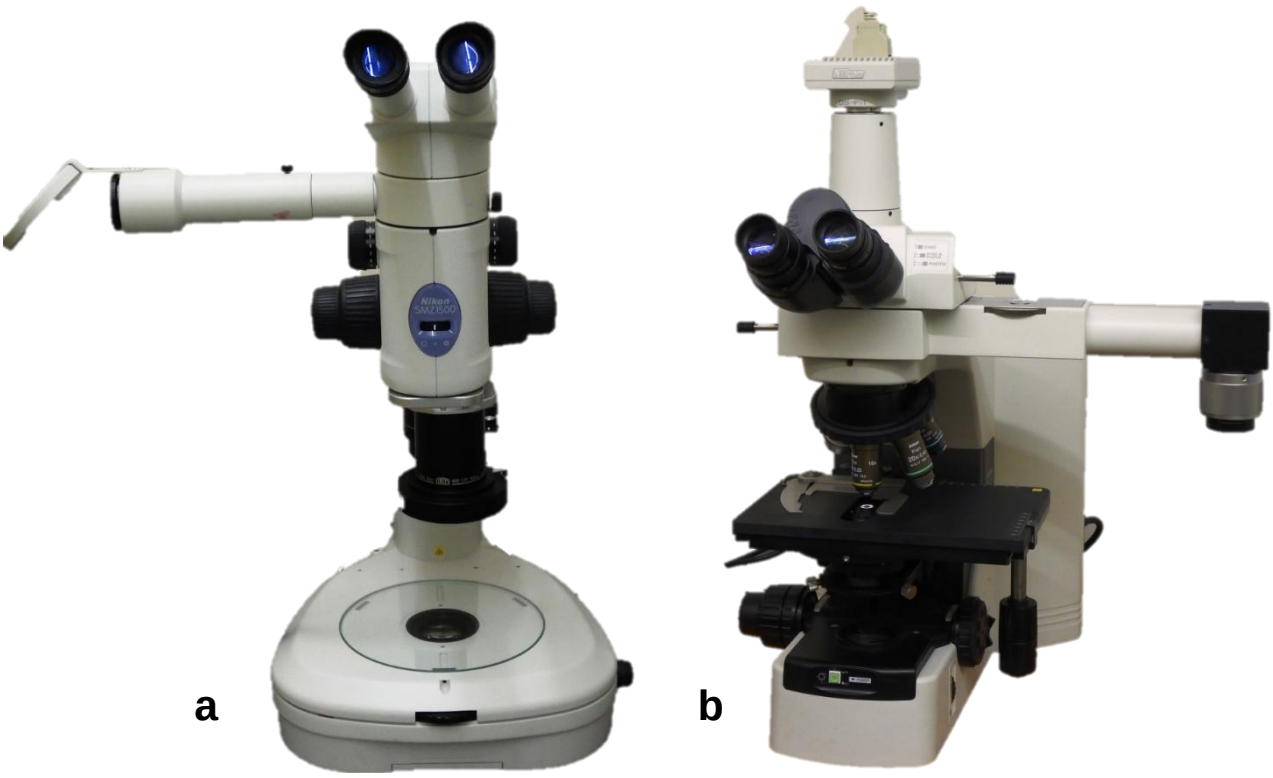

Figure 2.6: **a** Nikon SMZ1500 Stereo Microscope. **b** Nikon Eclipse80i Compound Microscope, both equipped with drawing tubes, used for morphological analysis.

It was important to ensure that minimal harm was done to the rest of the cymothoid during dissection so that it may still be preserved for future examination and museum deposition. Material borrowed from national museums was not permitted to be dissected or damaged in any way. These specimens were morphologically analysed by illustrating body parts and views without any dissection.

Mouthparts and small appendages were stained with lignin pink to obtain a better visual of the overlapping layers. Lignin pink (BioQuip products, Inc), purchased as abysmal peach blossom powder, was mixed with absolute lactic acid (Amresco, ACS Grade) to obtain a deep pink coloured liquid (approximately 2.0g of lignin pink with 10ml lactic acid). The dissected body parts were submerged in this mixture for approximately three hours. This pink stain is absorbed into the tissue of the isopod, resulting in a better visualisation of structures. After three hours, the body parts were removed from the staining mixture and cleared with lactic acid. They were then placed onto a cavity slide with 70% ethanol for viewing under a higher magnification.

Isopods were submerged or dampened with 70% ethanol throughout the illustration process to prevent the tissue from drying out, becoming fragile and susceptible to disintegration. After examination, each specimen was returned to its collection container with a complete

label containing information on collection location, date, collector's name, host detail (if available) and possible species or genus name. Dissected body parts were individually stored in 0.2ml micro-vials (Quality Scientific products) with a label containing the cymothoid detail as well as the name of the dissected part. These vials were stored inside the container of the accompanying cymothoid specimen.

Hand-drawn illustrations were scanned onto a computer to obtain a digital version thereof. These files were copied onto the editing software program Adobe Illustrator CS6, where the illustrations were digitally and identically inked. The files were then printed out as hard copies to be used for various measurements and morphological comparisons. They were also compared to published illustrations of known cymothoid species, especially from the sub-Sahara African region.

The software package DELTA© (Descriptive Language for Taxonomy) (see Coleman et al., 2010) was used to record taxonomic character information of each specimen into an electronic taxonomic database following a general Cymothoidae character dataset, originally developed by Hadfield et al. (2013) and recently updated (Hadfield et al., 2016b). The datasets produced on DELTA© were exported and translated into natural language RTF files, generating the species descriptions in text format.

These descriptions include the presence, shape and sizes of all morphological features, as well as various measurements that were recorded to determine the ratios of specific segments and body parts for morphological comparison (see Figures 2.7–2.8). Ratios and measurements for the descriptions were made using the maximum length / width values of segments, except when stated that it was measured from the medial or anterior part of the specific segment. All proportional measurements were rounded off to one decimal place. Some of these morphological features are largely variable within and among Cymothoidae species.

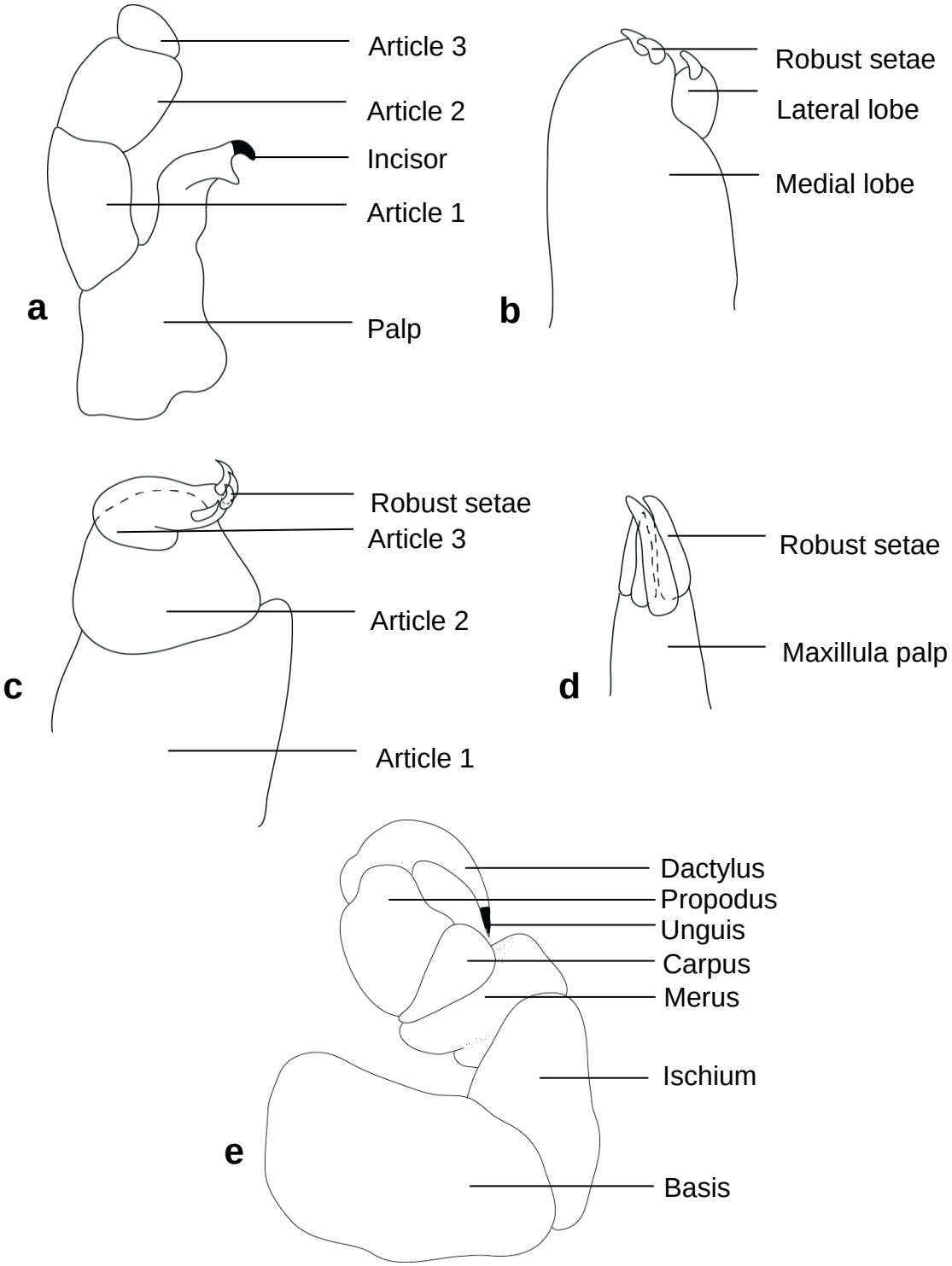

Figure 2.7: Cymothoid isopod mouthpart and pereopod morphological structures. Illustrations of a *Norileca* Bruce, 1990 specimen collected during the present study. **a** Mandible. **b** Maxilla. **c** Maxilliped. **d** Maxillula. **e** Pereopod.

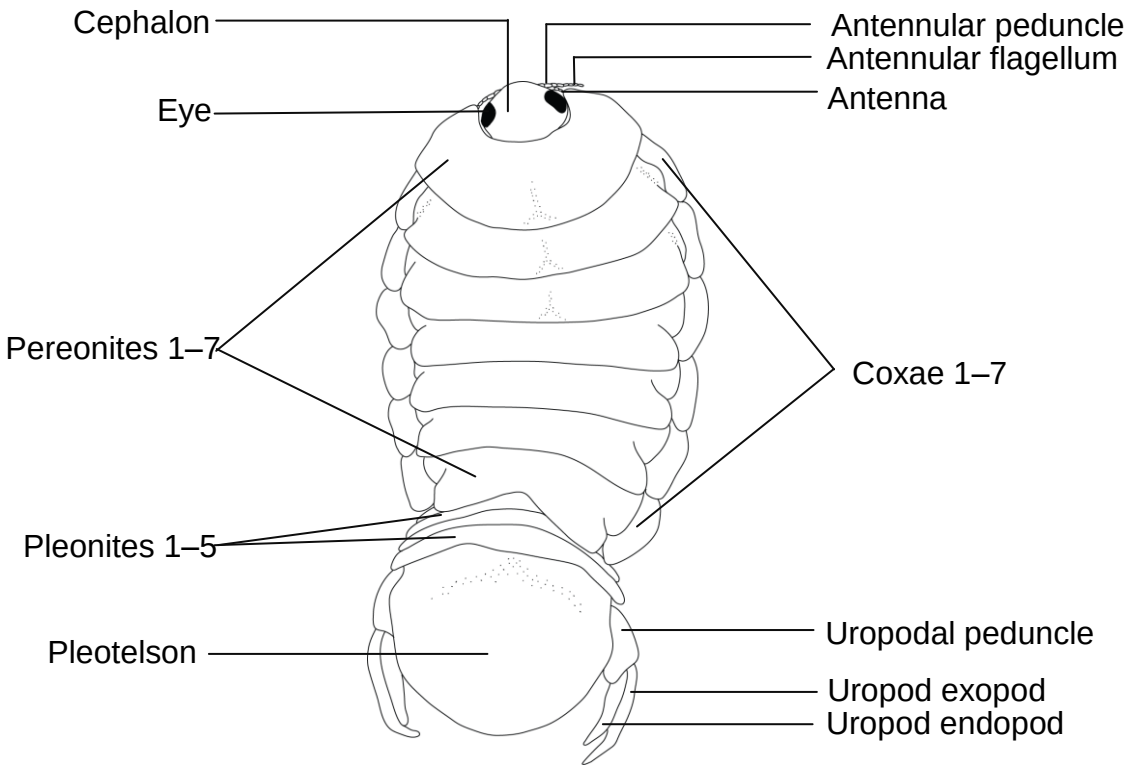

Figure 2.8: Dorsal view schematic representation of a cymothoid isopod body plan with descriptive morphological features. Illustration of a *Mothocya* Costa, 1851 specimen collected as part of the present study.

2.4 Molecular characterisation

Genomic DNA was extracted from pereopods and pleopods of isopod specimens following the protocol for animal tissue extraction of the GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, USA). Tissue was cut as finely as possible, approximately 25mg of tissue, and placed into a 1.5 ml microcentrifuge tube. Buffer T1 (180 µl) and proteinase K (25 µl) were added as lysis solution to each tube, after which it was incubated overnight at 55–60°C using a shaking incubator (Provocell™ Shaking Micro Incubator, ESCO Life Science laboratory Equipment Solutions). After incubation, Buffer B3 was added (200 µl). This step was preceded and followed by vortexing. Samples were then incubated at 70°C for 10 min. DNA binding conditions were then adjusted by adding 210 µl of 96–100% ethanol to each sample, followed by vortexing. To bind the DNA, a NucleoSpin Tissue Column was placed inside a collection tube, to which each sample was transferred to. This step was followed by centrifuging (ORTO ALRESA centrifuge for laboratory, SA) for 1 min at 11 000 x g. The flow through was discarded with the collection tube and a new collection tube was added to the column. A two-step process was used to wash the silica membrane: first, Buffer BW (500 µl) was added to each column followed by centrifuging at the same conditions as with binding the DNA. Secondly, Buffer B5 (600 µl) was added to the column, followed by

the same centrifuging conditions. Drying the silica membrane of residual ethanol was done by centrifuging the column for a last time at 11 000 x g for 1 min. To obtain the highly pure extracted DNA, each column was placed into a 1.5 ml microcentrifuge tube, to which Buffer BE (100 µl) was added and incubated at room temperature for 1 min. The DNA samples were centrifuged for the final time at the above mentioned conditions.

A targeted part of the mitochondrial cytochrome c oxidase-1 (COI) gene of each specimen was subjected to Polymerase Chain Reactions (PCR) amplification with the aid of the ProFlex™ PCR thermal cycler from Applied Biosystems (Life Technologies) and universal invertebrate primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (see Folmer et al., 1994). The COI gene was selected as the gene of interest, which, according to Folmer et al. (1994), is because of the highly conservative nature of this gene in comparison to other protein-coding genes that are present within animal mitochondrial genomes, and in particular, metazoan invertebrates. This characteristic of the COI gene makes it the most appropriate gene to analyse evolutionary relationships. The PCR protocol followed Ketmaier et al. (2008), and was prepared under a 4Ft Airvolution fume hood (G7 Lab Solutions). PCR mixtures were prepared using 12.5 µl Thermo Scientific DreamTaq PCR master mix (2× DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM MgCl2); 1.25 µl of primers LCO1490 and HC02198 respectively; 5 µl of PCR-grade nuclease free water (Thermo Scientific, Vilnius, Lithuania); and 5 µl of DNA. The 0.2 ml PCR tubes with PCR mixture were briefly spun to remove any bubbles that may alter the PCR results. Conditions for this PCR consisted of denaturation (1x cycle) for 5 min at 94.0°C followed by annealing (36x cycles) for 30 sec at 94.0°C, 50 sec at 47.0°C and 2 min at 72.0°C. Final elongation was done for 10 min at 72.0˚C.

The amplified COI gene fragments were made visible under ultraviolet light by performing 1% Agarose Gel Electrophoresis using 60 ml TAE buffer with 0.6 g Agar powder (Thermo Scientific TopVision Agarose). A total of 1 µl loading dye (EZ-VISION® Blue Light DNA Dye, Biotechnology) was mixed with 2 µl of each of the DNA amplicons, and inserted into the agar. A 1kb DNA ladder (Thermo Scientific GeneRuler 1kb, 0.1 µg/µl) was placed in a separate lane (1 µl loading dye and 2 µl of the ladder). The electrophoresis was run at 80 Volts for 45 minutes. Visualisation of the amplicons was done with the aid of Enduro™ Gel Documentation system (Labnet International, Inc.).

PCR products were sequenced in both directions by *Inqaba Biotechnical Industries* (Pty) Ltd, Pretoria, South Africa. The bioinformatics software platform, Geneious R9.1 (created by Biomatters, available from http://www.geneious.com), was used to generate assembled, trimmed and aligned consensus sequences. The GenBank® analysis program BLAST

(Basic Local Alignment Search Tool) was used to search and compare the generated COI sequences with those of published material. This heuristic tool measures specific similarities within an assortment of sequences to reveal biological relationships and relevance between sequences. By comparing sequences, researchers are able to recognise the function of certain genes and proteins, identify new genes and gain a better understanding of evolutionary relationships (Madden, 2013). MEGA®7 (Molecular Evolutionary Genetics Analysis) bioinformatics software program was used to construct a Maximum Likelihood (ML) phylogenetic tree for the generic placement of the identified specimens in relation to closely related species within the Cymothoidae family. Sequences were deposited into the NCBI (National Center for Biotechnology Information) GenBank® database (http://www.ncbi.nlm.nih.gov/genbank/).

Figure 2.9: Equipment used for molecular analysis. **a** Provocell™ Shaking Micro Incubator. **b** ORTO ALRESA centrifuge. **c** ProFlex™ PCR thermal cycler. **d** Enduro™ Gel Documentation system. **e** PCR protocol followed.

CHAPTER 3: REVIEW OF *NORILECA INDICA* (MILNE EDWARDS, 1840) FROM MOZAMBIQUE

3.1 Introduction

Species of *Norileca* Bruce, 1990 inhabit the branchial cavity of fish hosts and are commonly recorded from pelagic fishes (Rameshkumar et al., 2015). There are three known species: *Norileca borealis* Javed & Yasmeen, 1999, *N. triangulata* Richardson, 1910 and *N. indica* (Milne Edwards, 1840). *Norileca borealis* was originally described from the northern Arabian Sea (Javed and Yasmeen, 1999), parasitising the Indian mackerel, *Rastrelliger kanagurta* Cuvier, 1817. No other recordings of this species have been reported since its original description. *Norileca triangulata* was first recorded from Tanimdao Island, the Philippines, without mention of a fish host (Richardson, 1910). Specimens of *N. triangulata* have since been recorded from Australia (from Cape York, Great Barrier Reef and south-eastern Queensland), from the branchial cavity of the sailfin flyingfish, *Parexocoetus brachypterus* Richardson, 1846 and *Sardinella gibbosa* Bleeker, 1849 (Bruce, 1990). More recent recordings are from the Parangipettai coastal waters on the south-east coast of India, from the goldstripe sardinella, *Sardinella gibbosa* Bleeker, 1849 (Rameshkumar and Ravichandran, 2015). *Norileca indica* was originally described by Milne Edwards, 1840 as *Livoneca indica*. It was later redescribed by Bruce (1990) and transferred to the genus *Norileca*. Bruce's (1990) redescription was based on non-ovigerous females and included short notes on the male. This chapter has been published in *African Zoology* (Van der Wal et al., 2017).

3.2 Specific materials and methodology used

Norileca indica specimens were collected during November 2013 from the bigeye scad, *Selar crumenophthalmus* Bloch, 1793, with the assistance of local subsistence fishermen in Maputo Bay, Mozambique. This material was donated by Dr Wynand Vlok. Methods follow general methodology (see Chapter 2.3). A targeted part of the mitochondrial cytochrome oxidase-1 (COI) gene (approximately 660 bp) was sequenced for *Norileca*. Molecular characterisation follows Chapter 2.4.

3.3 Taxonomy

Suborder Cymothoida Wägele, 1989
Superfamily Cymothooidea Leach, 1814
Family Cymothoidae Leach, 1814

Genus *Norileca* Bruce, 1990
Norileca Bruce, 1990: 289.—Bruce, Lew Ton and Poore 2002: 181.

Type species: *Livoneca indica* Milne Edwards, 1840, by original designation (Bruce, 1990).

3.4 Diagnosis

Cephalon posterior margin medially indented, weakly or not trilobed. Coxae narrow, shorter than, or as long as respective pereonites. Brood pouch with 4 pairs of alternately overlapping oostegites on coxae 2–5. Pleon not immersed in pereon; pleonite 1 widest; pleonites 1–5 becoming progressively narrower; pleonites 1 and 2 without ventrolateral processes. Uropods not extending beyond posterior margin of pleotelson. Pleopods 1–4 with laminar rami; peduncle articles 2–5 lateral margin with laminar lobe; endopods 3–5 folded proximomedial lobe present; endopod 5 distal margin medially indented, including 2 folded lobes. Pereopods lacking expanded lobes; without expanded carina on bases. Antennula shorter than, or subequal to antenna; bases of antennula wide apart. Mandible palp article 2 flattened, prominently expanded. Maxilliped lacking oostegital lobes.

3.5 Remarks

Norileca shares several characters with *Livoneca* Leach, 1818. Both genera are similar with regard to their pereopod morphology, all of which are robust and lacking an expanded carina on the base of the pereopods; the cephalon posterior margin is trilobed; and the pleon is not immersed in the pereon with pleonites 1–5 becoming progressively narrower. *Norileca* can be distinguished from *Livoneca* in having a weakly trilobed cephalon (versus strongly trilobed in *Livoneca*) and pleonites 1–3 lateral margins which are not bilobed. *Norileca* also has an expanded mandible palp article 3, pleopods 3–4 without folds on endopods, as well as an absence of branchiated pleopod peduncles (Bruce, 1990). It can be distinguished from other cymothoid genera by pleonite 1 being the widest of the pleonites, as well as its weakly twisted body shape (Hadfield, 2012).

3.6 Key to the species of *Norileca* Bruce, 1990

1. Pleonite 5 narrower than pleonite 1; uropods almost reaching posterior margin of pleotelson; pleotelson approximately 0.9 times as long as wide…….....**2**
   Pleonite 5 and pleonite 1 subequal; uropods two thirds the length of pleotelson; pleotelson approximately 1.0–1.2 times as long as wide…….....……***N. indica***

2. Body twisted to the side; maxilla medial lobe with 1 robust seta and lateral lobe with 4 robust setae ……...…….…..…..…..…... ***N. borealis***
   Body nearly straight; maxilla medial lobe with 2 robust setae and lateral lobe with 2 robust setae……..…….…….....…...…... ***N. triangulata***

3.7 *Norileca indica* Milne-Edwards, 1884

Livoneca indica Milne Edwards, 1840: 262.—Bleeker, 1857: 21, 28.—Gerstaecker, 1882: 261.—Schioedte & Meinert, 1884: 362–365, pl. 5, figs. 3–6; Richardson, 1910: 24.—Nierstrasz, 1915: 99–100.—Nierstrasz, 1931: 142–143, 145.—Borcea, 1933: 482.—Beumer, Ashburner, Burbury, Jette and Latham, 1982: 33.

Livoneca ornata Heller, 1868: 145–146, pl. 12, fig. 15.—Gerstaecker, 1882: 261.

Lironeca indica.—Trilles, 1976: 777–778, pl. 2, fig. 3.—Avdeev, 1978: 281–282.—Trilles, 1979b: 266.—Rokicki, 1982: 205–208, figs. 1–2.—Trilles, 1994: 178–179.

Norileca indica.—Bruce, 1990: 291–293.—Bruce, Lew Ton and Poore, 2002: 181.—Ghani, 2003: 219.—Yu and Li, 2003: 235–237, fig. 10.—Yamauchi, Ohtsuka and Nagasawa, 2005: 25–27.—Nagasawa and Petchsupa, 2009: 131–133.—Rameshkumar, Ravichandran and Sivasubramanian, 2013b: 99–105.—Rameshkumar, Ravichandran, Sivasubramanian and Trilles, 2013c: 42–46.—Argente, Narido, Palla and Celedonio, 2014: 3–8.—Neeraja, Tripathi and Shameem, 2014: 49–56.—Rameshkumar and Ravichandran, 2015: 33–36.—Rameshkumar, Ramesh, Ravichandran, Trilles and Subbiah, 2015: 712–715.—Aneesh, Kappalli, Kottarathil, Gopinathan and Trilles, 2015: 42.—Behera, Ghosh and Pattnaik, 2016: 856–862.—Jithin, Swapna, Kumar, Venu, Helna and Sudha, 2016: 47–53.—Cruz-Lacierda and Nagasawa, 2017: 60–63.

Type material: Holotype held at the Museum Nationale d'Histore Naturelle, Paris (MNHN-IU-2007-4159).

Type locality: Sumatra Island, Indonesia (Milne-Edwards, 1840).

Type host: Unknown.

3.7.1 Material examined

A total of eight specimens were collected and examined. Five ♀ (26.0–33.0 mm TL; 13.0–17.0 mm W); two ♀ (non-ovigerous, 28.0–30.0 mm TL; 14.0–18.0 mm W), and one ♂ (11.0 mm TL; 3.0 mm W), collected at Maputo Bay, Mozambique during 2013. The fish host was identified as the bigeye scad *Selar crumenophthalmus* (Bloch, 1793). This material was collected and donated by Wynand Vlok and deposited at SAM (SAMC-A089028). Illustrated material: One ♀ (ovigerous, 33.0 mm TL, 16.0 mm W); one ♀ (non-ovigerous, 28.0 mm TL, 14.0 mm W) and one ♂ (11.0 mm TL, 3.0 mm W).

3.7.2 Description

***Norileca indica* Milne-Edwards, 1884 ♀**

Figs. 3.1–3.4

Body twisted to the right side, 2.2 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 4, most narrow at pereonite 1. Pleon 0.2 times as long as total body length. Pereonite lateral margins posteriorly protruding. *Cephalon* 1.1 times longer than wide, visible from dorsal view, triangular. *Frontal margin* thickened and ventrally folded. *Eyes* oval with distinct margins, one eye 0.3 times the width of the cephalon, 0.3 times the length of the cephalon. *Pereonite 1* smooth, with anterior border slightly concave and anterolateral angle weakly produced, broadly rounded, extending to middle of eyes. Coxae 2–3 wide with posteroventral angles rounded; coxae 4–7 acute, posteriorly pointed, not extending past pereonite margin. Pereonites 6 and 7 narrower than pereonites 1–5; 3–5 subequal. *Pleon* with pleonite 1 slightly wider than other pleonites, visible in dorsal view; pleonites posterior margin straight with slight indents, curved laterally. Pleonite 2 partially overlapped by pereonite 7 posterolateral margin; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4. *Pleotelson* as long as anterior width; triangular; dorsal surface smooth; lateral margins weakly convex, posteriorly narrow; posterior margin converging to caudomedial point.

Antennula consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 1.1 times as long as article 1; article 3 1.6 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2; flagellum with 5 articles, extending to posterior margin of eye with tufts of simple setae on articles 3–6 and 8. *Antenna* consists of 9 articles; peduncle article 3 1.0 times as long as article 2; article 4 2.2 times as long as wide, 1.4 times as long as article 3; article 5 twice as long as wide, 0.7 times as long as article 4. Antenna flagellum with 6 articles, terminal article with 1–5 short simple setae, extending to anterior

margin of pereonite 1. *Mandibular molar process* present, ending in acute incisor, without simple setae; mandible palp article 2 and 3 without setae. *Maxillula* simple with 4 terminal robust setae. *Maxilla* medial lobe partly fused to lateral lobe; medial lobe with 2 recurved robust setae, lateral lobe with 1 large recurved robust setae. *Maxilliped* palp article 2 without setae; article 3 with 4 recurved robust setae.

Pereopod 1 basis 1.8 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.1 times as long as wide; dactylus slender, 3.8 as long as propodus, 3.8 times as long as basal width. *Pereopods* 3–6 similar to pereopod 2, gradually increasing in size towards posterior, all without setae. *Pereopod 7* basis without carina, 0.6 times as long as greatest width; ischium 0.8 as long as basis, without protrusions; merus proximal margin with large bulbous protrusion; 0.4 times as long as wide, 0.3 as long as ischium; carpus 0.4 times as long as wide, 0.2 as long as ischium, with slight bulbous protrusion; propodus as long as wide, 0.4 as long as ischium; dactylus slender, 1.9 as long as propodus, 2.7 times as long as basal width.

Pleopods without setae, exopod larger than endopod. *Pleopod 1* exopod 1.1 times as long as wide, lateral margin weakly convex, distally broadly rounded, medial margin strongly convex; endopod 1.2 times as long as wide, lateral margin convex, distally broadly rounded, medial margin straight; peduncle 0.3 times as wide as long. Pleopods 2–5 similar to pleopod 1. *Pleopods 3–5* with fleshy folds present, increasing in size from pleopod 3–5. Peduncle lobes present, increasing in size from pleopod 1–5.

Uropod more than half the length of pleotelson; peduncle 0.7 times longer than rami, lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. *Endopod* 2.3 times as long as greatest width, without setae. *Exopod* not extending to end of endopod, 3 times as long as greatest width, without setae.

Antennula more stout than antenna, longer than antenna; consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 as long as article 1; article 3 1.4 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2; flagellum with 5 articles, extending to anterior of pereonite 1, with tufts of setae on articles 3–8.

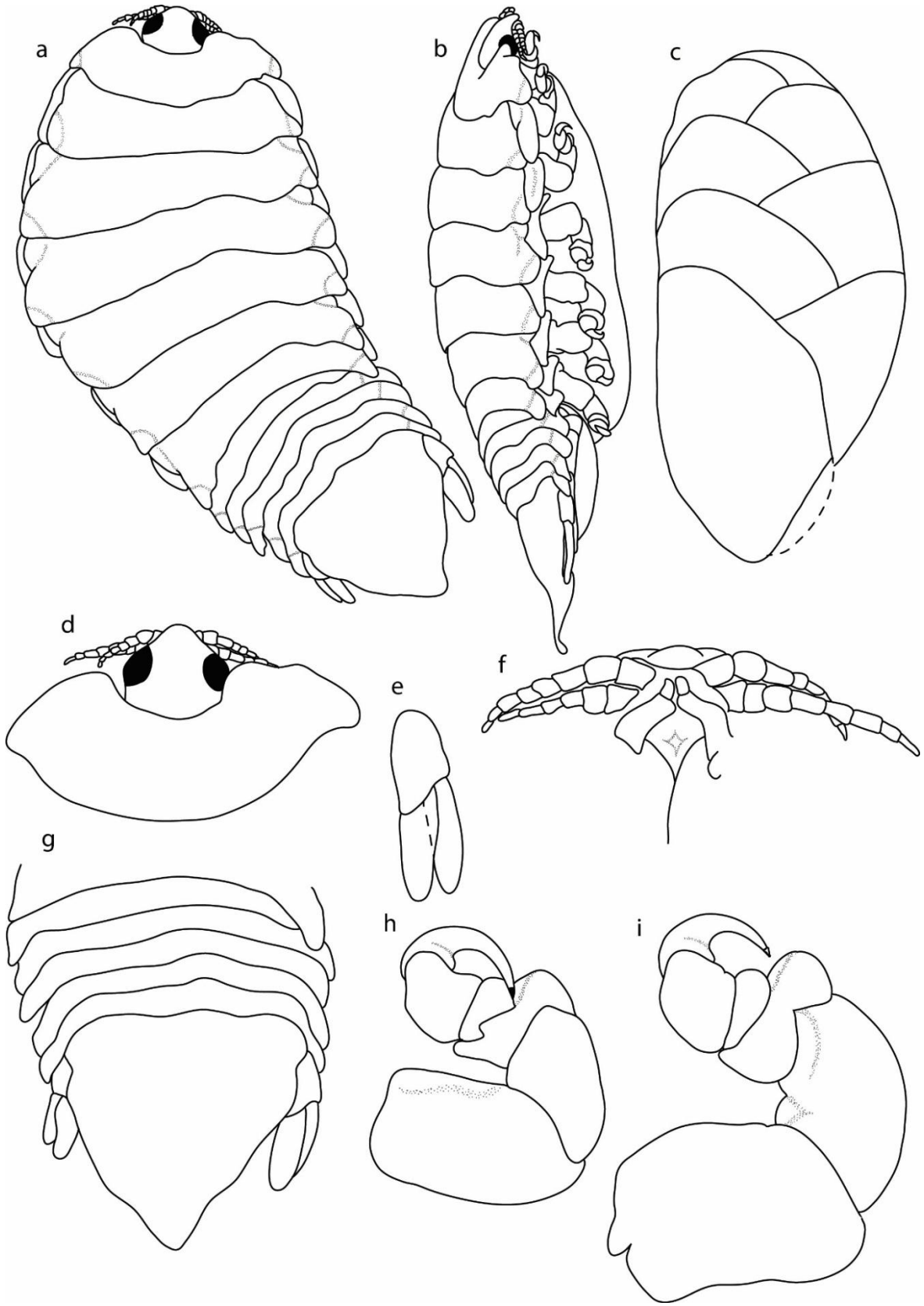

Figure 3.1: *Norileca indica* (Milne Edwards, 1840) ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon and pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Dorsal view of pleon. **h** Pereopod 1. **i** Pereopod 7.

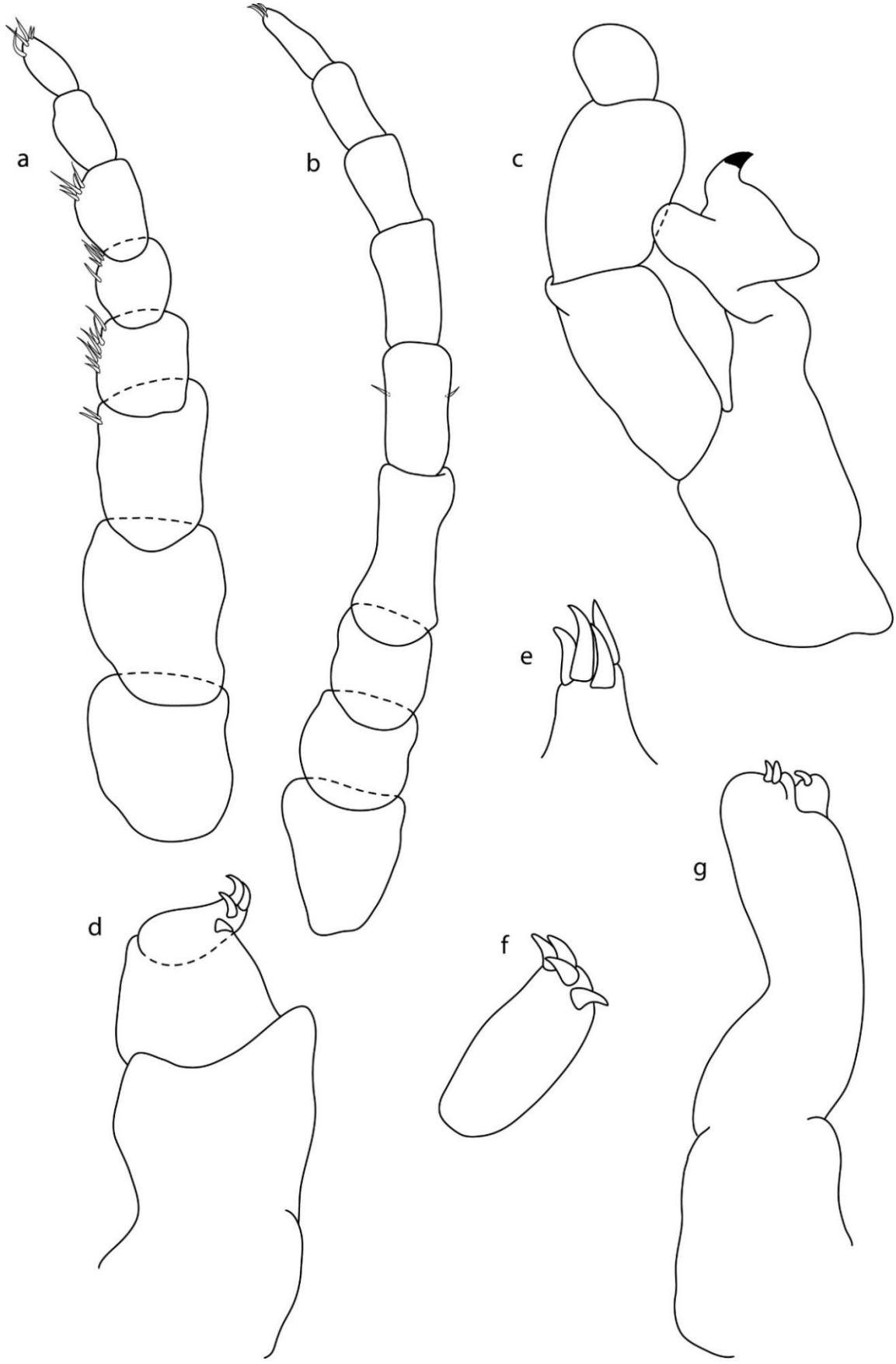

Figure3.2: *Norileca indica* (Milne Edwards, 1840) ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028**). a** Antennula. **b** Antenna. **c** Mandible. **d** Maxilliped. **e** Tip of maxillula. **f** Tip of maxilliped article 3. **g** Maxilla.

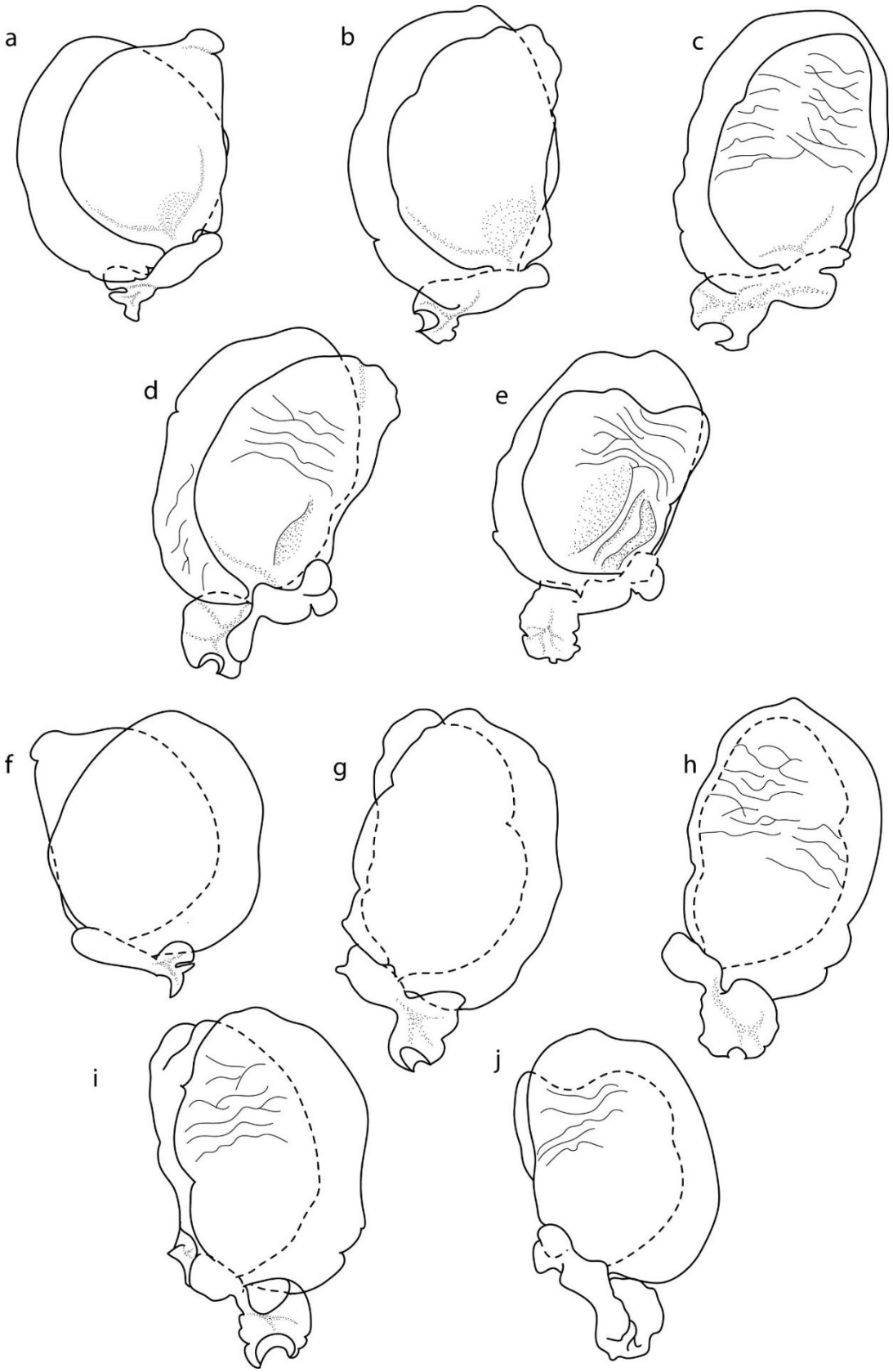

Figure 3.3: *Norileca indica* (Milne Edwards, 1840) ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Pleopod 1 ventral view. **b** Pleopod 2 ventral view. **c** Pleopod 3 ventral view. **d** Pleopod 4 ventral view. **e** Pleopod 5 ventral view. **f** Pleopod 1 dorsal view. **g** Pleopod 2 dorsal view. **h** Pleopod 3 dorsal view. **i** Pleopod 4 dorsal view. **j** Pleopod 5 dorsal view.

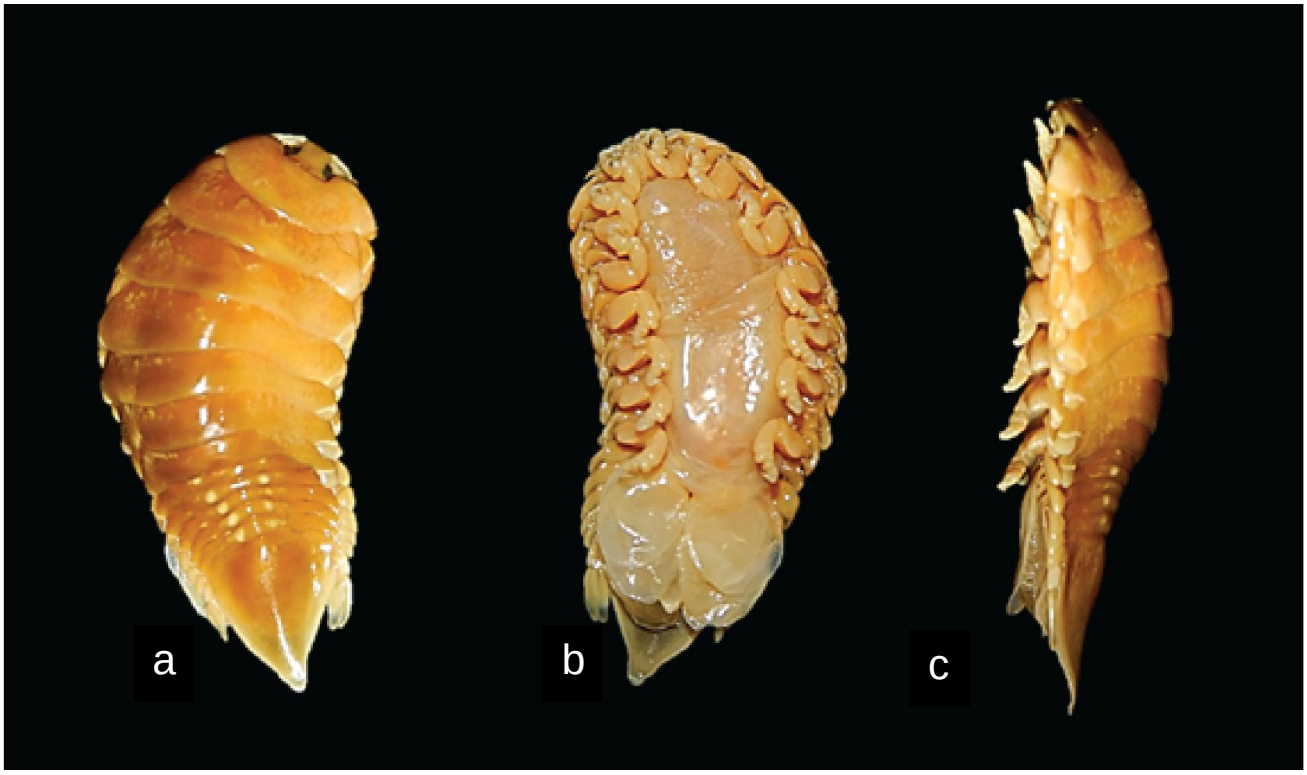

Figure 3.4: Photos of *Norileca indica* (Milne Edwards, 1840) ♀ (ovigerous, 33.0 mm TL, 16 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Dorsal view. **b** Ventral view. **c** Lateral view.

***Norileca indica* Milne-Edwards, 1884 ♂**

Figs. 3.5–3.7

Body straight, not twisted, 2.7 times as long as greatest width, widest at pereonite 5, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate. *Cephalon* 0.79 times longer than wide, visible from dorsal view, triangular, not immersed in pereonite 1. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.6 times length of cephalon. *Pereonite 1* smooth, anterior border indented; anterolateral angle weakly produced, extending past the posterior margin of eyes. Posterior margins of pereonites smooth and straight. Coxae 2–3 wide; with posteroventral angles rounded; 4–7 acute, posteriorly pointed; not extending past pereonite margin. Pereonites 6 and 7 becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 largely concealed by pereonite 7; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4. *Pleotelson* as long as anterior width; dorsal surface smooth; lateral margins weakly convex, posterior margin converging to caudomedial point.

Antenna consists of 9 articles; peduncle article 3 1.1 times as long as article 2; article 4 1.5 times as long as wide, 1.3 times as long as article 3; article 5 1.6 times as long as wide, as long as article 4. Antenna flagellum with 7 articles, terminal article terminating in 1–5 short simple setae, extending to anterior margin of pereonite 1. *Mandibular molar process* present; palp article 2 with 3 distolateral setae, article 3 with 6 simple setae. *Maxilla* medial lobe not fused to lateral lobe; medial lobe with 2 recurved robust setae, lateral lobe with 1 large recurved robust setae. *Maxilliped* consists of 3 articles; palp article 2 without setae; article 3 with 4 recurved robust setae.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin with slight bulbous protrusion; carpus with rounded proximal margin; propodus 1.3 times as long as wide; dactylus slender, 1.8 as long as propodus, 3.8 times as long as basal width. *Pereopod 7* basis 1.6 times as long as greatest width; ischium 0.9 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, 0.6 times as long as wide, 0.3 as long as ischium; carpus 0.6 times as long as wide, 0.2 as long as ischium, without bulbous protrusion; propodus 1.4 times as long as wide, 0.5 as long as ischium; dactylus slender, 1.7 as long as propodus, 3.6 times as long as basal width.

Pleopod exopod larger than endopod. *Pleopod 1* exopod 1.3 times as long as wide, lateral margin weakly convex, distally narrowly rounded, medial margin weakly oblique; endopod 1.6 times as long as wide, lateral margin slightly straight, distally narrowly rounded, medial margin straight; peduncle 0.3 times as wide as long, without retinaculae. *Pleopod 2* appendix masculina with parallel margins, 0.8 times as long as endopod, distally acute. *Pleopod 5* with fleshy folds present. Peduncle lobes present, increasing in size from pleopod 1 to 5.

Uropod same length as pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, marginal setae absent, apices narrowly rounded. *Endopod* 2.5 times as long as greatest width, without setae. *Exopod* 2.4 times as long as greatest width, without setae.

Penes prominent, 2.3 times as long as basal width, tubercules connecting at base.

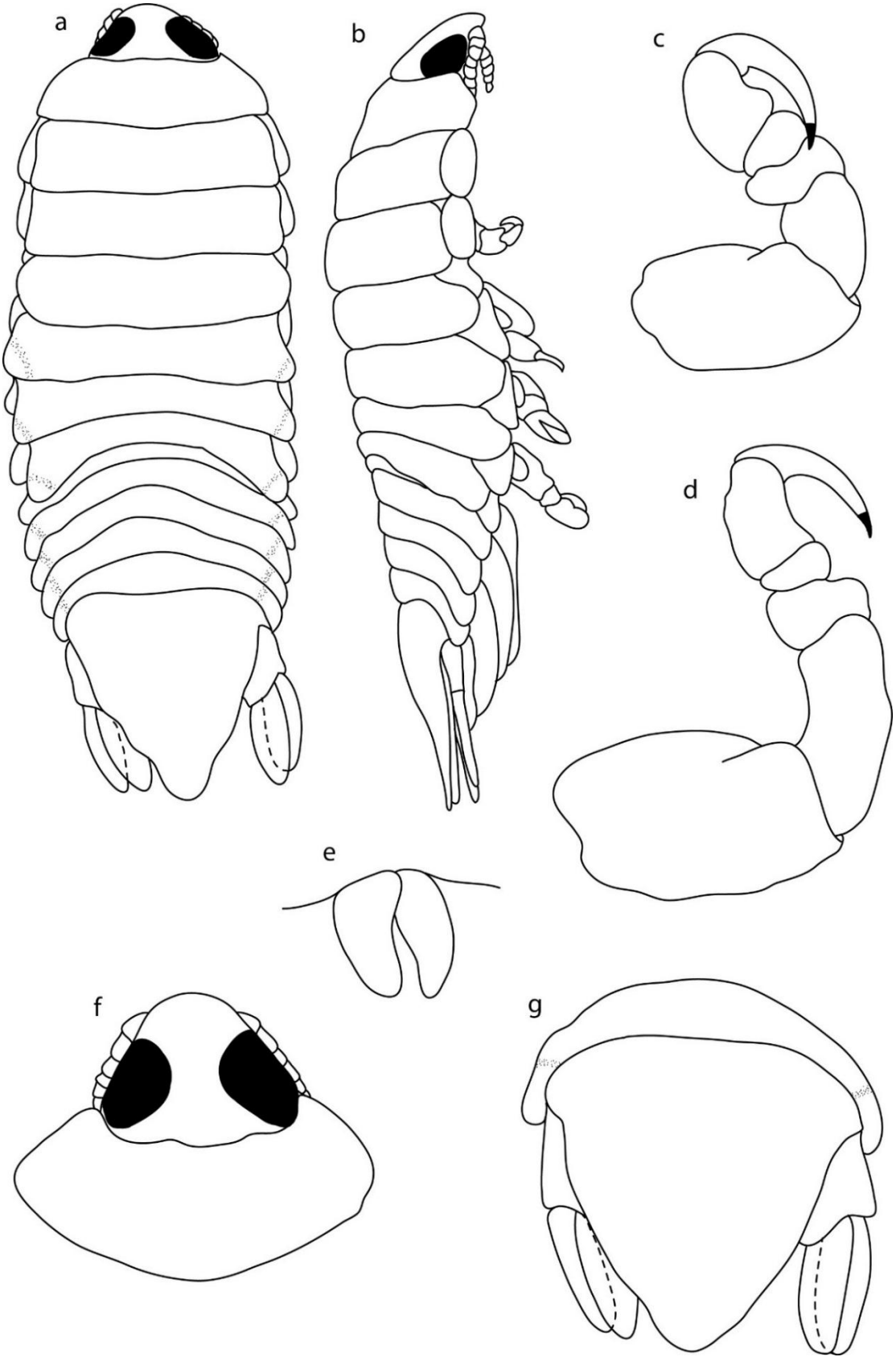

Figure 3.5: *Norileca indica* (Milne Edwards, 1840) ♂ (11.0 mm TL, 3.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Dorsal body. **b** Lateral body. **c** Pereopod 1. **d** Pereopod 7. **e** Penes. **f** Dorsal view of cephalon with pereonite 1. **g** Dorsal view of pleotelson.

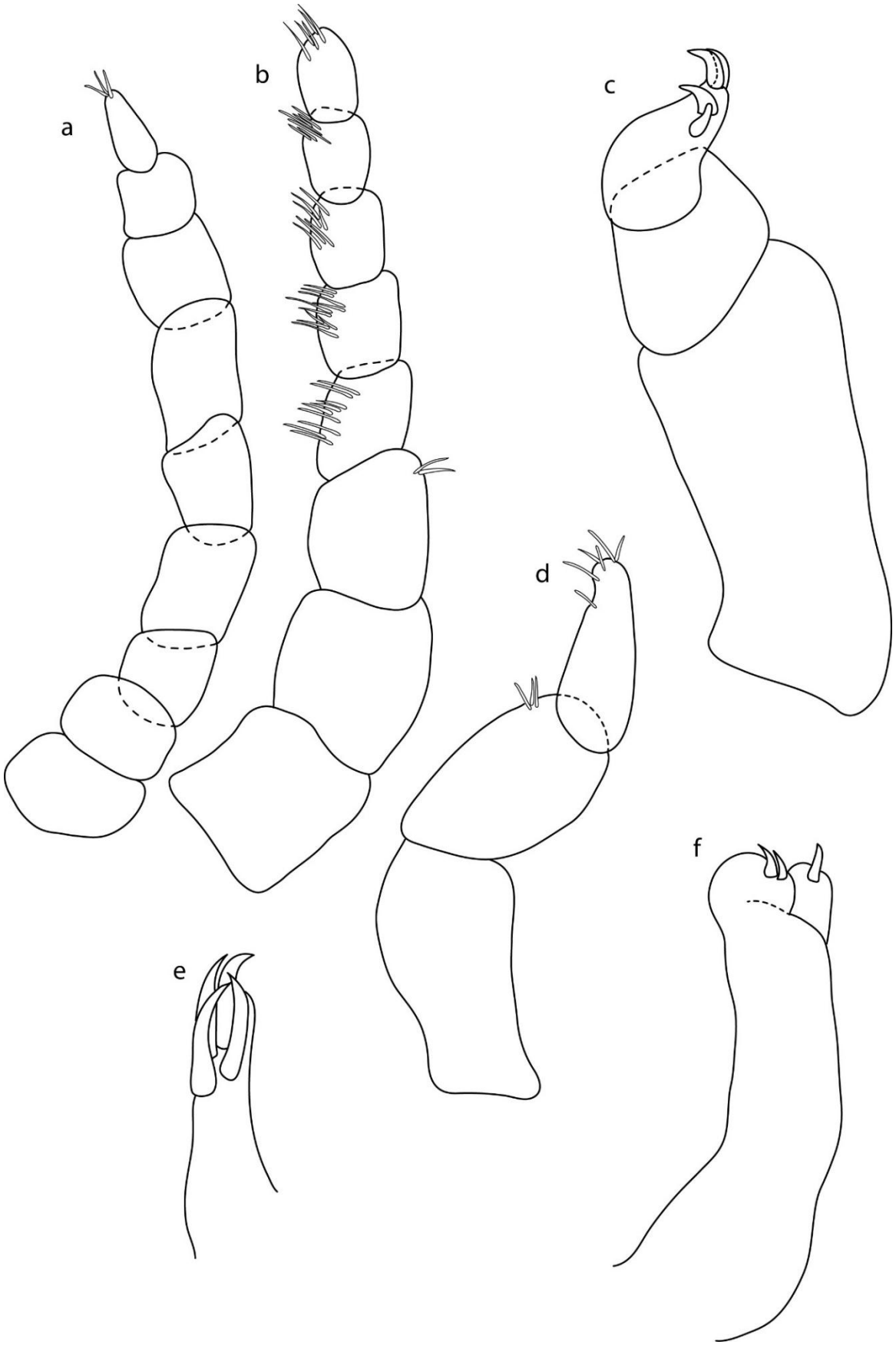

Figure 3.6: *Norileca indica* (Milne Edwards, 1840) ♂ (11.0 mm TL, 3.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Antenna. **b** Antennula. **c** Maxilliped. **d** Mandibular palp. **e** Tip of maxillula. **f** Maxilla.

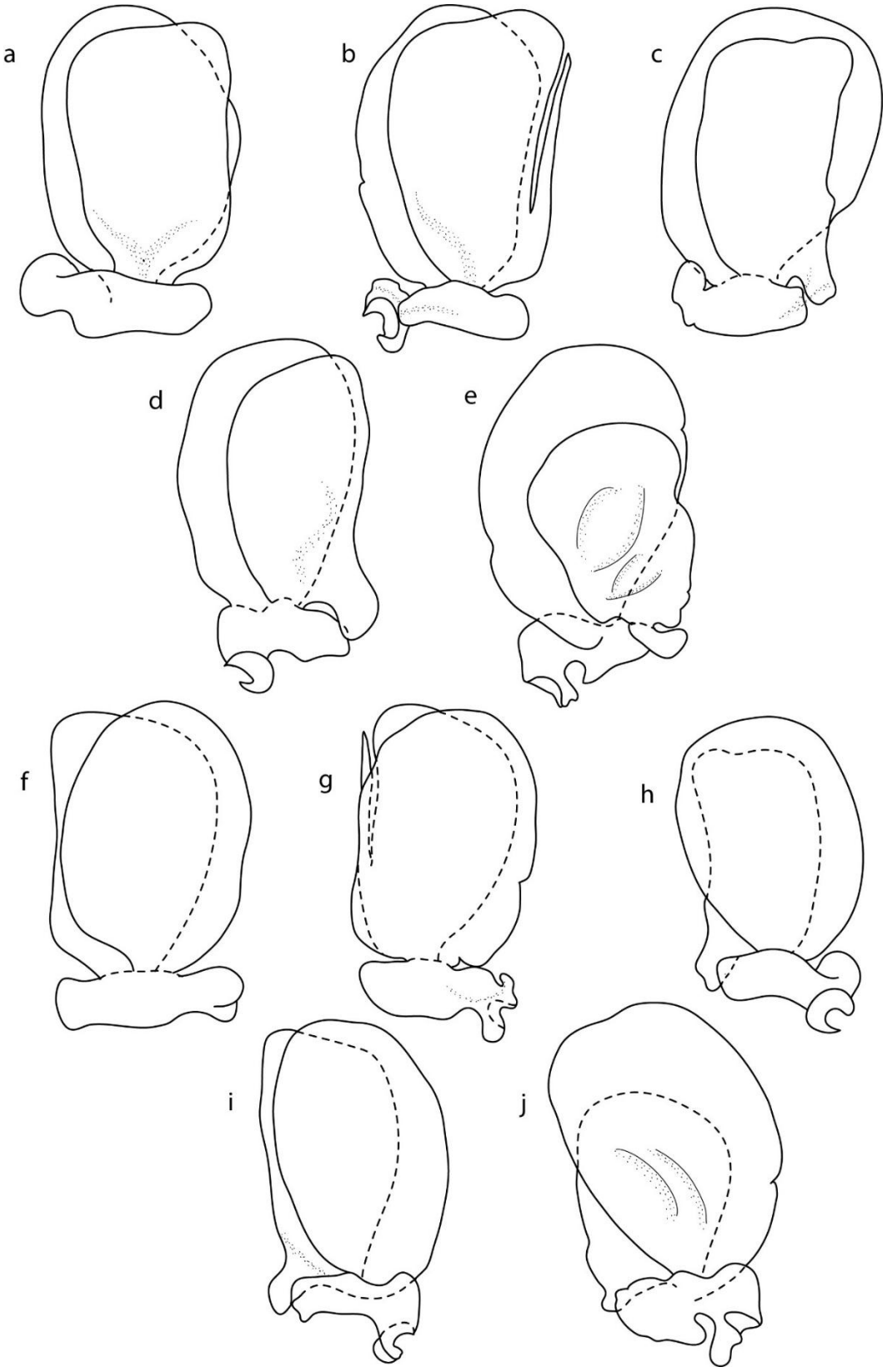

Figure 3.7: *Norileca indica* (Milne Edwards, 1840) ♂ (11.0 mm TL, 3.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Pleopod 1 ventral view. **b** Pleopod 2 ventral view. **c** Pleopod 3 ventral view. **d** Pleopod 4 ventral view. **e** Pleopod 5 ventral view. **f** Pleopod 1 dorsal view. **g** Pleopod 2 dorsal view. **h** Pleopod 3 dorsal view. **i** Pleopod 4 dorsal view. **j** Pleopod 5 dorsal view.

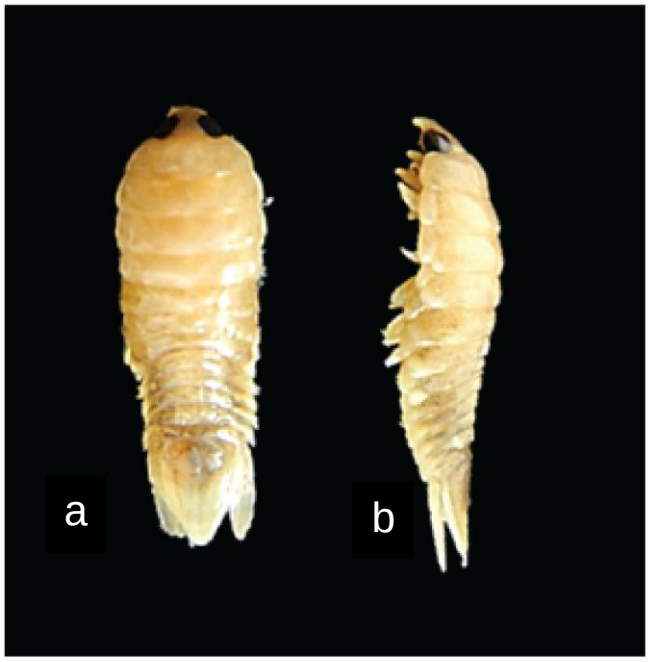

Figure 3.8: Photos of *Norileca indica* (Milne Edwards, 1840) ♂ (11.0 mm TL, 3.0 mm W) from Maputo Bay, Mozambique (SAMC-A089028). **a** Dorsal view. **b** Lateral view.

3.7.3 Distribution

Vilanandro, north-western coast of Madagascar (previously Cape Saint André) (Rokicki, 1982); Mayotte Island (Trilles, 1976); Pakistan (Behera et al., 2016); India (Rameshkumar et al., 2013c, 2015) as well as Indian eastern coast and Visakhapatnam (north-west Bay of Bengal) (Behera et al., 2016); Thailand (Nagasawa and Petchsupa, 2009) and Ko Khram (Schioedte and Meinert, 1884); Indonesia (Milne Edwards, 1840; Trilles, 1979b); China (Yu and Li, 2003); Mariveles and Luzon Islands (Schioedte and Meinert, 1884; Trilles, 1976; Yamauchi et al., 2005) and Panay Gulf, Province of Iloilo, the Philippines (Cruz-Lacierda and Nagasawa, 2017); Arafura Sea, off the Northern Territory coast of Australia (Bruce, 1990); and north-western Australia (Avdeev, 1978).

The only previous record of *N. indica* from Mozambique was by Rokicki (1982), off the Zambezi river estuary. This paper presents the first report of *N. indica* from Maputo Bay, Mozambique, representing the most southern distribution in the Indian Ocean for this species. From the distribution data, it is evident that most *N. indica* recordings have been made from the eastern regions of the Indian Ocean. The localities from this study, as well as from Trilles (1976) and Rokicki (1982), provide the only evidence of the presence of *N. indica* from the western region of the Indian Ocean. Records of *N. indica* correspond to the distribution pattern of their fish hosts.

3.7.4 Hosts

Hosts are usually pelagic and demersal marine teleosts with a preference towards schooling fish, especially those from the family Carangidae. Hosts include smallmouth scad, *Alepes apercna* Grant, 1987 (see Trilles, 1976b); Indian mackerel, *Rastelliger kanagurta* Cuvier, 1816 (see Avdeev, 1978; Rokicki, 1982; Ghani, 2003; Rameshkumar et al., 2015); blackfin scad, *Alepes melanoptera* Swainson, 1839 (previously as *Atule malam* Bleeker, 1851) (see Avdeev, 1978); bigeye scad *Selar crumenophthalmus* Bloch, 1793 (see Rokicki, 1982; Bruce, 1990; Nagasawa and Petchsupa, 2009; Neeraja et al., 2014; Cruz-Lacierda and Nagasawa, 2017; present study); *Herklotsichthys* sp. (see Bruce, 1990; Ghani, 2003; Yu and Li, 2003); and *Decapterus* sp. including the Indian scad, *D. russelli* Ruppell, 1830 (see Ghani, 2003). Other recent host records include the pugnose ponyfish, *Secutor insidiator* Bloch, 1787 (see Behera et al., 2016), and the redtail scad *Decapterus kurroides* Bleeker, 1855 (see Cruz-Lacierda and Nagasawa, 2017). Yamauchi et al. (2005) obtained *N. indica* from the stomach of the common dolphinfish, *Coryphaena hippurus* Linnaeus, 1758 where the natural host would have been eaten by the dolphinfish.

Behera et al. (2016) recorded *N. indica* from Randall's threadfin bream, *Nemipterus randalli* Russell, 1986, which is a doubtful host record. It is the first and only host record from the family Nemipteridae and a photo provided by Behera et al. (2016) (see their fig. 5c) does not appear to represent *N. indica.*

3.8 Molecular characterisation

Two ovigerous females were sequenced (forward and reverse). One ovigerous female (30.0 mm TL, 17 mm W) produced a 686 bp contig of the COI gene (accession number: MF628258), and another (26.0 mm TL, 13 mm W) produced a 687 bp and 679 bp contig of the COI gene (accession numbers: MF628259 and MF628260) respectively. These COI gene sequences are the first sequences to be generated for *N. indica* and the genus *Norileca* (see Appendix A, Sequence A1–A3). The generic placement of *N. indica* forms a seperate clade among various Cymothoidae genera (Figure 4. 15).

3.9 Remarks

Norileca indica attaches to the ventral part of the host branchial cavity, with the cephalon to the anterior end of the host and with its ventral side (abdomen/brood pouch) directed

outwards, facing the branchial operculum (Bruce, 1990; Neeraja et al., 2014; Rameshkumar et al., 2015; Behera et al., 2016). Its asymmetrical body is twisted to the left when it occupies the right branchial cavity and it is twisted to the right when it occupies the left branchial cavity (Nagasawa and Petchsupa, 2009; Neeraja et al., 2014).

Norileca indica can be recognised by its twisted body, with a 2.1–2.5 length to width body ratio and the oval eyes have distinct margins. The pleotelson of *N. indica* is approximately as long as it is wide and the uropods are two–thirds the length of the pleotelson length. Furthermore, pleonite 5 is about as wide as pleonite 1.

Males are similar in appearance to females but smaller in size and with a straight body shape. Ovigerous females differ from non-ovigerous females in having a larger body length to width ratio as they become wider as eggs develop in the brood pouch. Both Rokicki (1982) and Bruce (1990) reported that *N. indica* is ventrally positioned in the host gill cavity with the cephalon facing the anterior end of the host and the abdomen outwards toward the operculum (in a lateral position). Four of the currently collected specimens were also ventrally positioned; however, two were positioned with the dorsal surface to the operculum.

Norileca indica can be distinguished from *N. triangulata* by being larger than the latter, with its body twisted to the side, a straight sided pleon, smaller eyes, as well as shorter uropods and a shorter mandible palp article 3 (Bruce, 1990). It differs from *N. borealis* in having shorter uropods, larger eyes, and a larger length to width ratio of the pleotelson. Pleonite 1 and 5 of *N. indica* are more equal in width than that of *N. borealis* where pleonite 5 is narrower than pleonite 1. In addition, *N. borealis* has a medial lobe with 4 recurved robust setae (2 recurved robust setae on *N. indica*), and article 3 of the maxilliped with 3 recurved robust setae (4 recurved robust setae on *N. indica*) (Javed and Yasmeen, 1999). Even though *N. borealis* and *N. triangulata* are more similar to each other than to *N. indica*, they can be distinguished from each other by body shape, ventral margin of the cephalon, as well as pleon and pleopod morphology.

Since the redescription of *N. indica* in 1990, records of this species from the Indian subcontinent have mainly been made due to the catchment of its fish hosts by local fisherman. Many host species, including *Selar crumenophthalmus* and *Decapterus kurroides,* are considered to be commercially important fish species as they are regularly caught for human consumption (Argente et al., 2014; Cruz-Lacierda and Nagasawa, 2017). Other publications provide new host or locality information on *N. indica* as well as some ecological data including prevalence, mean intensity and abundance (Neeraja et al., 2014; Rameshkumar et al., 2015; Behera et al., 2016; Jithin et al., 2016). Despite this species

being frequently collected, limited work has been done recently on the morphology and taxonomy of this species.

3.10 Discussion

The redescription of *N. indica* by Bruce (1990) was based on non-ovigerous females. In this chapter the first detailed redescription of an ovigerous female specimen is presented. Ovigerous females display diagnostic characteristics and structures that may not be present or as well developed in non-ovigerous females and males.

Cymothoid isopods are protandrous hermaphorodites, making *N. indica* difficult to identify during different developmental stages. As part of the female development, the pleotelson becomes wider and other structures (such as the gonopod, eye- and uropod perimeter, and the first antenna) become shorter in length (Cook and Munguia, 2015). Males tend to be morphologically similar to one another; therefore, accurate and comprehensive descriptions of males are essential to ensure species identification can potentially be made in the absence of ovigerous females.

Norileca indica is widely distributed and morphological analyses combined with molecular analyses will provide a better understanding of this species. These analyses will confirm species identity, even during the natatory stage of development (see Jones et al., 2008). It is essential to combine genetic characterisation of a species, with an accompanying description of that species based on the same material in order to verify the identification of the species. Only when the identification of the species is accurate, can phylogenetic analysis be effective, providing useful information without having the confusion of misidentified species as is currently the problem with cymothoids.

The use of combining molecular and morphological data for phylogenetics is becoming more prominent within taxonomic publications. This combination improves the resolution, internal support and overall quality of phylogenetic studies (Caddick et al., 2002; Scotland et al., 2003). In many instances, there exists a lack of either morphological or molecular data (Giribet et al., 2001). This is also the case with *N. indica,* as no other publication is available that provides a comprehensive dataset of both morphological and molecular results of this species. The use of molecular techniques seems to eliminate morphological bias as well as over- and under-estimations of biodiversity that is occasionally associated with traditional morphological analyses (Lefébure, 2006).

CHAPTER 4: REVIEW OF *ELTHUSA* SCHIOEDTE AND MEINERT, 1884, FROM SOUTH AFRICA

4.1 Introduction

The type species for the genus *Elthusa*, recorded as *Livoneca emarginata* Bleeker, 1857 was first described from Jakarta in Indonesia (previously known as Batavia). Information regarding the site of attachment and hosts were unavailable, unknown or just not recorded. In 1884, Schioedte and Meinert redescribed this species and placed it into a new genus, *Elthusa,* based on specimens found in Indonesia. It was the only species of this genus at the time (Hadfield et al., 2017d). The description of the genus *Elthusa*, and the transfer of *Livoneca emarginata* to this genus, was based on the level of immersion of the pleon. *Livoneca* was described with having an immersed pleon, while *Elthusa* was described with having a less immersed pleon. This redescription added valuable information on the genus including morphological features, host species and a new locality, but was still insufficient with regards to the description of distinguishing characteristics, separating the *Elthusa* from other related genera. Following its original description in 1884, *Elthusa* received no futher attention until it was fully redescribed by Bruce (1990) based on one of Bleeker's syntypes from 1857.

To date, there are 31 known and accepted *Elthusa* species according to the World Register of Marine Species (Hadfield et al., 2017a). They represent one of the most speciose genera within the Cymothoidae family (Kumar and Bruce, 1997). Many *Elthusa* species still need to be studied and redescribed as a result of the high morphological variability that exists within this genus (Hadfield et al., 2017d). Most species from the genus *Elthusa* represent branchial cavity inhabiting cymothoids, with the exception of two species. *Elthusa neocytta* (Avdeev, 1975) ovigerous females has been recorded from the buccal cavity of the spiky oreo, *Neocyttus rhomboidalis* Gilchrist, 1906 (Stephenson, 1987) and *Elthusa splendida* (Sadowsky & Moreira, 1981) has been recorded from the buccal cavity of the spiny dogfish *Squalus cubensis* Rivero, 1936. Several early publications provide records of *Elthusa* species attaching to the buccal- or external surface of a host (Richardson, 1909; Richardson, 1910; Yamauchi, 2009). These records are questionable, and are most likely a result of host abandonment or trawl transfer.

Elthusa is considered to be cosmopolitan, except for polar waters (Bruce et al., 2002; Rocha-Ramírez et al., 2005; Hadfield et al., 2017d), and is predominantly recorded from the Pacific and Indo-Pacific region, with occasional records of species from the Atlantic Ocean

(Kensley and Brusca, 2001). Trilles and Justine (2006) provide the general global distribution of *Elthusa* species. *Elthusa raynaudii* (Milne Edwards, 1840) is the only *Elthusa* species that has been described from sub-Sahara Africa, and in particular from South Africa. These records were made at the Cape of Good Hope, Hout Bay and Table Bay (Kensley, 1978) from the rocksucker, *Chorisochismus dentex* (Pallas, 1769), as well as at Durban (Kensley, 1976) from the striped trumpeter, *Latris lineata* (Forster, 1801).

Species from the genus *Elthusa* can be distinguished from other genera by having a slightly vaulted body shape with a wide pleon; a cephalon posterior margin which is not trilobed and lamellar pleopods. Smaller diagnostic characteristics include antennulae that are shorter than antennae; a slender maxilliped palp article 3, with setae present; as well as pereopods with relatively short dactyli (Bruce, 1990). Confusion on the distinction between genera is mostly due to the incomplete or insufficient descriptions of type species, including those described by Schioedte and Meinert (1884). Bruce (1990) transferred many *Livoneca* species to the genus *Elthusa* based on previous descriptions and the examination of type material.

4.2 Specific materials and methodology used

Fourteen preserved specimens representing that of the genus *Elthusa* were examined. Specimens were collected from South Africa. Additional preserved material (also originally collected from South Africa) from the National Museum of Natural History, Paris, France (MNHN) and the Iziko South African Museum, Cape Town (SAM) were included in the examination. Methods follow the general methodology (see Chapter 3.3–3.4). MEGA®7 bioinformatics software program was used to align sequences and construct a Maximum likelihood (ML) phylogenetic tree based on the Kimura 2-parameter model.

4.3 Taxonomy

Suborder Cymothoida Wägele, 1989
Superfamily Cymothooidea Leach, 1814
Family Cymothoidae Leach, 1814

Genus *Elthusa* Schioedte and Meinert, 1884

Elthusa Schioedte and Meinert, 1884: 337.—Nierstrasz, 1915: 96.—Nierstrasz, 1931: 128.—Bruce, 1990: 254.—Trilles 1994: 164.—Bruce, Lew Ton and Poore, 2002: 176.—

Trilles and Justine, 2004: 213.—Trilles and Justine, 2006: 58-59.—Trilles and Justine, 2010: 181.—Trilles and Randall, 2011: 453–454.—Hadfield, Tuttle and Smit, 2017d: 3.

Type species: *Livoneca emarginata* Bleeker, 1857 by monotypy (Schioedte and Meinert, 1884). A single female syntype examined by Bleeker (1857), is deposited at the Rijksmuseum von Natuurlijke Historie, Leiden, under the registration number RMNH 66 (Bruce, 1990). The specimen examined by Schioedte and Meinert, 1884, is held in the MNHN, Paris, under registration number 241 (Trilles, 1976).

4.4 Diagnosis of female

Body weakly vaulted, approximately 1.8–2.3 times as long as greatest width, twisted to the side or symmetrical. Cephalon moderately or deeply immersed in pereonite 1; anterior margin weakly or moderately produced, rostrum subtruncate, posterior margin not trilobed. Pleon wide, moderately to deeply immersed in pereonite 7; pleonite 1 as wide as, or somewhat narrower than pleonite 2. Antennula shorter than antenna or subequal; rarely consisting of less than 8 articles, bases never in contact. Antenna rarely consisting of more than 8–12 articles. . Mandible palp slender, setae present on article 3 or 2 and 3. Pereopod bases with carina; pereopods 1–3 with carpus cleft. Oostegites 1–4 present, oostegites 5–6 sometimes present. Pleopods simple, lamellar, without setose lobes and folds, decreasing in size from 1–5. Pleopod 5 endopod medial margin never concave. Uropods not extending to the posterior margin of pleotelson, rami typically subequal.

4.5 Remarks

Bruce (1990) provided a provisional diagnosis of the genus. Trilles and Randall (2011) and Hadfield et al. (2017d) later provided further diagnoses. It has become clear that most of the originally described *Lironeca* Leach, 1818 species belong to the genus *Elthusa* as their generic descriptions correspond to those of *Elthusa*.

Intra-specific variation in morphological characters are evident within *Elthusa*, including the distance between antennula bases; the anterior margin of the cephalon; width of the pleon in relation to pereonite 7; width of pleonite 5; and the maxilliped of ovigerous females with or without oostigital lobe. These characters should be taken into account with the generic

characters to provide a comprehensive description and accurately identify and describe species.

4.6 Key to the species of *Elthusa* from southern Africa

1. Pereonite 1 anterior margin straight; coxae 7 not extending past posterior margin of pereonite 7; pleonite 5 lateral margins visible; pleonites sub-equal in width; uropods more than half the length of pleotelson……..……..……**2**
   Pereonite 1 anterior margin with medial point; coxae 7 extending past posterior margin of pereonite 7; pleonite 5 lateral margins largely concealed by pleonite 4; pleonite 1 widest; uropods short, less than half the length of pleotelson…….***Elthusa* sp. 2**

2. Anterior margin of cephalon narrowly truncate; uropod rami apically rounded; pleotelson shape semi-oval……..***Elthusa raynaudii***
   Anterior margin of cephalon pointed; uropod rami apically pointed; pleotelson rectangular shape……..…***Elthusa* sp. 1**

4.7 *Elthusa raynaudii* (Milne Edwards, 1840)

Livoneca Raynaudii Milne Edwards, 1840: 262.—Krauss, 1843: 66.—Bleeker, 1857: 30.—Schioedte and Meinert, 1884: 367, pl. 12, figs 9–13.—Thielemann, 1910: 42.—Hale 1926: 215–217, figs. 10 a–j.

Cymothoa Novae–Zealandia White, 1847: 110, *nomen nudum.*

Lironeca novae–zealandia Miers, 1874: 228.—Miers, 1876: 106, pl. III, fig. 2.—Miers, 1881: 64, 67.

Lironeca laticauda Miers, 1877: 677, pl. 69, fig. 5.—Ellis, 1981: 124.

Livoneca Raynaudi.—Gerstaecker, 1882: 259.

Livoneca Novae Zelandiae.—Gerstaecker, 1882: 263.

Lironeca Stewarti Filhol, 1885: 450, pl. 4, fig. 6.

Lironeca neo-zelanica.—Thomson and Chilton, 1886: 154.

Livoneca raynaudii.—Whitelegge, 1902: 236.—Stebbing, 1910: 125.—Chilton, 1909: 606.—Chilton, 1911: 309.—Chilton, 1912: 135.—Young, 1926: 283.—Hale, 1926: 215, fig. 10.—Hale, 1929: 261, figs 253, 259.—Hale, 1940: 303.—Barnard, 1940: 491.—Hurley, 1961: 268.—Hewitt and Hine, 1972: 108.—Sivertsen and Holthuis, 1980: 34.—Beumer, Ashburner, Burbury, Jette, Latha, 1982: 33.

Livoneca epimerias Richardson, 1909: 88, fig. 13.—Kussakin, 1979: 301, figs. 69, 170.

Livoneca raynaudi.—Nierstrasz, 1915: 97.—Nierstrasz, 1931: 145.—Barnard, 1920: 358.—Pillai, 1954: 16.

Livoneca laticauda.—Nierstrasz, 1931: 143.

Lironeca raynaudii.—Brian and Dartevelle, 1949: 176.—Avdeev, 1975: 250.—Avdeev, 1978: 281.—Trilles, 1976: 778, pl.1, fig. 4.—Poore, 1981: 341.

Lironeca raynaudi.—Menzies, 1962: 115, fig. 36A, B.—Kensley, 1978: 80, fig. 33B.—Moreira and Sadowsky, 1978: 111.

Lironeca magna Mañé-Garzón, 1979: 18, figs. 1–5.

Elthusa raynaudii—Bruce, 1990: 263.—Bruce, Lew Ton and Poore, 2002: 177.—Williams, Bunkley-Williams and Ebert, 2010: 99–101.

Elthusa raynaudi—Ghani 2003: 218.

Type material: Type material held at the Museum National d'Histoire Naturelle, Paris (No.255).

Type locality: Cape of Good Hope, South Africa.

Type host: Unknown.

4.7.1 Material examined

Syntype. Female (ovigerous, 26.7 mm TL, 14.1 mm W), *Livoneca raynaudii* Milne Edwards, 1840, collected from the Cape of Good Hope, South Africa (MNHN–IU–2016–9885).

Other material. Female (ovigerous, 20.0 mm TL, 12.0 mm W), from RV *Dr Fridtjof Nansen* trawl (269 m), southern African West coast (fish sorting table), during February 2010 (SAMC – A089957). No host detail recorded. Female (ovigerous, 26.0 mm TL, 15.0 mm W), *Lironeca raynaudii*, collected from RV *Dr Fridtjof Nansen* trawl (291 m), southern African West coast (Station NAN401T062), during January 2007 (SAMC – A47881), by L Atkinson. No host recorded. Female (ovigerous, 26.0 mm TL, 14.0 mm W), from RV *Africana* trawl (no trawl depth available), southern African South coast (fish sorting table), during April 2003. No host detail was recorded. (In the collection of the authors at NWU) (see Figure 4.4).

4.7.2 Descriptions

***Elthusa raynaudii* (Milne Edwards, 1840)** ♀

Figs. 4.1–4.3

Body ovoid, slightly twisted to the left, 1.7 times as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1; pereonite lateral margins mostly posteriorly ovate, medially indented. *Cephalon* 0.9 times longer than wide, visible from dorsal view, sub-truncate with blunt anterior margin. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.2 times width of cephalon, 0.4 times length of cephalon. *Pereonite 1* smooth, anterior border medially straight, curved laterally, anterolateral angle narrowly rounded, extend to the medial region of eyes. Posterior margins of pereonites smooth, slightly curved laterally. *Coxae* 2–3 wide, with posteroventral angles rounded; coxae 4–7 with rounded point, not extending past pereonite posterior margin. Pereonites 2–5 subequal, becoming more progressively rounded posteriorly; 6 and 7 slightly narrower. *Pleon* 0.4 times as long as total body length, with pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin mostly concave. *Pleonite 2* partially overlapped by pereonite 7. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 widest, with posterolateral angles narrowly rounded, posterior margin straight. *Pleotelson* 0.6 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins weakly convex, posterior margin evenly rounded.

Antennula shorter than antenna, consists of 8 articles; antennula peduncle articles 1 and 2 distinct and articulated, extending to anterior of pereonite 1. *Antenna* consists of 11 articles, extending to middle of pereonite 1.

Pereopod 1 basis 1.6 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.4 times as long as wide; dactylus slender, 1.6 times as long as propodus, 2.9 times as long as basal width. *Pereopod* 3 similar to pereopod 2, all without robust or simple setae. *Pereopod 7* basis with carina, 2.5 times as long as greatest width; ischium 0.5 times as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, 0.7 times as long as wide, 0.4 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 0.8 times as long as wide, 0.3 times as long as ischium; dactylus slender, 2.3 times as long as propodus, 3.5 times as long as basal width.

Pleopods simple, exopod larger than endopod. *Pleopod 1* exopod 1.3 times as long as wide, lateral margin weakly convex, distally narrowly rounded, mesial margin straight; peduncle 2.3 times as wide as long.

Uropod more than half the length of pleotelson, peduncle 0.5 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, apices broadly rounded. *Endopod* apically rounded, 2.7 times as long as greatest width, terminating without setae. *Exopod* extending to end of endopod, 2.2 times as long as greatest width, apically rounded, terminating without setae.

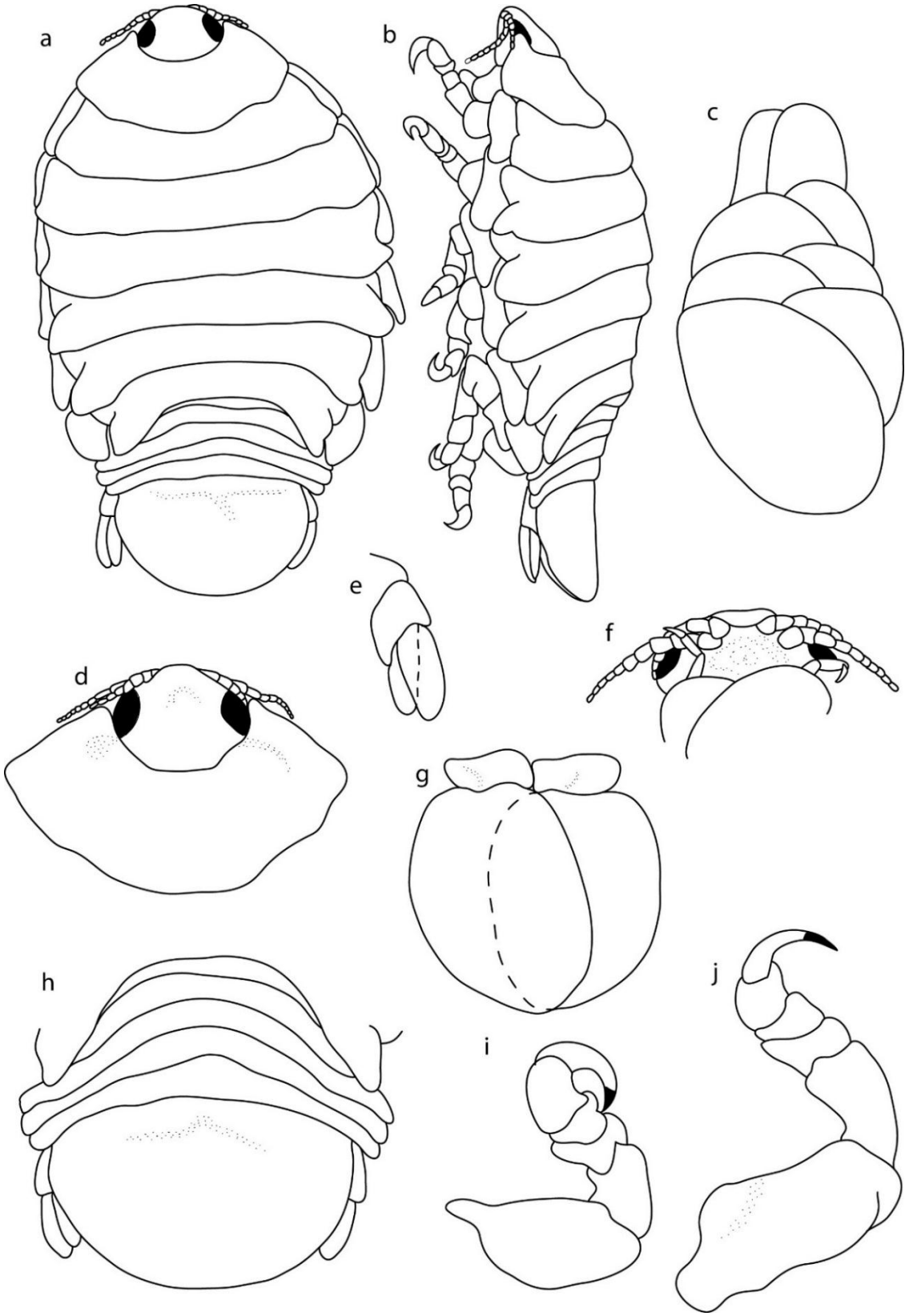

Figure 4.10: *Elthusa raynaudii* (Milne Edwards, 1840) ♀ (ovigerous, 20.0 mm TL, 12.0 mm W) from *Dr Fridtjof Nansen* research vessel. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon and pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Pleopod 1. **h** Dorsal view of pleon. **i** Pereopod 1. **j** Pereopod 7.

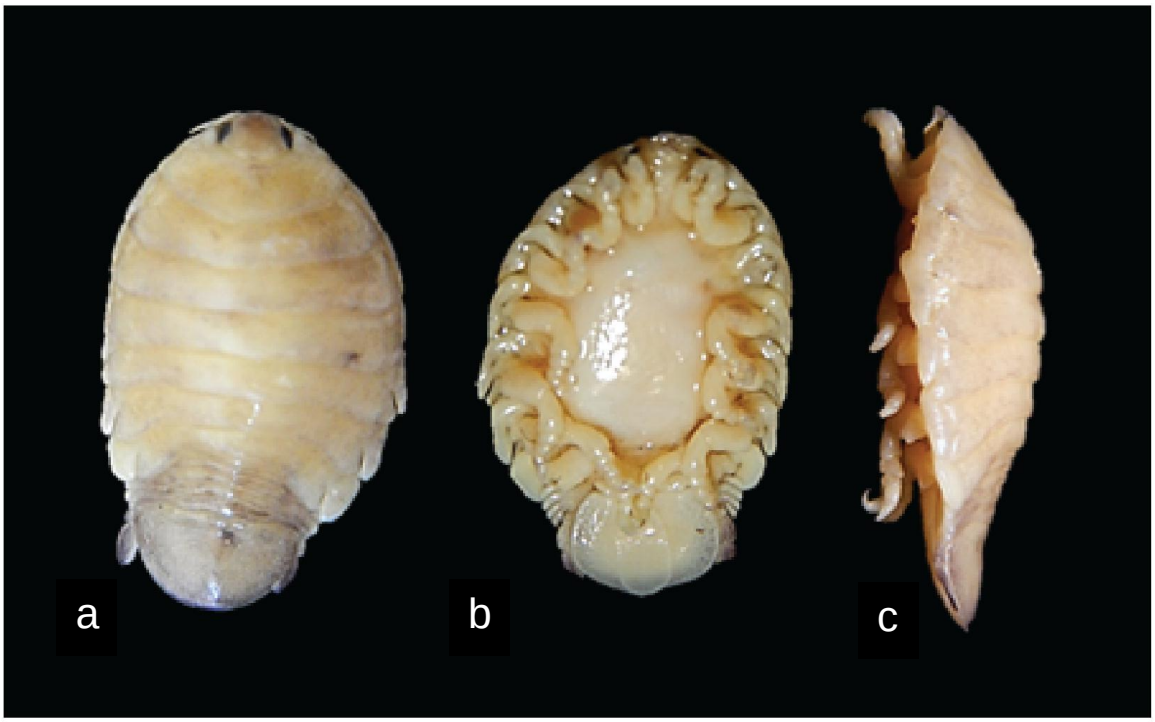

Figure 4.2: Photos of *Elthusa raynaudii* (Milne Edwards, 1840) ♀ (ovigerous, 20.0 mm TL, 12.0 mm W) from *Dr Fridtjof Nansen* research vessel. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

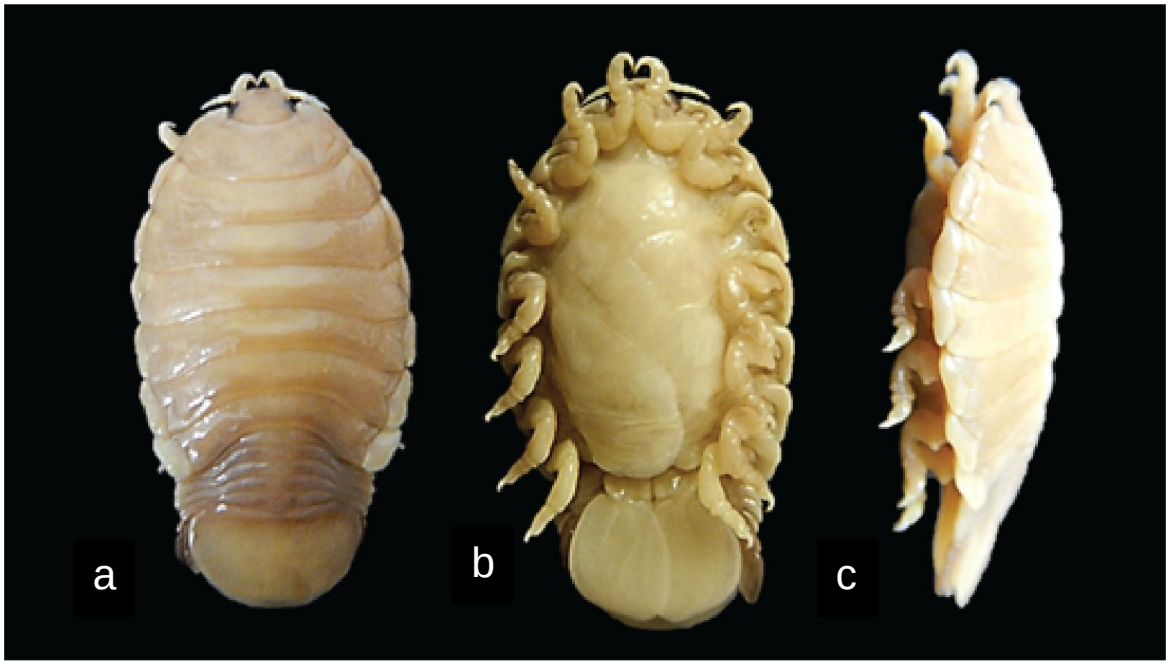

Figure 4.3: Photos of *Elthusa raynaudii* (Milne Edwards, 1840) ♀ (ovigerous, 26.0 mm TL, 14.0 mm) from *Africana* research vessel. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

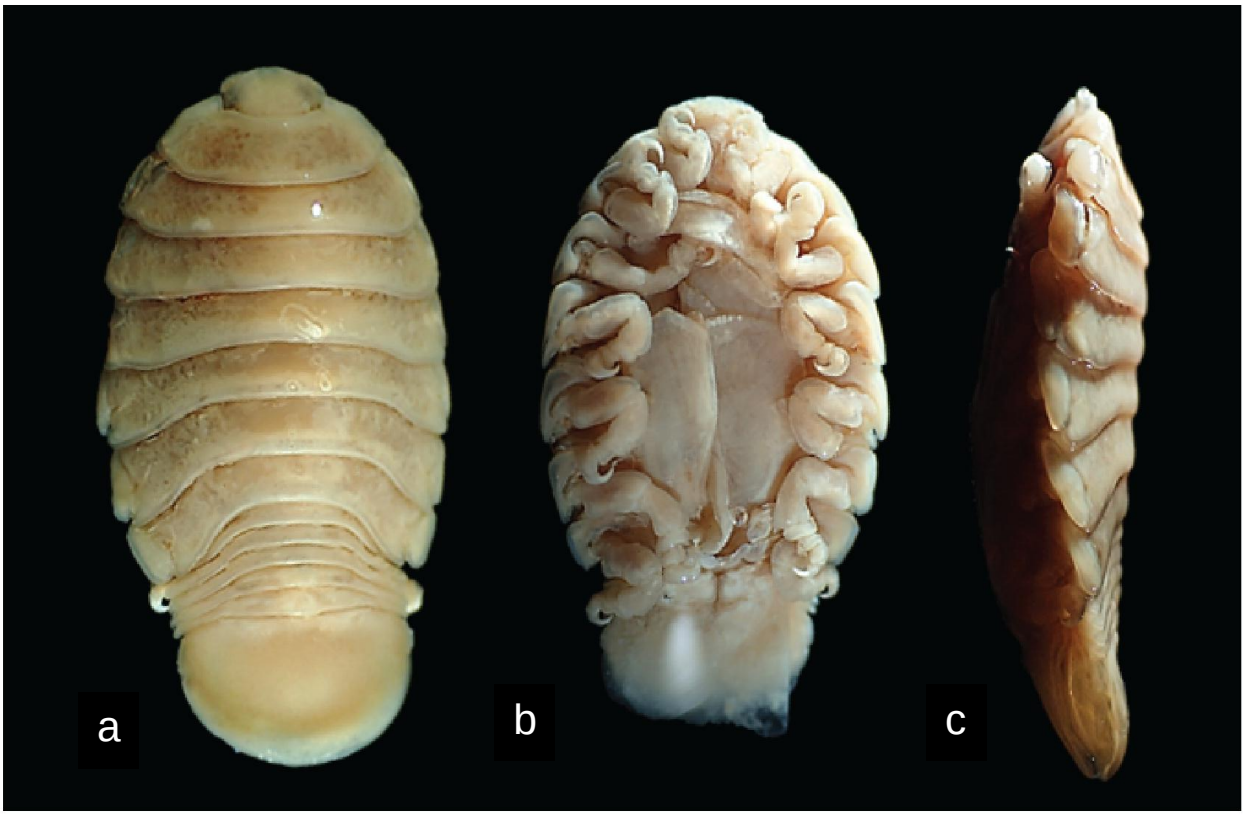

Figure 4.4: Photos of syntype material MNHN–IU–2016–9885 (MNHN–Is692) *Livoneca raynaudii* Milne Edwards, 1840 ♀ (ovigerous, 26.7 mm TL, 14.1 mm W). **a** Dorsal view. **b** Ventral view. **c** Lateral view.

4.7.3 Variation

Some degree of morphological variation among the examined *E. raynaudii* specimens was noted. These intra-specific variations can cause difficulty in the identification process and should be taken into consideration. Body shape may vary from being completely ovoid to elongated ovoid; body widest at pereonite 4 or 5; pereonite 1 anterior margin may be slightly concave; pleonite 5 posterior margin range between begin straight, slightly concave or with a slight medial point.

4.7.4 Distribution

Records listed from West to East.

South America: Punta Quillaipe (Menzies, 1962) and Strait of Magellan (Nierstrasz, 1931), Chile; Uruguay (Mañé-Garzón, 1979). **South Africa:** Hout Bay and Table Bay (Kensley, 1978); Cape of Good Hope (Milne Edwards, 1840); Durban (Kensley, 1976). **India**:

Travancore (Pillai, 1954). **Australia:** South Australia: Cape Wiles and Port Adelaide (Hale, 1926). Queensland: south-eastern Queensland; Flinder's Reef; Skua Point (Bruce, 1990). New South Wales: Cape Three points (Whitelegge, 1901; Bruce, 1990), Sydney and Terrigal off Botany Bay (Hale, 1926); Wattamolla lagoon ("Wata Mooli") (see Whitelegge, 1901); east of Coogee, Long Reef, Shoalhaven Bight, north-east of Wollongong, and Port Jackson (Bruce, 1990). Victoria: south-east of Gabo Island, south-south-west of McCann, Bruni Island, and off Tasman Head (Bruce, 1990); Hobart (Schioedte and Meinert, 1884). Tazmania: Eaglehawk Neck, Murdunna, Dunally, Taranna, north-east of East Sister Island, and off Strahan (Bruce, 1990). **New Zealand:** Schioedte and Meinert (1884) mentioned New Zealand; Stewart Island (Filhol, 1885), Snares Islands (Bruce, 1990), Antipode Islands (Chilton, 1909), Norfolk Island (Hurley, 1961), Akaroa (Nierstrasz, 1915). **Japan**: Hale (1929) mentioned Japan; Yokohama (Schioedte and Meinert, 1884). **North Pacific Ocean:** St. Paul Island in the Bering Sea (Kensley, 1976). **Southern Indian Ocean:** Amsterdam Island (Kensley, 1976). **South Atlantic Ocean:** Saint Helena and Tristan da Cunha (Sivertsen and Holthuis, 1980).

4.7.5 Hosts

Elthusa raynaudii has been recorded from various fish hosts of multiple orders and families. These hosts are: *Chelidonichthys kumu* (Cuvier, 1829) (see Avdeev, 1978); *Chorisochismus dentex* (Pallas, 1769) (see Barnard, 1920); *Cyttus australis* (Richardson, 1843) (see Avdeev, 1978, 1984; Bruce, 1990); *Cyttus novaezelandiae* (Arthur, 1885) (see Avdeev, 1978, 1984); *Cyttus traversi* Hutton, 1872, previously *Cyttoidops mccullochi* (Whitley, 1947) (see Avdeev, 1984; Bruce, 1990); *Genypterus blacodes* (Bloch and Schneider, 1801) (see Hewitt and Hine 1972); *Gnathanacanthus goetzeei* Bleeker, 1855 (see Bruce, 1990); *Hyporhamphus intermedius* (Cantor, 1842) (see Powell, 1959; Stephenson, 1969); *Latris lineata* (Forster, 1801) (see Kensley, 1976); *Meuschenia freycineti* (Quoy and Gaimard, 1824) (see Bruce, 1990); *Mustelus antarcticus* Günther, 1870 (see Hewitt and Hine, 1972); *Nemadactylus monodactylus* (Carmichael, 1819), previously *Acantholatris monodactylus* (Carmichael, 1819) (see Sivertsen and Holthuis, 1980); *Nematalosa nasus* (Bloch, 1795) (see Ghani, 2003); *Notacanthus sexspinis* Richardson, 1846 (see Avdeev, 1978, 1984); *Notothenia microlepidota* Hutton, 1875, previously *Notothenia colbecki* (see Chilton, 1909; Hewitt and Hine, 1972; Avdeev, 1978, 1984); *Notolabrus tetricus* (Richardson, 1840), previously *Pseudolabrus tetricus* (see Bruce, 1990); *Paranotothenia magellanica* (Forster, 1801), previously *Notothenia macrocephala* (see Avdeev, 1978); *Ilisha melastoma* (Bloch and Schneider, 1801) previously *Pellona brachysom*a (see Pillai, 1954); *Pelotretis flavilatus*

Waite, 1911 (see Chilton, 1911); *Pseudophycis bachus* (Forster, 1801) previously *Physiculus bachus* (see Hewitt and Hine, 1972); *Physiculus* sp. (see Bruce, 1990); *Physiculus barbatus* (Günther, 1863), previously *Physiculus barbatus* (see Bruce, 1990); *Pseudolabrus miles* (Schneider and Forster, 1801) (see Poore, 1981; Bruce, 1990); *Pseudophycis bachus* (Forster, 1801) (see Chilton, 1911; Bruce, 1990); *Rexea solandri* (Cuvier, 1832) (see Bruce, 1990); *Rhombosolea* sp. (see Hewitt and Hine, 1972); *Sardinops sagax* (Jenyns, 1842), previously *Clupea neopilchardus* Steindachner, 1879 (see Chilton, 1911); *Scorpaena cardinalis* Solander and Richardson, 1842 (see Poore, 1981); *Sebastes capensis* (Gmelin, 1789), previously *Sebastichthys capensis* (Gmelin, 1789) (see Sivertsen and Holthuis, 1980); *Stolephorus commersonnii* Lacepède, 1803 (see Pillai, 1954); *Thyrsites atun* (Euphrasen, 1791) (see Sivertsen and Holthuis, 1980); *Zenopsis nebulosa* (Temminck and Schlegel, 1845), previously *Zenopsis nebulosus* (see Bruce, 1990); *Zeus faber* Linnaeus, 1758 (see Hale, 1926; Avdeev, 1984). Unidentified by scientific names: banded perch (Serranidae), flathead (Platycephalidae) (see Bruce, 1990).

4.7.6 Remarks

Elthusa raynaudii was originally described from the Cape of Good Hope in South Africa, from an unknown host. It is named after its collector, M. Raynaud. Since it was first described in 1840, *E. raynaudii* has been recorded numerous times from a wide range of localities within the Indo-Pacific region. Numerous fish hosts have also been recorded for this species, providing evidence of low host specificity (see Table 4.2).

Bruce (1990) provides defining characteristics for *E. raynaudii*: ovoid body shape; narrowly truncate cephalon anterior margin; straight anterior margin of pereonite 1; antenna longer than antennula; short pleon and pleotelson; carina present on pereopods 5-7 basis; short uropods; and large size (22–67 mm TL). The material examined in this study, including the syntype, border on the lower size range provided by Bruce (1990). Although uropods are described as being short, they measure to more than half the length of the pleotelson. These characteristics corresponded with those of the syntype material from MNHN.

Since *E. raynaudii* is the only *Elthusa* species that has been described from sub-Sahara Africa, it could not be compared to other species from this region. Kensley (1978) provides the only previous illustration of *E. raynaudii* from this region (Hout Bay and Table Bay, South Africa), from which some degree of variation with the material from this study can be noted. The *E. raynaudii* illustration provided by Kensley (1978) presents very sharp uropodal rami

apices, with a more pointed cephalon anterior margin compared to the specimens from this study. Further comparisons can be made with *E. raynaudii* from Australia, which with the aid of illustrations provided by Bruce (1990). These specimens present almost identical morphological features to those from this study. Minor variation was noted with the presence of simple setae on the uropod peduncle and rami from the Australian specimens. *Elthusa sigani* Bruce, 1990 seems to have the most morphological similarities to *E. raynaudii*, but can be distinguished by having an evenly concave pereonite 1 anterior margin; a flat, straight cephalon anterior margin; and coxae 7 that extend part the posterior margin of pereonite 7.

4.8 *Elthusa* sp. 1

4.8.1 Material examined

Holotype. Female (ovigerous, 34.0 mm TL, 17.0 mm W), from Alexander Bay, South African West coast, during July 1993 from the super klipfish, *Clinus superciliosus* (Linnaeus, 1758) (SAMC – A089958).
Paratype. Male (8.0 mm TL, 4.0 mm W), same data as holotype (SAMC - A089959).

4.8.2 Descriptions

***Elthusa* sp. 1 holotype** ♀
Figs. 4.5–4.6

Body slightly twisted to the left, elongated ovoid, twice as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate, medially indented. *Cephalon* 0.8 times longer than wide, visible from dorsal view, semi-oval with anterior point. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.1 times width of cephalon, 0.3 times length of cephalon. *Pereonite 1* smooth, anterior border slightly concave, anterolateral angle rounded, anteriorly projections, extend to the medial region of eyes. Posterior margins of pereonites smooth, slightly curved laterally. Coxae 2–3 narrow with posteroventral angles narrowly rounded; coxae 4–7 with rounded point, not extending past pereonite margin. Pereonites 2–5 subequal, 6 and 7 slightly narrower. *Pleon* 0.4 times as long as total body length, with pleonite 1 same width as other pleonites, lateral margins concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin smooth, slightly curved laterally. *Pleonite 2* partially overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 widest, free, not overlapped by lateral margins of pleonite 4, with posterolateral angles narrowly rounded,

posterior margin with 3 indented points. *Pleotelson* 0.6 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins convex, posterior margin evenly rounded, slightly damaged.

Antennula shorter than antenna, consists of 8 articles; antennula peduncle articles 1 and 2 distinct and articulated, extending to anterior of pereonite 1. *Antenna* consists of 11 articles, extending to past anterior margin of pereonite 1.

Pereopod 1 basis 1.8 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.8 times as long as wide; dactylus slender, 0.8 times as long as propodus, 2.3 times as long as basal width. *Pereopods* 3 similar to pereopod 2, all without robust or simple setae. *Pereopod 7* basis without carina, 1.5 times as long as greatest width; ischium 0.92 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 0.6 times as long as wide, 0.3 times as long as ischium; carpus 0.9 times as long as wide, 0.5 times as long as ischium, with slight bulbous protrusion; propodus as long as wide, 0.4 times as long as ischium; dactylus slender, 1.9 times as long as propodus, 3.1 times as long as basal width.

Pleopods simple, exopod larger than endopod. *Pleopod 1* exopod 1.1 times as long as wide, lateral margin strongly convex, distally broadly rounded, mesial margin weakly convex; peduncle 2.8 times as wide as long.

Uropod more than half the length of pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, apices narrowly rounded. Endopod apically rounded, 2.5 times as long as greatest width, lateral margin weakly convex, mesial margin straight, terminating without setae. Exopod extending beyond end of endopod, twice as long as greatest width, apically rounded, lateral margin weakly convex, mesial margin straight, terminating without setae.

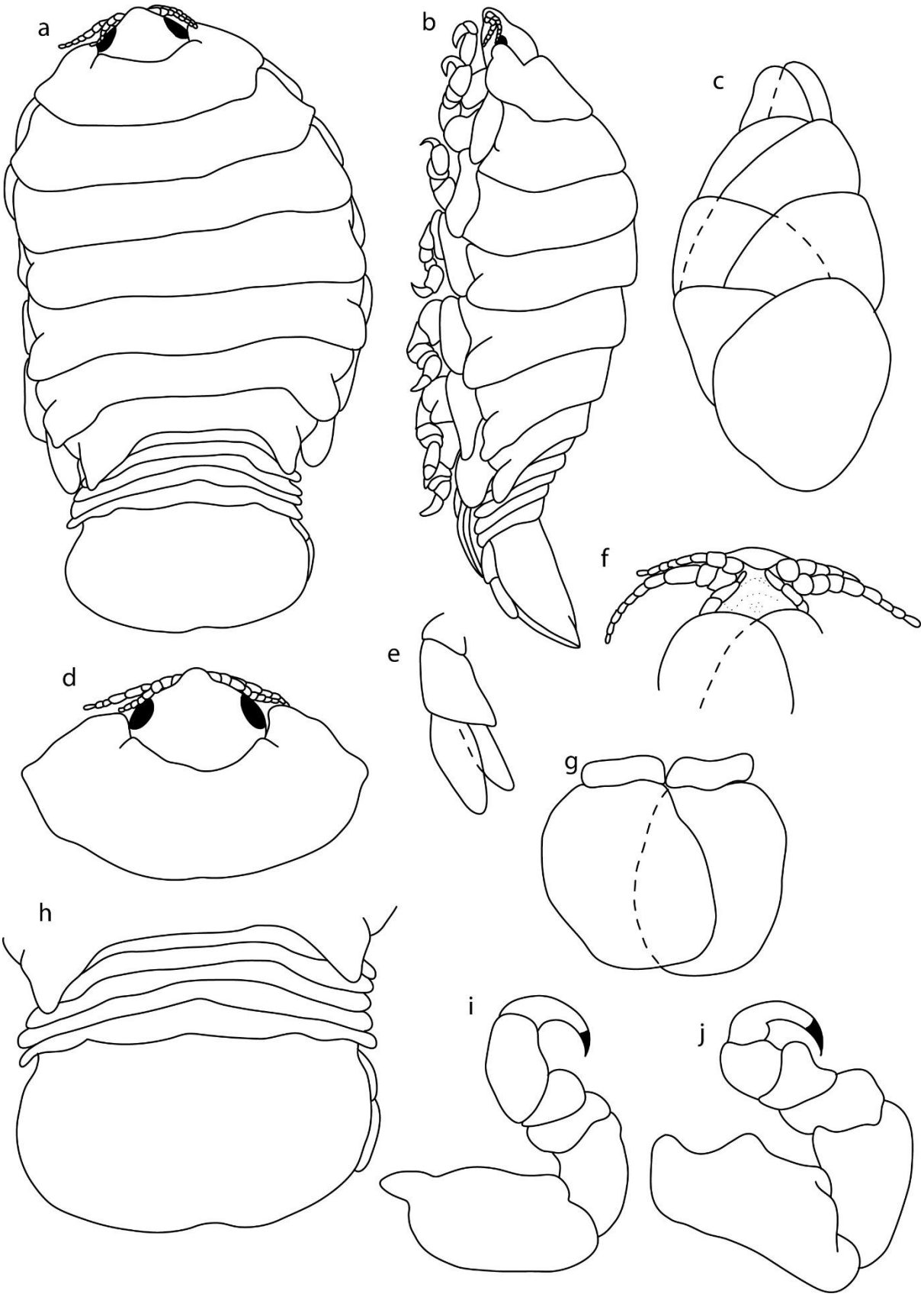

Figure 4.5: *Elthusa* sp. 1 holotype ♀ (ovigerous, 34.0 mm TL, 17.0 mm W) from Alexander Bay, South Africa. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon and pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Pleopod 1. **h** Dorsal view of pleon. **i** Pereopod 1. **j** Pereopod 7.

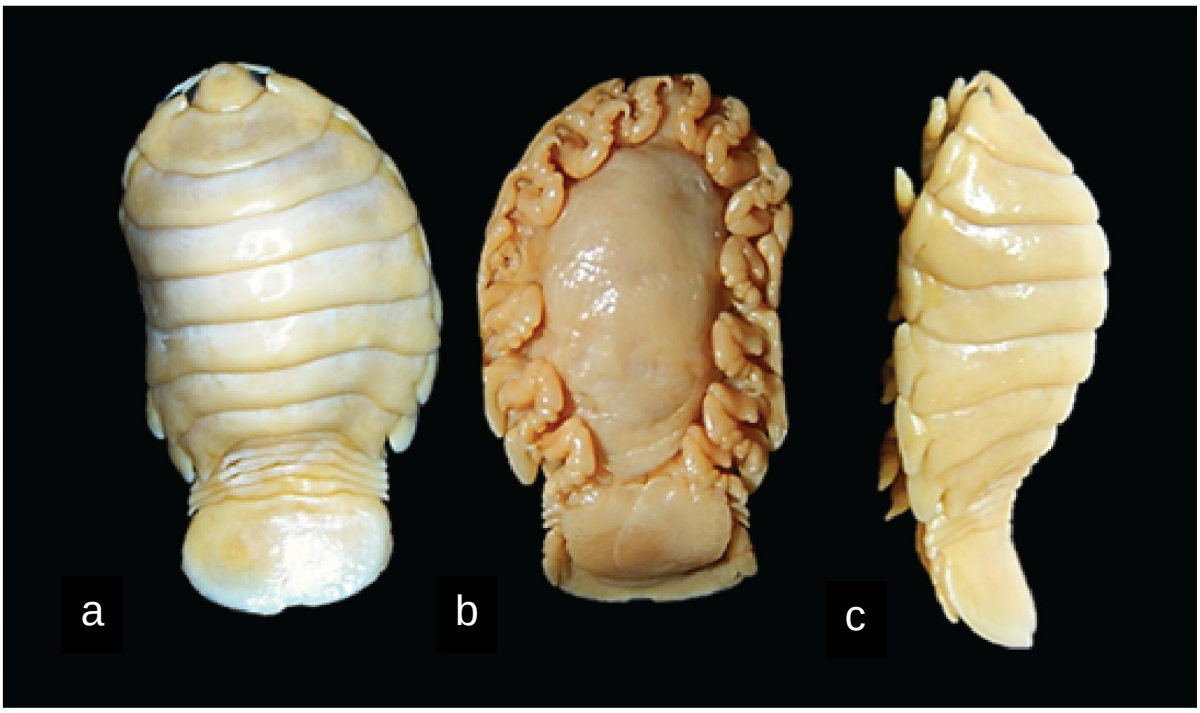

Figure 4.6: Photos of *Elthusa* sp. 1 holotype ♀ (ovigerous, 34.0 mm TL, 17.0 mm W) from Alexander Bay, South Africa. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

***Elthusa* sp. 1 paratype ♂**

Fig. 4.7–4.8

Specimen midmoult. *Body* rectangular or straight, not twisted, twice as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, pereonite lateral margins mostly subparallel. *Cephalon* 0.7 times longer than wide, visible from dorsal view, subtriangular with blunt anterior point. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct margins; one eye 0.2 times width of cephalon; 0.5 times length of cephalon. *Pereonite 1* smooth, anterior border concave, anterolateral angle rounded, extending past base of cephalon. Posterior margins of pereonites smooth and straight, except pereonite 4 and 5. Coxae 2–3 wide, with posteroventral angles rounded; 4–7 rounded, not extending past pereonite margin. Pereonites 6 and 7 narrower, becoming more progressively rounded posteriorly. *Pleon* 0.3 times as long as total body length, with pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin 1–3 posteriorly concave, smooth and slightly curved laterally. *Pleonite 2* partially overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 overlapped by lateral margins of pleonite 4, with posterolateral angles narrowly rounded, posterior margin straight. *Pleotelson* 0.8 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins straight or weakly convex, posterior margin broadly truncate.

Antennula shorter than antenna, consists of 8 articles; antennula peduncle articles 1 and 2 distinct and articulated; extending to anterior of pereonite 1. *Antenna* consists of 10 articles, extending to middle of pereonite 1.

Pereopod 1 basis twice as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.6 times as long as wide; dactylus slender, 1.1 times as long as propodus, 3 times as long as basal width. *Pereopods* 3 similar to pereopod 2, all without robust or simple setae. *Pereopod 7* basis with carina, twice as long as greatest width; ischium 0.7 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 0.7 times as long as wide, 0.4 times as long as ischium; carpus 0.7 times as long as wide, 0.4 times as long as ischium, without bulbous protrusion; propodus 1.3 times as long as wide, 0.6 as long as ischium; dactylus slender, 1.4 times as long as propodus, 2.7 times as long as basal width.

Pleopods simple; exopod larger than endopod. *Pleopod 1* exopod 1.2 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin straight; endopod 2.1 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin straight, peduncle 2.2 times as wide as long. *Pleopod 2* appendix masculina with parallel margins, 1.1 times as long as endopod, distally narrowly rounded.

Uropod same length or slightly longer than the pleotelson, peduncle 0.4 times longer than rami, peduncle lateral margin without setae; rami extending slightly beyond pleotelson, marginal setae absent, apices narrowly rounded. *Endopod* apically slightly pointed, 3 times as long as greatest width, lateral margin weakly convex, mesial margin straight, terminating without setae. *Exopod* extending beyond end of endopod, 2.6 times as long as greatest width, apically rounded, lateral margin weakly convex, mesial margin straight, terminating without setae.

Penes medially united, penial process 0.7 times as long as basal width.

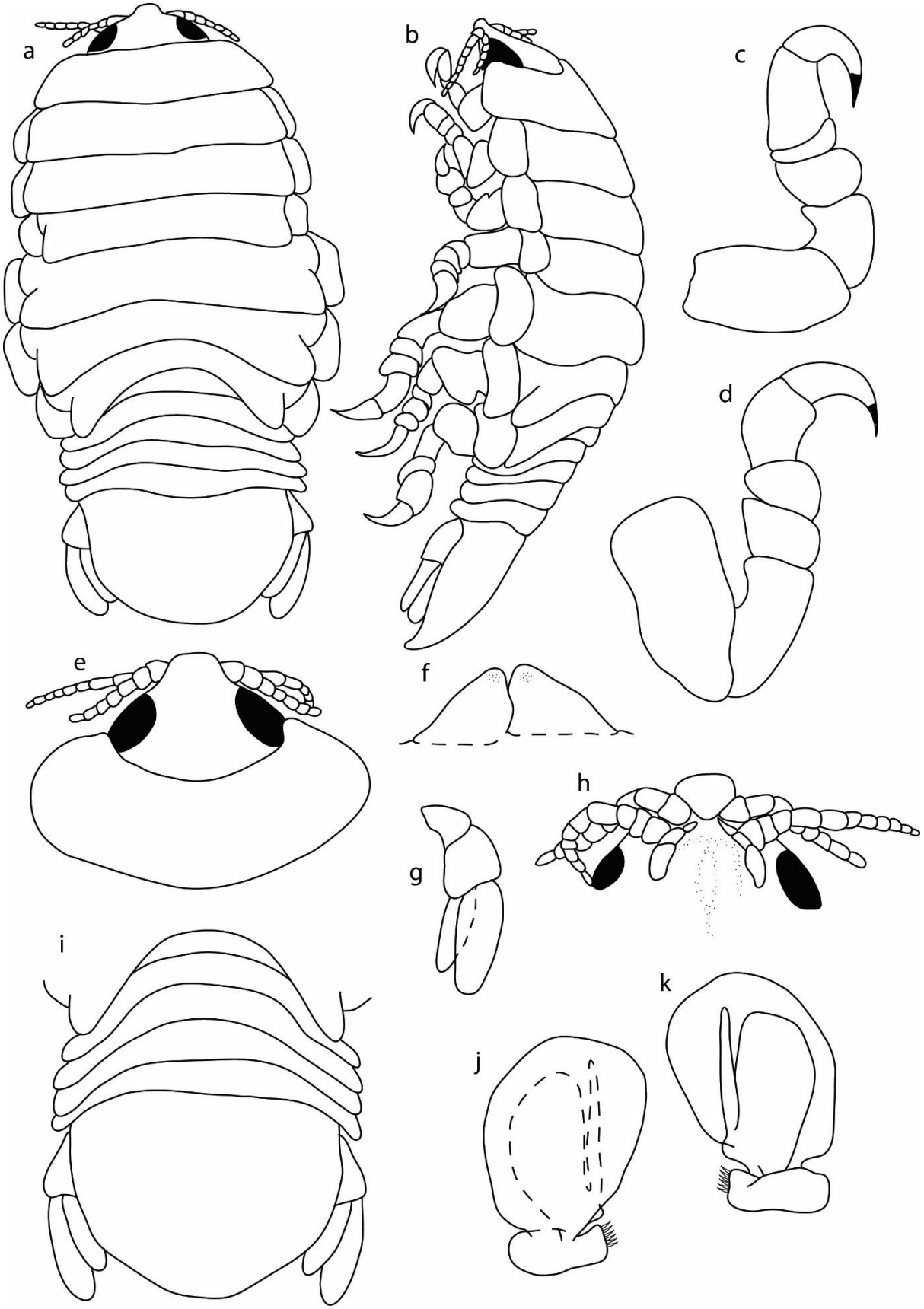

Figure 4.7: *Elthusa* sp. 1 paratype ♂ (8 mm TL, 4 mm W) from Alexander Bay, South Africa. **a** Dorsal body. **b** Lateral body. **c** Pereopod 1. **d** Pereopod 7. **e** Dorsal view of cephalon. **f** Penes. **g** Uropod. **h** Ventral cephalon. **i** Dorsal view of pleon. **j** Pereopod 1. **k** Pereopod 7.

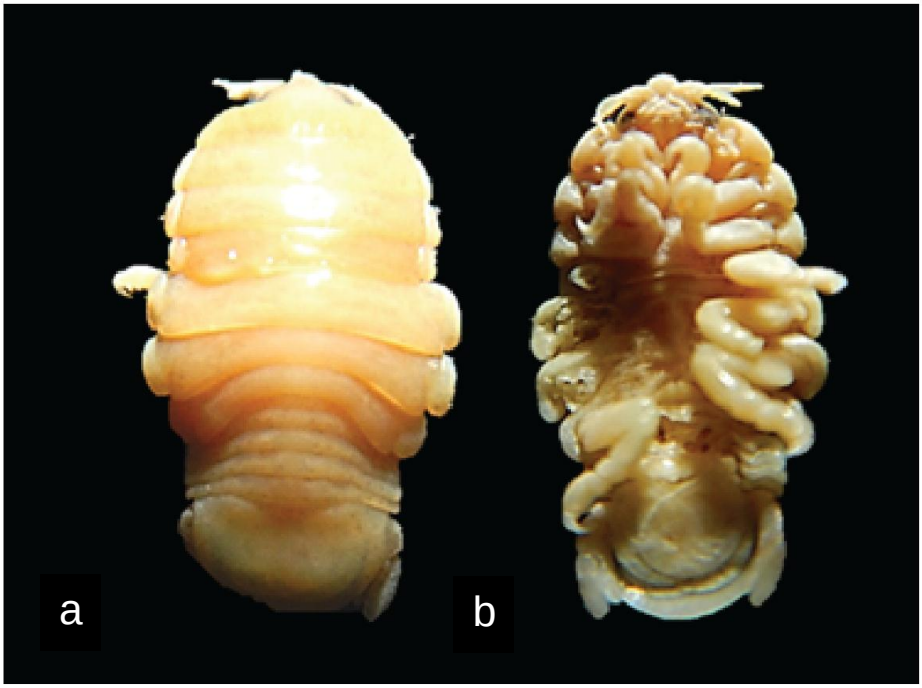

Figure 4.8: Photos of *Elthusa* sp. 1 paratype ♂ (8.0 mm TL, 4.0 mm W) from Alexander Bay, South Africa. **a** Dorsal view. **b** Ventral view.

4.8.3 Remarks

Elthusa sp. 1 female can be identified by having an elongated, ovoid body shape; coxae 7 that does not extending past the posterior margin of pereonite 7; a pointed anterior margin of the cephalon; evenly rounded, slightly concave anterior margin of pereonite 1; uropod rami that are apically pointed and extend to more the half the length of pleotelson; pleonite 5 posterior margin with a medial indentation; and a short, rectangular pleotelson.

This is the first record of *Clinus supercilious,* the intertidal super klipfish, as fish host of an *Elthusa* species. This host belongs to the fish order Perciformes, and is endemic to the Southeast Atlantic Ocean, from northern Namibia to the Kei River of South Africa (Smith and Heemstra, 1986).

Elthusa sp. 1 can be distinguished from *E. raynaudii* by having a pointed cephalon anterior margin, compared to the narrowly truncate margin from that of *E. raynaudii*. Other differences include the shape of the pleon and pleotelson, which is wide and short for *Elthusa* sp. 1, and ovoid for *E. raynaudii*; as well as the concave shape of *Elthusa* sp. 1 pereonite 1 anterior margin versus the straight margin from that of *E. raynaudii.* See Table 4.1 for morphological variation and comparisons.

4.9 *Elthusa* sp. 2

4.9.1 Material examined

Holotype. Female (ovigerous, 39.0 mm TL, 19.0 mm W) from RV *Africana*, collected during April 2003 (SAMC - A089960).
Paratypes. Females 3x (ovigerous, 28.0–30.0 mm TL, 15.0–17.0 mm W), same data as holotype (SAMC - A089961).
Other material. Female (ovigerous, 29.0 mm TL, 17.0 mm W), same data as holotype (dissected). (In the collection of the authors at NWU). Females 4x (non-ovigerous, 19.0–24.0 mm TL, 10.0–14.0 mm W), same data as holotype. (In the collection of the authors at NWU). Female (ovigerous, 40.0 mm TL, 19.0 mm W), (257 m) during January 1999 (SAMC – A19448). Female (ovigerous, 30.0 mm TL, 15.0 mm W), (234 m) during January 1999 (SAMC – A19458). Females 3x ovigerous, 6x non-ovigerous (15.0–40.0 mm TL, 8.0–19.0 mm W), (213 m), during January 1999 (SAMC – A19450).

4.9.2 Descriptions

***Elthusa* sp. 2 holotype** ♀
Fig. 4.9–4.11

Body slightly twisted to the right, elongated ovoid, 2.1 times as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 4, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate, medially indented. *Cephalon* 0.4 times longer than wide, visible from dorsal view, semi-oval with anterior point. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.2 times width of cephalon, 0.4 times length of cephalon. *Pereonite 1* smooth, anterior border medially produced point, with 2 indentations, anterolateral angle rounded, anteriorly projections, extend to posterior margin of eyes. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2–3 wide; with posteroventral angles rounded; 4–7 with rounded point. Coxae 7 extending slightly past pereonite posterior margin. Pereonites 2–5 subequal, becoming more progressively rounded posteriorly. *Pleon* 0.4 times as long as total body length, with pleonite 1 widest, lateral margins concealed by pereonite 7, visible in dorsal view; pleonites posterior margin smooth and slightly curved laterally.

Pleonite 2 partially overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 overlapped by lateral margins of pleonite 4, posterior margin straight, with slight medial point. *Pleotelson* 0.7 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins weakly convex, posterior margin evenly rounded, with slight medial indent.

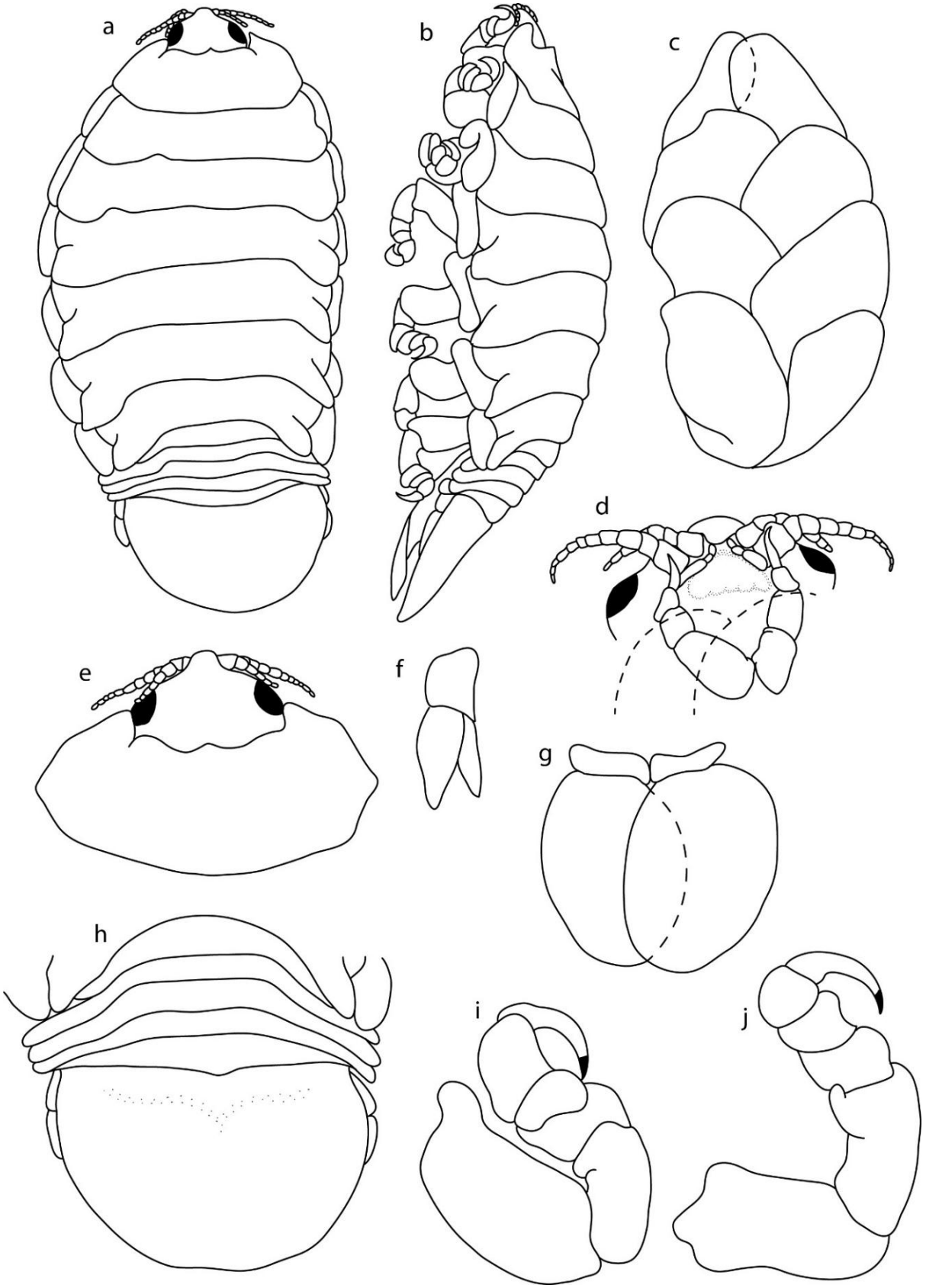

Figure 4.9: *Elthusa* sp. 2 holotype ♀ (ovigerous, 39.0 mm TL, 19.0 mm W) from *Africana* research vessel. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Ventral cephalon. **e** Dorsal view of cephalon and pereonite 1. **f** Uropod. **g** Pleopod 1. **h** Dorsal view of pleon. **i** Pereopod 1. **j** Pereopod 7.

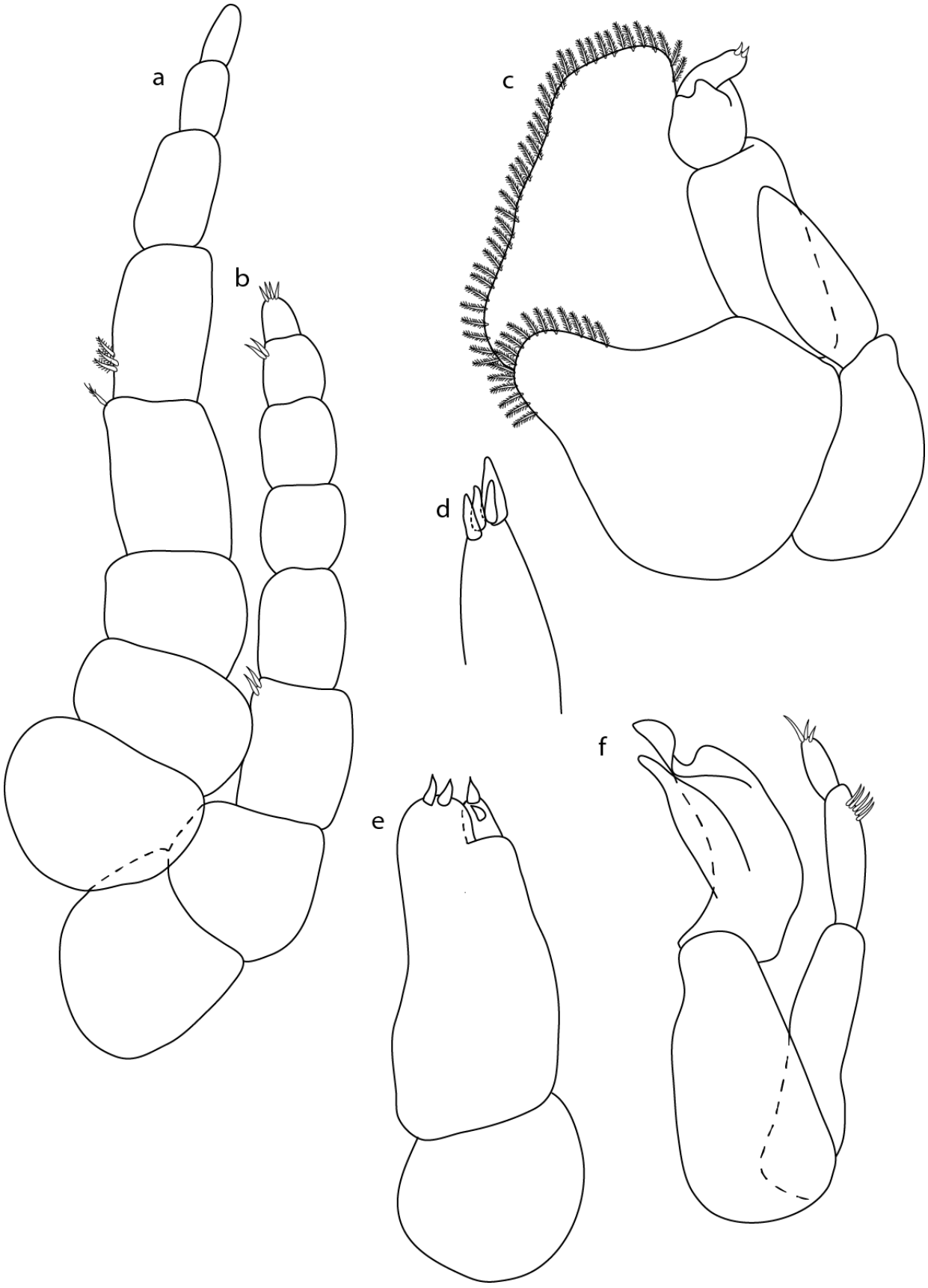

Figure 4.10: *Elthusa* sp. 2 paratype ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from *Africana* research vessel. **a** Antennula. **b** Antenna. **c** Maxilliped. **d** Maxillula. **e** Maxilla. **f** Mandible.

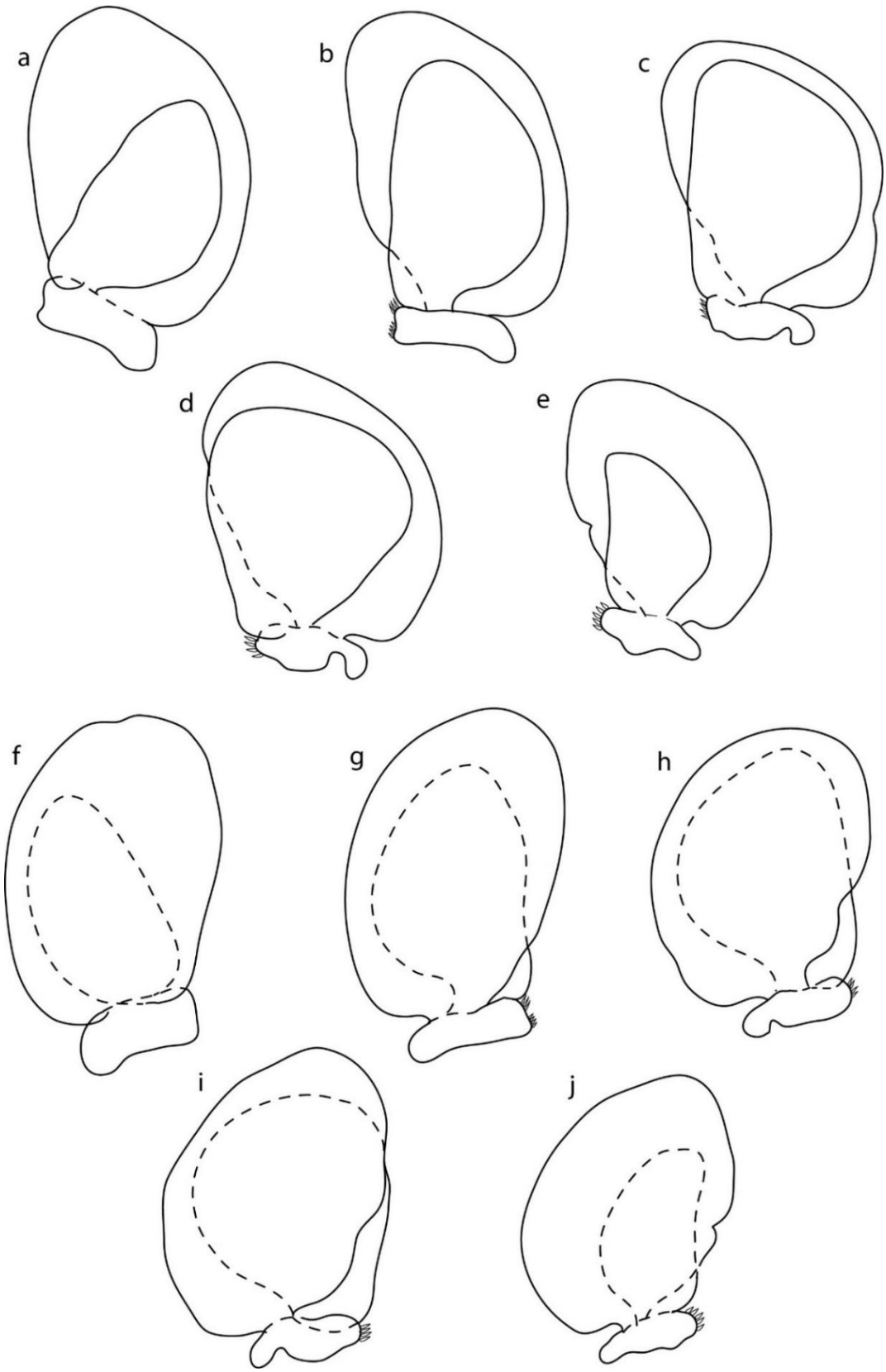

Figure 4.11: *Elthusa* sp. 2 paratype ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from *Africana* research vessel. **a** Pleopod 1 ventral view. **b** Pleopod 2 ventral view. **c** Pleopod 3 ventral view. **d** Pleopod 4 ventral view. **e** Pleopod 5 ventral view. **f** Pleopod 1 dorsal view. **g** Pleopod 2 dorsal view. **h** Pleopod 3 dorsal view. **i** Pleopod 4 dorsal view. **j** Pleopod 5 dorsal view.

Antennula shorter than antenna, consists of 8 articles; antennula peduncle articles 1 and 2 distinct and articulated; article 2 0.9 times as long as article 1; article 3 1.4 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2; antennula flagellum with 5 articles, extending to middle of eye, with tufts of setae on articles 1–3 and article 8. *Antenna* consists of 11 or 12 articles. *Antenna* peduncle article 3 1.3 times as long as article 2; article 4 1.3 times as long as wide, 1.2 times as long as article 3; article 5 1.5 times as long as wide, 1.1 times as long as article 4. Antenna flagellum with 6 articles, terminal article terminating in 1–5 short simple setae, extending to past anterior margin of pereonite 1. Mandible palp article 2 with 5 distolateral setae, and article 3 with 3 simple setae. *Maxillula* simple with 4 terminal robust setae. *Maxilla* mesial lobe not fused to lateral lobe; lateral lobe without simple setae, 2 recurved robust setae; mesial lobe without simple setae, and 2 large recurved robust setae. *Maxilliped* consists of 3 articles, with lamellar oostegite lobe or second, smaller oostegite lobe on basal part of article, palp article 2 without simple setae, article 3 with 3 recurved robust setae. Oostegites margin covered in numerous plumose setae.

Pereopod 1 basis 1.9 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with slight bulbous protrusion; carpus with rounded proximal margin; propodus 1.1 times as long as wide; dactylus slender, 1.3 as long as propodus, 3 times as long as basal width. *Pereopods* 3 similar to pereopod 2, all without robust or simple setae. *Pereopod 7* basis 1.9 times as long as greatest width; ischium 0.9 times as long as basis, with slight bulbous protrusion; merus proximal margin without bulbous protrusion, 0.6 times as long as wide, 0.3 as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, with slight bulbous protrusion; propodus 1 times as long as wide, 0.3 times as long as ischium; dactylus slender, 1.9 times as long as propodus, 3.3 times as long as basal width.

Pleopods simple; exopod larger than endopod, with 4–7 simple setae on peduncle of pleopods 2–5. *Pleopod 1* exopod 1.3 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin straight; peduncle 3 times as wide as long. *Endopod* 1.6 times as long as wide, lateral margin convex, distally narrowly rounded, mesial margin straight, peduncle 2.4 times as wide as long. *Pleopods* 2–5 similar to pleopod 1, mesial margins becoming more strongly produced, peduncle lobes absent.

Uropod less than half the length of the pleotelson, peduncle 0.7 times longer than rami, peduncle lateral margin without setae, marginal setae absent, apices narrowly rounded. *Endopod* apically slightly pointed, 3.4 times as long as greatest width, lateral margin weakly convex, mesial margin straight, terminating without setae. *Exopod* extending to end of endopod, 2.3 times as long as greatest width, apically rounded, lateral margin distally convex, mesial margin straight, terminating without setae.

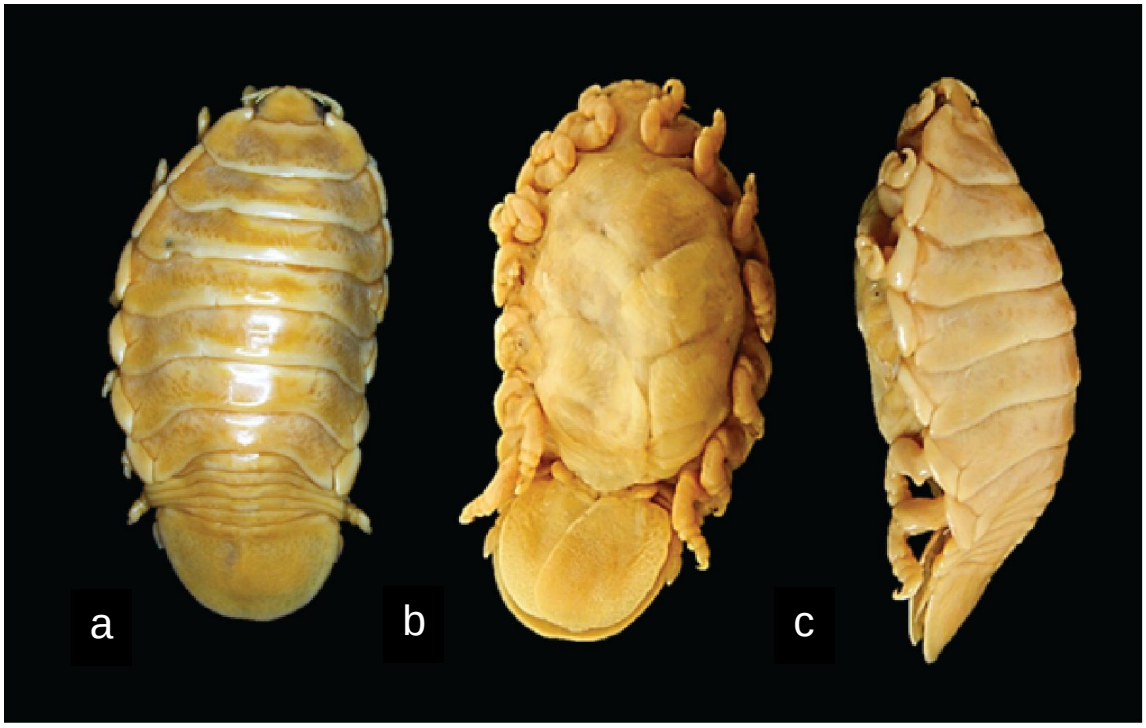

Figure 4.12: Photos of *Elthusa* sp. 2 holotype ♀ (ovigerous, 39.0 mm TL, 19.0 mm W) from *Africana* research vessel. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

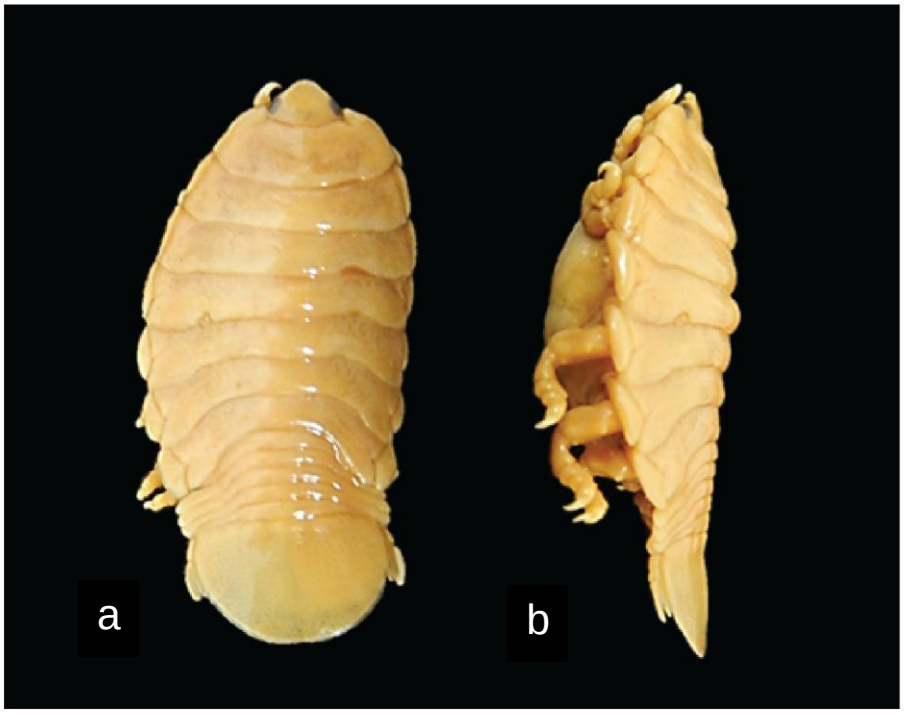

Figure 4.13: Photos of *Elthusa* sp. 2 paratype ♀ (ovigerous, 33.0 mm TL, 16.0 mm W) from *Africana* research vessel. **a** Dorsal view. **c** Lateral view.

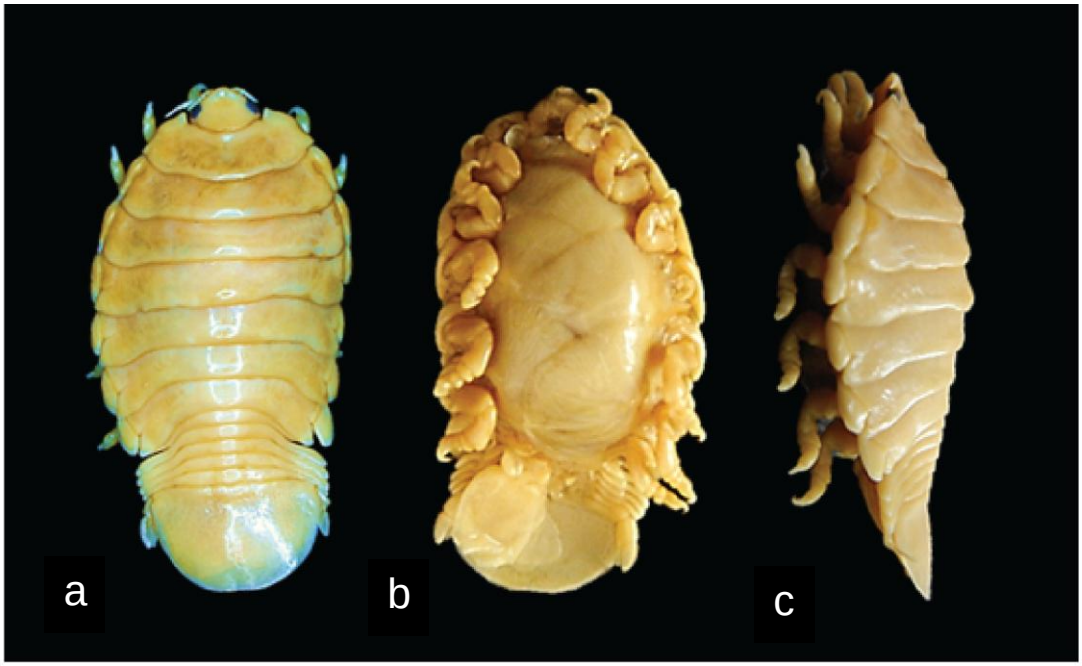

Figure 4.14: Photos of *Elthusa* sp. 2 paratype ♀ (ovigerous, 31.0 mm TL, 15.0 mm W) from *Africana* research vessel. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

4.9.3 Variation

Intra-specific variation was observed among the examined specimens of *Elthusa* sp. 2. This indicated that intra-specific variation may even occur in specimens within the same life stage, as the illustrated specimens were all ovigerous. The size of the medial point formed at the anterior margin of pereonite 1, may vary. Some specimens portrayed a very obvious, sharp medial point, while others only had a noticeable medial projection of the anterior margin of pereonite 1. Variation in the length of the uropods is slight, but some specimens such as *Elthusa* sp. 2 paratype ♀ had uropod rami extending to half the length of the pleotelson, while all the others specimens' uropods were remarkably short. The overlapping of pleonite 5 lateral margins by pleonite 4 was consistent, except with one of the other examined paratype females, where pleonite 5 lateral margins are slightly visible. Some variation was noted in the width of pleonite 1.

4.9.4 Remarks

Elthusa sp. 2 can be identified by its elongated, ovoid body shape; very short, apically pointed, uropod rami, which extends to less than half of the length of the pleotelson; coxae 7 that extend past the posterior margin of pereonite 7; a pointed anterior margin of the

cephalon; anterior margin of pereonite 1 with medial point; pleonite 5 lateral margins that are largely concealed by pleonite 4; pleonite 5 posterior margin with a medial point; pleonite 1 being the widest of the pleonites; and pleopod 5 endopod approximately half the size of exopod.

Several characteristics are considered to differentiate between *Elthusa* sp. 2 and *E. raynaudii.* The most obvious differences were seen with the cephalon and pereonite 1 morphology, as well as with the uropods and pleon. *Elthusa* sp. 2 has a prominent, pointed cephalon anterior margin with a medially pointed pereonite 1 anterior margin compared to the straight anterior margin of *E. raynaudii* cephalon and pereonite 1. Pleon differences include the longer pleotelson of *Elthusa* sp. 2 with pleonite 1 widest and pleonite 5 lateral margins concealed by those of pleonite 4. *Elthusa* sp. 2 has short uropods that do not extend to the half of the pleotelson length, whereas those of *E. raynaudii* reach to, or slightly past, the half of the pleotelson length. Table 4.1 provides a summary of these differences and characteristics.

Morphological analyses yielded three distinct species from the genus *Elthusa*. *Elthusa raynaudii*, the only known *Elthusa* species from South Africa, was identified along with two new species from this genus. *Elthusa raynaudii* seemed to be more similar to *Elthusa* sp. 1 than to *Elthusa* sp. 2, based on morphological analysis (see Table 4.1 and species descriptions). *Elthusa* sp. 1 and *Elthusa* sp.2 are more similar to each other with regards to the shape of the cephalon anterior margin and rostrum. Species of *Elthusa* are known to exhibit intra-specific morphological variability (Bruce, 1990; Trilles and Randall, 2011; Hadfield et al., 2017d), and should therefore be identified with caution.

Table 4.1: Morphological variation between *Elthusa raynaudii* (Milne Edwards, 1840), *Elthusa* sp. 1 and *Elthusa* sp. 2.

Morphological feature	***E. raynaudii* (Milne Edwards, 1840)**	***Elthusa* sp. 1**	***Elthusa* sp. 2**
Body shape	Ovoid	Elongated ovoid	Elongated ovoid
Coxae 7 posterior margin	Not extending past posterior margin of pereonite 7	Not extending past posterior margin of pereonite 7	Extending past posterior margin of pereonite 7
Shape of anterior margin of cephalon	Narrowly truncate	Pointed	Pointed
Uropod length	More than half the length of pleotelson	Pointed, more the half the length of pleotelson	Short, pointed, less than half the length of pleotelson
Pleonite 5 lateral margins	Visible	Visible	Largely concealed by pleonite 4
Shape and size of pleonites	Sub-equal	Pleonite 5 medially indented	Pleonite 1 widest
Pereonite 1 anterior margin	Straight	Evenly curved	Medially pointed
Pleopod 5 endopod	Slightly smaller than exopod	Pleopods not dissected	Half the size of exopod

4.10 Molecular phylogeny

Three *Elthusa* COI gene sequences were obtained, one for each identified *Elthusa* species. *Elthusa raynaudii* (Milne Edwards, 1840) ♀ (20.0 mm TL, 12.0 mm W) produced a 694 bp contig; *Elthusa* sp. 1 ♀ (34.0 mm TL, 17.0 mm W) produced a 671 bp contig; and *Elthusa* sp. 2 ♀ (29.0 mm TL, 17.0 mm W) produced a 693 bp contig of the COI gene (see Appendix A, Sequence A4–A6).

These consensus sequences were aligned with related cymothoid amplicons from personal, unpublished sequence collections from the NWU WRG (without accession numbers), as well as two *Elthusa* sequences from Japan from GenBank. A maximum likelihood (ML) phylogenetic tree was constructed using MEGA®7 and 21 nucleotide COI sequences, based on the Kimura 2-parameter model. For ML analysis sequences, see Appendix B, Sequence

B1–B14. *Rocinela angustata* Richardson, 1904 (EF432739), from the family Aegidae, was chosen as the outgroup (see Fig. 4.15). Table 4.2 contains the number of base pair differences between the three identified *Elthusa* species from the southern African region as well as the percentage intra-specific divergence based on p–distances.

Table 4.2: Matrix showing the number of base pair differences (above diagonal) and inter-specific divergence based on the p–distance (in percentage, below diagonal) among *Elthusa raynaudii*, *Elthusa* sp. 1 and *Elthusa* sp. 2 COI gene sequence amplicons.

	Elthusa raynaudii	*Elthusa* sp. 1	*Elthusa* sp. 2
Elthusa raynaudii	0	43	9
Elthusa sp. 1	6.3	0	45
Elthusa sp. 2	1.2	6.7	0

Brusca (1981), Ketmaier et al. (2008), Bruce (1990) and Thangaraj et al. (2014) suggests that cymothoids from different attaching positions (buccal, branchial and external), evolved independently from three different true evolutionary lineages. The ML phylogeny form Fig. 4.15 contradicts this interpretation, as it does not present three distinct evolutionary lineages. Jones et al. (2008) theorised that the branchial and buccal cavity attaching genera are the most likely ancestral form of Cymothoidae, from which the externally attaching genera have evolved. Hata et al. (2017) presented similar results, suggesting that buccal and externally attaching genera are derived from the branchial attaching genera. Molecular analysis by Hata et al. (2017) did not generate a monophyletic *Elthusa* group, leading them to state that the *Elthusa* is indeed a non-monophyletic group with three distinct clades. By comparing the ML analysis from this study to those of Hata et al. (2017), the actual *Elthusa* clade can be identified as those containing the Japanese *Elthusa* species. The other two *Elthusa* species mentioned in Hata et al. (2017), *Elthusa sacciger* (Richardson, 1909) and *Elthusa vulgaris* (Stimpson, 1857), group separately and could possibly be misidentified.

The generated ML analysis from Fig. 4.15, is better interpreted in terms of the clade formation and sister relationships. Within this analysis (Fig. 4.15), genera of the family Cymothoidae form monophyletic clades. Two discrete clusters/ major clades were formed, one containing the *Elthusa*, as basal clade to the cymothoids, and the other containing the the larger clade of branchial cavity attaching *Mothocya* and *Norileca*; the buccal attaching *Cymothoa* and *Cinusa;* and the externally attaching *Anilocra*. Studies done by Hata et al. (2017) similarly present the *Elthusa* as a basal clade to the cymothoids. Ketmaier et al. (2008) provide an ML analysis where branchial cavity attaching cymothoids cluster as the basal clade with the buccal attaching genera as the most derived. This clade formation

suggests that the externally attaching genera, such as the *Anilocra*, evolved within this larger clade. In addition, *Elthusa* forms a sister taxa to the remaining cymothoids from the ML analysis in this study.

The three *Elthusa* species from this study, appear to be related to the *Elthusa* species from Japan (Hata et al., 2017) (LC159567 *Elthusa* sp. 2 and LC159565 *Elthusa* sp. 1) (see Fig. 4.15). Although these species cluster together, they appear to be distinct.

According to Costa et al. (2007), genetic divergence in COI sequences can be a useful tool to distinguish between different species and genera from the Subphylum Crustacea. They found that the level of intra-specific variation of the Crustacea is averaged at 0.47%, whereas the inter-specific variation is averaged at 17.16%. Gouws (2004) agrees that genetic divergence values can be useful to provide an estimate of the taxonomic status of an isopod species. In the study done by Gouws (2004), inter-specific divergence between isopod species ranged between 0.93% and 11.01%, while intra-specific divergences ranged between 0.1% and 1.88%. The divergence ranges for the Cymothoidae have recognised intra-specific divergence at less than 1%, and inter-specific divergence at more than 4% (Welicky et al., 2017). These levels of divergence are unique to different groups of organisms. Table 4.2 presents the inter-specific divergence between the three identified *Elthusa* species, showing that *Elthusa* sp. 1 has a 6.3% divergence to *E. raynaudii*, whereas *Elthusa* sp. 2 has a 1.2% divergence to *E. raynaudii*. Although *Elthusa* sp. 2 and *E. raynaudii* have a small genetic divergence, morphological analysis provided several constant characteristics to distinguish between these two species (see Table 4.1 and description illustrations). The divergence values for the *Elthusa* species suggest that these genetic divergences are inter-specific. It further provides evidence that *Elthusa* sp. 2 and *E. raynaudii* are more closely related. The results from this study confirm that the three identified *Elthusa* species are morphologically and molecularly different.

4.11 Summary of *Elthusa* species

Table 4.3 contains detail on host and distribution records of all 31 accepted *Elthusa* species. Type host and localities are provided along with the site of attachment of each species, where this information is available. Original descriptions were often made with insufficient information available, especially with regards to fish host species, in which case the type host is “unknown”. The discovery of two new *Elthusa* species from southern Africa, more than double the known records of *Elthusa* from this region.

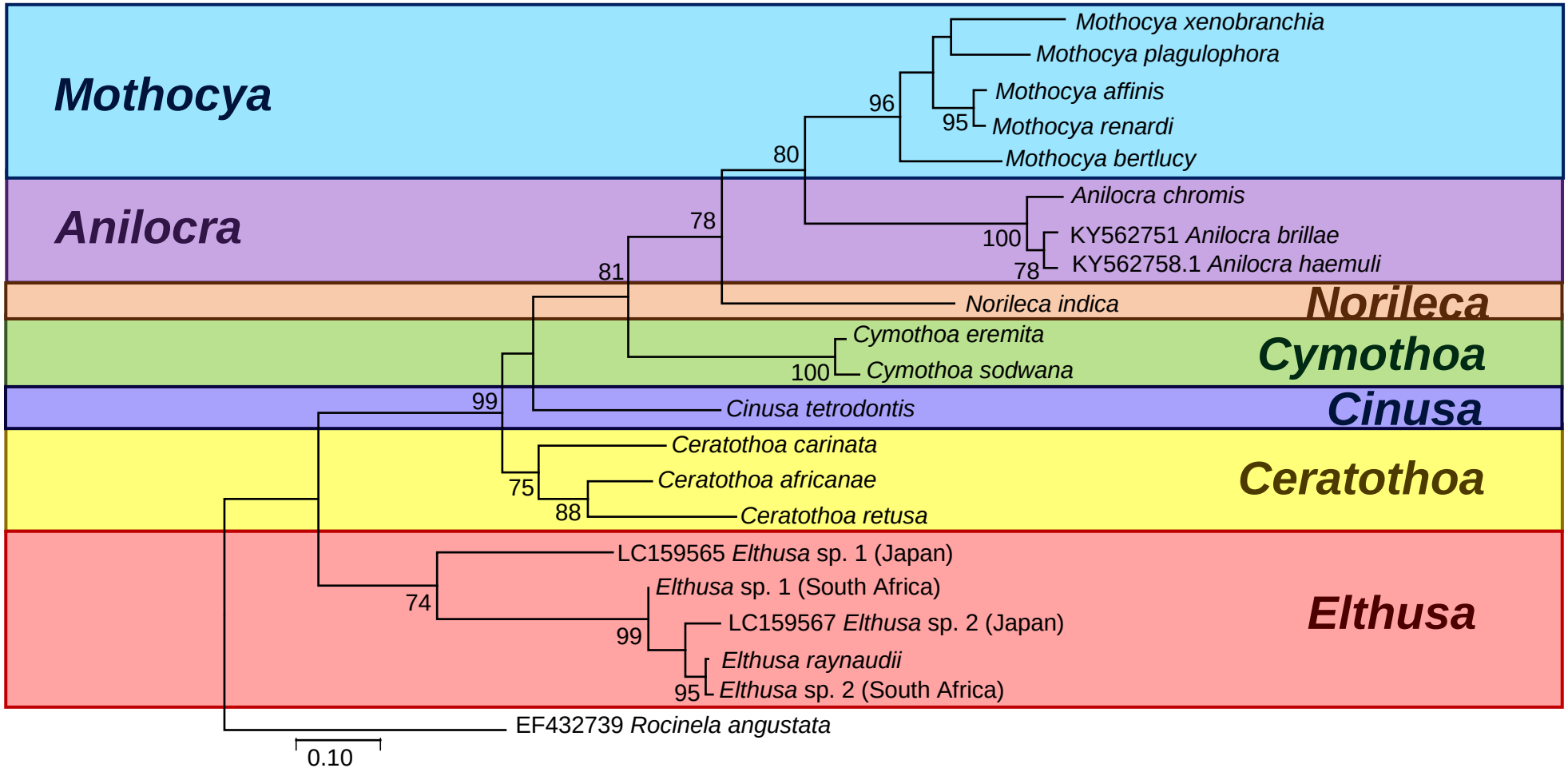

Figure 4.15: Molecular Phylogenetic analysis by Maximum Likelihood (ML) method based on the Kimura 2-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2796)). ML bootstrap values (provided adjacent to branches) provide nodal support, where only values above 70% are shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 21 nucleotide sequences and was implemented with the aid of MEGA®7. This ML includes the new *Elthusa* species from southern Africa and related cymothoid species from personal sequence collections as well as two *Elthusa* sequences from Japan. *Rocinela angustata* (EF432739) is chosen as the outgroup. The different Cymothoidae clades are illustrated in various colours as indicated.

Table 4.3: Summary of the hosts and distribution of all species from the genus Elthusa Schioedte and Meinert, 1884.

Species	Distribution	Hosts	Attachment	References
Elthusa alvaradoensis Rocha-Ramírez, Chávez-López & Bruce, 2005	**Type locality:** Alvarado, Veracruz, Mexico.	**Type host:** *Synodus foetens* (Linnaeus, 1766).	Branchial	Rocha-Ramírez et al. (2005)
Elthusa arnoglossi Trilles & Justine, 2006	**Type locality:** Chesterfield Islands, New Caledonia.	**Type host:** *Arnoglossus* sp. (either *Arnoglossus polyspilus* (Günther, 1880) or *A. japonicus* (Hubbs, 1915).	Branchial	Trilles & Justine (2006)
Elthusa atlantniroi (Kononenko, 1988) **Synonym:** *Lironeca atlantniroi* Kononenko, 1988	**Type locality:** Bay of Biscay, northeast Atlantic Ocean.	**Type host:** *Cepola macrophthalma* (Linnaeus, 1758).	Branchial	Kononenko (1988)
Elthusa californica (Schioedte & Meinert, 1884) **Synonym:** *Livoneca californica* Schioedte & Meinert, 1884	**Type locality:** California, near San Francisco. **Other localities:** Pacific coast from Canada to Peru; Alaska, USA to Punta Eugenio, Mexico; Mexico, and West coast of Baja California; California including Campbell Cove of Bodega Bay; Lake Merrit, Silver Strand (was Coronado Strand), San Diego Bay.	**Type host:** *Holconoti* sp. **Other hosts:** *Alosa sapidissima* (Wilson, 1811); *Amphistichus argenteus* Agassiz, 1854; *Apodichthys flavidus* Girard, 1854; *Artedius lateralis* (Girard 1854); *Atherinops affinis* (Ayres, 1860); *Aulorhynchus flavidus* Gill, 1861; *Brachyistius frenatus* Gill, 1862; *Citharichthys stigmaeus* Jordan & Gilbert, 1882; *Clevelandia ios* (Jordan & Gilbert, 1882); *Clinocottus analis* (Girard 1858); *Clupea harengus* Linnaeus, 1758; *Cymatogaster aggregata* Gibbons, 1854; *Engraulis mordax* Girard, 1854; *Enophrys bison* (Girard, 1854); *Fundulus parvipinnis* Girard, 1854; *Gasterosteus aculeatus* Linnaeus, 1758; *Gibbonsia metzi* Hubbs, 1927; *Hexagrammos decagrammus* (Pallas, 1810); *Hypomesus pretiosus* (Girard, 1854); *Lepidogobius lepidus* (Girard, 1858); *Leptocottus armatus* Girard,	Branchial	Bennett (1993); Brusca (1981); Brusca et al. (2001); Gamble et al. (2013); Hatch (1947); Iverson (1974); Keys (1928); Miller (1975); Olson (1972); Schioedte and Meinert (1884); Waugh et al. (1989)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
		1854; *Leuresthes tenuis* (Ayres, 1860); *Lucania parva* (Baird & Girard, 1855); *Micrometrus minimus* (Gibbons, 1854); *Morone saxatilis* (Walbaum, 1792); *Mugil cephalus* Linnaeus, 1758; *Oligocottus maculosus* (Girard 1856); *Oligocottus snyderi* Greeley, 1898; *Parophrys vetulus* Girard, 1854; *Phanerodon furcatus* Girard, 1854; *Pholis ornata* (Girard, 1854); *Quietula y-cauda* (Jenkins & Evermann, 1889); *Scorpaenichthys marmoratus* (Ayres 1854); *Sebastes caurinus* Richardson, 1844; *Sebastes flavidus* (Ayres, 1862).		
Elthusa caudata (Schioedte & Meinert, 1884) **Synonym:** *Livoneca caudata* Schioedte & Meinert, 1884	**Type locality:** Laponica islands, Japan. **Other localities:** New Zealand.	**Type host:** Unknown. **Other hosts:** *Genypterus blacodes* (Forster, 1801).	Branchial	Avdeev (1978); Schioedte and Mienert (1884)
Elthusa emarginata (Bleeker, 1857) **Synonym:** *Livoneca emarginata* Bleeker, 1857	**Other localities:** East India; Malaysia; Indonesia including Ambon, Biak Island, Maumere Bay, Irian Jaya (Papua), Wahaai, Ternate.	**Type host:** Unknown. **Other hosts:** *Parupeneus barberinus* (Lacepède, 1801); *Parupeneus heptacanthus* (Lacepède, 1802); *Parupeneus indicus* (Shaw, 1803) previously *Upeneus russelii* Cuvier, 1829.	Branchial	Bleeker (1857); Miers (1880); Nierstrasz (1915); Schioedte & Mienert (1884); Trilles and Randall (2011)
Elthusa epinepheli Trilles & Justine, 2010	**Type locality:** Off Nouméa, New Caledonia.	**Type host:** *Epinephelus howlandi* (Günther, 1873).	Branchial	Trilles and Justine (2010)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
Elthusa foveolata (Hansen, 1897) **Synonym:** *Irona foveolata* Hansen, 1897	**Type locality:** Sri Lanka.	**Type host:** Unknown.	Branchial	Hansen (1897)
Elthusa frontalis (Richardson, 1910) **Synonym:** *Livoneca frontalis* Richardson, 1910	**Type locality:** Sablayan, Philippines.	**Type host:** *Balistes* sp.	Branchial	Richardson (1910)
Elthusa menziesi (Brusca, 1981) **Synonym:** *Lironeca menziesi* Brusca, 1981	**Type locality:** San Quintin Bays, Baja California, Mexico. **Other locality:** Mexico including Tastiota estuary, Sonora, Bahía Todos Santo, Ensenada Melpomene, Guadalupe Island, Alijos Rocks, Western Baja California, the Gulf of California, the offshore Pacific Baja islands of Coronados, San Quintin Bay	**Type host:** *Clinocottus analis* (Girard, 1858). **Other hosts:** *Clinocottus analis* (Girard, 1858); *Eisenia sp.; Gibbonsia elegans* (Cooper, 1864) ; *Gibbonsia montereyensis* Hubbs, 1927; *Girella nigricans* (Ayres, 1860); *Gobiesox maeandricus* (Girard, 1858); *Hermosilla azurea* Jenkins & Evermann, 1889; *Hypsoblennius gilberti* (Jordan, 1882); *Leuresthes tenuis* (Ayres, 1860); *Paraclinus integripinnis* (Smith, 1880); *Rimicola eigenmanni* (Gilbert, 1890).	Branchial	Brusca (1981); Espinosa-Pérez & Hendrickx (2001); Ruiz-Campos et al. (1986); Wetzer et al. (1991)
Elthusa methepia (Schioedte & Meinert, 1884)	**Type locality:** Rio de Janeiro, Brazil.	**Type host:** *Achirus* sp.	Branchial	Schioedte and Mienert (1884)
Elthusa moritakii Saito & Yamauchi, 2016	**Type locality:** Honshu and east China Sea coast of Kyushu, Japan.	**Type host:** *Ereunias grallator* Jordan & Snyder, 1901.	Branchial	Saito and Yamauchi (2016)
Elthusa myripristae Bruce, 1990	**Type locality:** Escape Reef, outer Barrier Reef, Australia	**Type host:** *Myripristis violaceus* Bleeker, 1851	Branchial	Bruce (1990)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
Elthusa nanoides (Stebbing, 1905) **Synonym:** *Irona nanoides* Stebbing, 1905	**Type locality:** Galle, Sri Lanka (old Ceylon). **Other localities:** Gulf of Suez, Red Sea.	**Type host:** Unknown. **Other hosts:** *Dentex* Cuvier, 1814; *Leiognathus* Lacepède, 1802; *Fistularia* Forsskål, 1775 *nomen dubium*; *Scorpenidae* sp.	Branchial	Monod (1933); Stebbing (1905); Trilles (1976)
Elthusa neocytta (Avdeev, 1975) **Synonym:** *Lironeca neocytta* Avdeev, 1975	**Type locality:** New Zealand coastal region. **Other locality:** Tasmania including Maria island and off Strahan, north-east of East Sister Island; south-east New Zealand including the Chatham Rise, Pukaki Rise and Bounty Plateau.	**Type host:** *Neocyttus rhomboidalis* Gilchrist, 1906. **Other hosts:** *Allocyttus* sp. (Stephenson, 1987); *Cyttus traversi* Hutton, 1872; *Neocyttus rhomboidalis* Gilchrist, 1906; *Pseudocyttus maculatus* Gilchrist, 1906; *Rastrelliger kanagurta* (Cuvier, 1816); *Zenopsis nebulosa* (Temminck & Schlegel, 1845) previously *Zenopsis* nebulosus (Temminck & Schlegel, 1845).	Branchial Buccal	Avdeev (1975); Avdeev (1984); Bruce (1990); Stephenson (1987)
Elthusa nierstraszi Hadfield, Bruce & Smit, 2016 **Synonym:** *Lironeca parva* Nierstrasz, 1915. E*lthusa parva* (Nierstrasz, 1915) (Junior homonym of *Elthusa parva* (Richardson, 1910)	**Type locality:** Kisar Island, Moluccas, Indonesia.	**Type host:** *Ereunias grallator* Jordan & Snyder, 1901.	Branchial	Avdeev (1984); Hadfield et al. (2016a); Nierstrasz (1915)
Elthusa ochotensis (Kussakin, 1979) **Synonym:** *Lironeca ochotensis* Kussakin, 1979	**Type locality:** Sea of Ochosk (near the city of Ayan), western Pacific Ocean.	**Type host:** Unknown.	Branchial	Kussakin (1979)
Elthusa parabothi Trilles & Justine, 2004	**Type locality:** New Caledonia, off Coëtlogon Bank.	**Type host:** *Parabothus kiensis* (Tanaka, 1918).	Branchial	Trilles and Justine (2004)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
Elthusa parva (Richardson, 1910) **Synonym:** *Ceratothoa parva* (Richardson, 1910)	**Type locality:** Opol, Mindanao, Philippines.	**Type host:** Unknown.	Branchial	Richardson (1910)
Elthusa philippinensis (Richardson, 1910) **Synonym:** *Livoneca philippinensis* Richardson, 1910	**Type locality:** Jolo Light, Philippines.	**Type host:** Unknown.	Branchial	Richardson (1910)
***Elthusa propinqua* (Richardson, 1904)** **Synonym: *Livoneca propinqua* Richardson, 1904**	**Type locality:** Port Heda, Japan. **Other locality:** Arabian Sea; Laccadive Islands; India; Maldives; Myeik, Myanmar; Ose Saki and Kobe to Yokohama, Japan; Sombrero Island, Philippines; off Moreton Island Queensland, Australia.	**Type host:** Unknown. **Other hosts:** *"chalinura"*; *"a macrurid"*, "*Macrurus*"; *Ventrifossa* cf. *nigrodorsalis.*	Branchial	Barnard (1936); Bruce (1990); Richardson (1904); Richardson (1910)
Elthusa raynaudii (Milne Edwards, 1840) **Synonym:** *Livoneca raynaudii* Milne Edwards, 1840	**Type locality:** Cape of Good Hope, South Africa. **Other localities: South America:** Punta Quillaipe and Strait of Magellan, Chile; Uruguay. **South Africa:** Hout Bay and Table Bay; Cape of Good Hope; Durban. **India:** Travancore. **Australia:** South Australia: Cape Wiles and Port Adelaide. Queensland: south-eastern Queensland; Flinder's Reef; Skua Point. New South Wales: Cape Three points, Sydney and Terrigal off Botany Bay; Wattamolla lagoon ("Wata Mooli); east of Coogee, Long Reef, Shoalhaven Bight, north-east of Wollongong, and Port Jackson.	**Type host:** Unknown. **Other hosts:** *Chelidonichthys kumu* (Cuvier, 1829); *Chorisochismus dentex* (Pallas, 1769); *Cyttus australis* (Richardson, 1843); *Cyttus* novaezelandiae (Arthur, 1885); *Cyttus traversi* Hutton, 1872 previously *Cyttoidops mccullochi* (Whitley, 1947); *Genypterus blacodes* (Bloch and Schneider, 1801); *Gnathanacanthus goetzeei* Bleeker, 1855; *Hyporhamphus intermedius* (Cantor, 1842); *Latris lineata* (Forster, 1801); *Meuschenia freycineti* (Quoy and Gaimard, 1824); *Mustelus antarcticus* Günther, 1870; *Nemadactylus monodactylus* (Carmichael, 1819) previously *Acantholatris*	Branchial	Avdeev (1978, 1984); Bruce (1990); Chilton (1909, 1911); Filhol (1885); Ghani (2003); Hale (1926); Hewitt and Hine (1972); Hurley (1961); Kensley (1976, 1978); Mañé-Garzón (1979);

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
	Victoria: south-east of Gabo Island, south-south-west of McCann, Bruni Island, and off Tasman Head; Hobart. Tazmania: Eaglehawk Neck, Murdunna, Dunally, Taranna, north-east of East Sister Island, and off Strahan. **New Zealand**: Stewart Island, Snares Islands, Antipode Islands, Norfolk Island, Akaroa. Japan: Yokohama. **Other islands:** St. Paul Island in the Bering Sea; Amsterdam Island, southern Indian Ocean; Saint Helena and Tristan da Cunha	*monodactylus* (Carmichael, 1819); *Nematalosa nasus* (Bloch, 1795); *Notacanthus sexspinis* Richardson, 1846; *Notothenia microlepidota* Hutton, 1875 previously *Notothenia colbecki*; *Notolabrus tetricus* (Richardson, 1840) previously *Pseudolabrus tetricus*; *Paranotothenia magellanica* (Forster, 1801) previously *Notothenia macrocephala*; *Parophrys vetulus* Girard, 1854; *Ilisha melastoma* (Bloch & Schneider, 1801); *Pelotretis flavilatus* Waite, 1911; *Pseudophycis bachus* (Forster, 1801) previously *Physiculus; Physiculus* sp; *Physiculus barbatus* (Günther, 1863) previously *Physiculus barbatus*; *Pseudolabrus miles* (Schneider and Forster, 1801); *Pseudophycis bachus* (Forster, 1801); *Rexea solandri* (Cuvier, 1832); *Rhombosolea sp.*; *Sardinops sagax* (Jenyns, 1842) previously *Clupea neopilchardus* Steindachner, 1879; *Scorpaena cardinalis* Solander & Richardson, 1842; *Sebastes capensis* (Gmelin, 1789) previously *Sebastichthys capensis* (Gmelin, 1789); *Stolephorus commersonnii* Lacepède, 1803; *Thyrsites atun* (Euphrasen, 1791); *Zenopsis nebulosa* (Temminck & Schlegel, 1845) previously *Zenopsis nebulosus*; *Zeus faber* Linnaeus, 1758. Unidentified by scientific names: banded perch *(Serranidae),* flathead *(Platycephalidae).*		Menzies (1962); Milne Edwards (1840); Nierstrasz (1915, 1931); Pillai (1954); Poore (1981); Powell (1959); Richardson (1904); Schioedte and Meinert (1884); Sivertsen and Holthuis (1980); Stephenson (1969); Whitelegge (1901)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
Elthusa sacciger (Richardson, 1909) **Synonym:** *Livoneca sacciger* Richardson, 1909	**Type locality:** Bungo Channel and inland Sea off Mizimoko Light, Japan and the south coast of Hokkaido. **Other localities:** North-western Pacific; New South Wales inlcuding off Sydney and the central coast; Japan including off the Pacific coast of Tohoku and Tokyo, the Pacific coast of northern Honshu; Pacific coast of Hokkaido and off Muroran.	**Type host:** *Synaphobranchus* sp. **Other hosts:** *Synaphobranchus brevidorsalis* Günther, 1887 previously *Synaphobranchus pinnatus*; *Synaphobranchus kaupii* Johnson, 1862; *Sebastolobus macrochir* (Günther, 1877).	Branchial	Avdeev (1984); Bruce (1990); Hata et al. (2017); Richardson (1910); Shiino (1951); Yamauchi (2009)
Elthusa samariscii (Shiino, 1951) **Synonym:** *Lironeca samariscii* Shiino, 1951	**Type locality**: Japan. **Other localities:** Kerala coast, India.	**Type host:** *Samariscus japonicus* Kamohara, 1936. **Other hosts:** *Samaris cristatus* Gray, 1831.	Branchial	Kumar and Bruce (1997); Shiino (1951)
Elthusa samoensis (Schioedte & Meinert, 1884) **Synonym:** *Livoneca samoensis* Schioedte & Meinert, 1884	**Type locality:** Samoa Islands (Samoenses islands).	**Type host:** Unknown.	Branchial	Schioedte and Mienert (1884)
Elthusa sigani Bruce, 1990	**Type locality:** North Stradbroke Island, Moreton Bay, southeastern Queensland, Australia.	**Type host:** *Siganus spinus* (Linnaeus, 1758).	Branchial	Bruce (1990)
Elthusa splendida (Sadowsky & Moreira, 1981) **Synonym:** *Lironeca splendida* Sadowsky & Moreira, 1981	**Type locality:** South Western Atlantic Ocean.	**Type host:** *Squalus cubensis* Howell Rivero, 1936.	Buccal	Sadowsky and Moreira (1981)
Elthusa tropicalis (Menzies & Kruczynski, 1983) **Synonym:** *Lironeca tropicalis* Menzies & Kruczynski, 1983	**Type locality:** off Egmont Key, Florida, USA.	**Type host:** *Ogcocephalus parvus* Longley & Hildebrand, 1940.	Branchial	Menzies and Kruczynski (1983)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
Elthusa turgidula (Hale, 1926) **Synonym:** *Livoneca turgidula* Hale, 1926	**Type locality:** Fremantle and Cottesloe, Western Australia. **Other localities:** One Tree Island, Great Barrier Reef.	**Type host:** Unknown. **Other hosts:** *Chlorurus sordidus* (Forsskål, 1775) previously *Scarus sordidus; Scarus* spp, *Scarus psittacus* Forsskål, 1775 previously *Scarus venosus.*	Branchial	Bruce (1990); Hale (1926)
Elthusa vulgaris (Simpson, 1857) **Synonym:** *Livoneca vulgaris* Stimpson, 1857	**Type locality:** San Francisco Bay, eastern Pacific Ocean; Tomales Bay; Monterey. **Other localities:** Off Puerto Madero, Chiapas, Mexico; Gulf of California; California including southern California; Campbell Cove, Bodega Bay; San Francisco Bay, Coos Bay; Oregon to Colombia (near Malpelo Island); eastern Pacific Ocean.	**Type host:** Unknown. **Other hosts:** *Amphistichus rhodoterus* (Agassiz, 1854); *Cetengraulis mysticetus* (Günther, 1867); *Citharichthys stigmaeus* (Gilbert & Jordan 1882); *Citharichthys sordidus* (Girard, 1854); Clevelandia ios (Jordan & Gilbert, 1882); *Embiotoca jacksoni* Agassiz, 1853; *Hexagrammos decagrammus* Pallas, 1810*; Hippoglossina stomata* Eigenmann & Eigenmann, 1890; *Hyperprosopon argenteum* Gibbons, 1854; *Hypsopsetta guttulata* (Girard, 1856); *Leptocottus armatus* Girard, 1854*; Morone saxatilis* (Walbaum, 1792); *Neoclinus blanchardi* Girard, 1858; *Ophiodon elongatus* Girard, 1854; *Oxylebius pictus* Gill, 1862; *Paralichthys californicus* (Ayres, 1859); *Phanerodon furcatus* Girard, 1854; *Paralabrax nebulifer* (Girard, 1854); Platichthys stellatus (Pallas, 1787); *Rhacochilus vacca* (Girard, 1855)*; Scorpaena guttata* Girard, 1854; *Scorpaenichthys marmoratus* (Ayres, 1854)*; Sebastes caurinus* Richardson, 1844; *Sebastiscus marmoratus* (Cuvier, 1829)*;*	Branchial	Bennett (1993); Brusca (1978, 1981); Espinosa-Pérez and Hendrickx (2001); Gamble et al. (2013); Hobson (1971); Richardson (1904); Stimpson (1857); Turner et al. (1969)

Table 4.3: Continued.

Species	Distribution	Hosts	Attachment	References
		Sebastes mystinus (Jordan & Gilbert, 1881); *Sebastes serranoides* (Eigenmann & Eigenmann, 1890); *Serranus aequidens* Gilbert, 1890; *Symphurus atricaudus* (Jordan & Gilbert, 1880); *Synodus lucioceps* (Ayres, 1855); *Trachurus symmetricus* (Ayres, 1855); “rock cod”, “flounder”, “lingcod.		
Elthusa winstoni Hadfield, Tuttle & Smit, 2017	**Type locality:** Hawaii.	**Type host:** *Ctenochaetus strigosus* (Bennett, 1828); *Acanthurus nigroris* Valenciennes, 1835.	Branchial	Hadfield et al. (2017d)

4.11.1 Discussion

Members from the genus *Elthusa* range in host specificity from being relatively general to highly specific (see Table 4.3). *Elthusa raynaudii, E. vulgaris, E. californica* and *E. menziesi* are examples of relatively general parasites. *Elthusa raynaudii* is known to be one of the *Elthusa* species with the weakest host specificity, parasitising 22 fish host families. These host families are Cheilodactylidae Bonaparte, 1850; Clupeidae Cuvier, 1816; Cyttidae Günther, 1860; Engraulidae Gill, 1861; Gnathanacanthidae Gill, 1892; Gobiesocidae Bleeker, 1859; Gempylidae Gill, 1862; Hemiramphidae Gill, 1859; Labridae Cuvier, 1816; Latridae Gill, 1862; Monacanthidae Nardo, 1843; Moridae Moreau, 1881; Notacanthidae Rafinesque, 1810; Nototheniidae Günther, 1861; Ophidiidae Rafinesque, 1810; Pleuronectidae Rafinesque, 1815; Pristigasteridae Bleeker, 1872; Scorpaenidae Risso, 1827; Sebastidae Kaup, 1873; Triakidae Gray, 1851; Triglidae Rafinesque, 1815; and Zeidae Rafinesque, 1815. *Elthusa vulgaris* has been recorded from 14 fish host families; *Elthusa californica* from 19 host families; and *Elthusa menziesi* from eight fish families. These are the more generalist *Elthusa* species.

Some *Elthusa* species are less host specific, such as *E. neocytta* and *E. nanoides* (which have both been recorded from three fish host families), and *E. sacciger* which has been recorded from two host families. The rest of the *Elthusa* species are limited to a single fish host family, or the host records are either unknown or scientifically unidentified. These records of host specificity cannot be conclusive, since many of these species have received little to no attention since being described, thus, host records may be limited due to the lack of study of these species and not as a result of host specificity.

The information summarised in Table 4.3 indicate that all *Elthusa* species are indeed branchial cavity inhabiting cymothoids, with the exception of *Elthusa neocytta* (Avdeev, 1975), that has been recorded from both the branchial cavity as well as the buccal cavity of some hosts, and *Elthusa splendida* (Sadowsky and Moreira, 1981) recorded mainly from the branchial cavity, with exceptional cases of males and juveniles in the branchial cavity. The recorded attachment position of *E. neocytta* in the buccal cavity of *Neocyttus rhomboidalis* Gilchrist, 1906 (Stephenson, 1987) can be confirmed as its regular attachment site, since its body shape is bilaterally symmetrical and cylindrical (Bruce, 1990), ideal to fit and attach to the buccal cavity of a fish host in addition to the branchial cavity. All buccal cavity attaching genera and species possess a slender, more elongated and cylindrical body shape in order to fit into the available space in the buccal cavity, or as a result of long-term attachment to the buccal cavity that caused the growth of the cymothoid to be altered and pressed into a certain shape.

Elthusa propinqua (Richardson, 1904) has been recorded from the buccal cavity (Richardson, 1910), and *Elthusa sacciger* (Richardson, 1909) from the buccal cavity (Richardson, 1909) and body surface of the host (Yamauchi, 2009) in addition to the branchial cavity. These attachment records of *E. propinqua* and *E. sacciger* are highly unlikely and are almost certainly examples of trawl transfer or the detachment of the cymothoid from its host, moving around in the attempt to escape a dying host or finding a new one. This suggestion can be supported by examining the morphological body shape of these parasites. *Elthusa propinqua* has a very wide and twisted body shape with a rather large brood pouch which would not be adapted to attach and survive in the buccal cavity of a host. *Elthusa sacciger* has a bilaterally symmetrical body shape, but distinctively ovoid with robust, bulbous coxae and a large brood pouch, which also makes it highly unlikely that it would attach to the buccal cavity or external surface of a host.

The phylogenetic relationships generated in this study from the ML tree (Figure 4. 15), contradicts the suggestion of Brusca (1981), that the Cymothoidae form three distinct evolutionary lineages.

CHAPTER 5: REVIEW OF *MOTHOCYA* COSTA, IN HOPE, 1851, FROM KENYA AND NIGERIA

5.1 Introduction

The genus *Mothocya* Costa, in Hope, 1851, contains 31 known and described species (Hadfield et al., 2017c). All *Mothocya* species except one, *Mothocya lineata* (Miers, 1876) (previously *Mothocya ihi* Bruce, 1986), which has been recorded and described from the mouth of its host (Bruce, 1986; Stephenson, 1969) are branchial attaching cymothoid parasites. *Mothocya* spp. are parasitic on atheriniform, beloniform and hemiramform fishes, and have recently been recorded from Beryciforme (Bruce, 1986; Elshahawy and Desouky, 2012; Hadfield et al., 2015; Aneesh et al., 2016a). *Mothocya* can be distinguished from some of the other cymothoid genera in having an antennula that is longer than the antenna; pereopods without carina and with long dactyli; laminal pleopods; and a maxilliped article 3 with 3–5 robust setae (Hadfield et al., 2014c).

Many *Mothocya* species have originally been placed within the genus *Irona* Schioedte and Meinert, 1884. Bruce (1986) synonymised and transferred many of these species from *Irona* during the revision of the genus *Mothocya* (Bruce, 1986; Hadfield et al., 2014c). *Mothocya* has a cosmopolitan distribution (Bruce et al., 2002). Records from eastern sub-Sahara Africa include five species. *Mothocya affinis* Hadfield, Bruce and Smit, 2015, which has only been recorded from north-eastern South Africa (Sodwana Bay) from the tropical halfbeak, *Hyporamphus affinis* (Günther, 1866). *Mothocya arrosor* Bruce, 1986, has a widespread distribution, within the Indo-west Pacific Ocean corresponding to where its only host, the ribbon halfbeak *Euleptorhamphus viridis* (van Hasselt, 1823), is found. The only sub-Sahara African record of *M. arrosor* has been from Kenya. *Mothocya collettei* Bruce, 1986, distributed in the Indo-west Pacific Ocean, has also only been recorded from sub-Sahara Africa in Kenya from the hound needlefish, *Tylosurus crocodilus crocodilus* (Péron & Lesueur, 1821). Other hosts of *M. collettei* are the flat needlefish, *Ablennes hians* (Valenciennes, 1846), the spotted long-tom, *Tylosurus punctulatus* (Günther, 1872) and the archerfish, *Toxotes* Cloquet, 1816 (Bruce, 1986).

Mothocya renardi (Bleeker, 1857) is a well-documented Indo-west Pacific and Indian Ocean distributed species that has been recorded from Kenya, Mozambique, Madagascar and South Africa in the sub-Sahara African region. *Mothocya renardi* is predominantly recorded from the banded needlefish, *Strongylura leiura* (Bleeker, 1850). *Mothocya plagulophora* (Haller, 1880) is similarly distributed in the Indo-west Pacific and Indian Ocean but has been recorded from sub-Sahara African regions such as Somalia, Kenya, Zanzibar, Mauritius,

Madagascar, Comoro Islands and Mozambique. *Mothocya plagulophora* has mainly been collected from the black-barred halfbeak, *Hemiramphus far* (Forsskål, 1775) with a single known record from the yellowtip halfbeak, *Hemiramphus marginatus* (Forsskål, 1775) (see Hadfield et al., 2015).

Records of *Mothocya* from western Africa include only two species. *Mothocya longicopa* Bruce, 1986 has only been recorded from the western African Gulf of Guinea and Guinea from the flat needlefish, *Ablennes hians* (Valenciennes, 1846) and the hound needlefish, *Tylosurus crocodilus crocodilus* (Péron & Lesueur, 1821). *Mothocya trillesi* (Rokicki, 1986) has only been recorded once, at the coastal region of Senegal from the garfish *Belone belone* (Linnaeus, 1760) (see Rokicki, 1986).

5.2 Specific materials and methodology used

Mothocya specimens from eastern and western Africa were examined. This preserved material was obtained by generous donations from international colleagues. Morphological analysis was performed following general methodology (see Chapter 2.3). Molecular characterisation of *Mothocya* specimens were attempted, but did not yield quality results possibly due to the age and fixation history of the specimens. *Mothocya affinis* has recently been described and therefore does not form part of the morphological analysis, but specimens were collected, along with its hosts, for the case study on the effects of the species on host health.

5.3 Taxonomy

Suborder Cymothoida Wägele, 1989
Superfamily Cymothooidea Leach, 1814
Family Cymothoidae Leach, 1814

Genus *Mothocya* Costa, in Hope, 1851

Mothocya Costa, in Hope, 1851: 48.—Trilles, 1968: 168.—Monod, 1971: 174.—Bowman and Tareen, 1983:25.—Bruce, 1986: 1092–1095.—Trilles, 1994: 197.—Hadfield, Sikkel and Smit, 2014c: 111.—Hadfield, Bruce and Smit, 2015: 148.

Irona Schioedte and Meinert, 1884: 381.—Stebbing, 1905: 27.—Richardson, 1905: 265.—Hale, 1926: 218.—Monod, 1971: 174.—Kussakin, 1979: 307.—Trilles, 1994: 166.

Type species: *Mothocya epimerica* Costa, in Hope, 1851; by subsequent designation (see Bruce, 1986).

5.4 Diagnosis

Twisted body (2.0–2.5 times as long as wide), widest at pereonite 5; a rounded cephalon anterior margin. Unsinuated, recessed pereonite 1 anterior margin, without lobes. Coxae large, round and wide, reaching the posterior margin, or extending beyond respective pereonite. Pereonite 7 partially overlapping pleon; pleonite 1 partially concealed by pereonite 7, lateral margins extending further on one lateral side. Pleon wide, often wider than pereonite 7. Robust pereopods without carina, subequal, increasing is size; with long dactyli. Lamellar, simple pleopods without setae; endopods 3–5 with proximomedial lobes. Pleopod peduncle articles with lateral lobe. Uropod with exopod apex extending beyond endopod apex. Oostegites in pairs of 4.

Antennae bases widely separated; stout antennulae, longer than antenna. Antennulae consist of 7–8 articles; antennae with 7–9 articles. Maxilliped lacking oostegital lobe, 3–5 robust setae on article 3. Mandible incisor thin, sharp; mandible palp lacking setae. Maxilla medial and lateral lobes partially fused. Maxillula simple, narrow, with 1 robust setae and 3 simple setae.

5.5 *Mothocya renardi* (Bleeker, 1857)

Livoneca Renardi Bleeker, 1857: 28–29, pl. 1, fig. 8.

Irona Renardi.—Schioedte and Meinert, 1884: 383–386, pl. XIV (Cym. XXXIV), figs. 10–15.

Livoneca Renardi.—Gerstaecker, 1882: 261.

Irona melanosticta.—Barnard, 1914: 373–374; 1955: 6.—Kensley, 1978: 80, fig. 33A (non *I. melanosticta* Schioedte and Meinert, 1884).

Irona renardi.—Nierstrasz, 1915: 104; 1931: 145.—Hale, 1926: 218–220, fig. 12.—Hale, 1929: 258, fig. 255.—Holthuis, 1959: 97, photo 11, figs. 4–9.—Monod, 1971: 173–174.—Monod, 1976: 863, figs. 30, 32.—Trilles, 1976: 785–786, pl. 11, fig. 10.—Trilles, 1979b: 266.—Beumer, Ashburner, Burbury, Jette and Latham, 1982: 32.

Irona robusta Nair, 1950: 66–70, figs. 1–12; 1956: 2.—Abraham, 1966: 23–42, figs. 28–54, photos 5–6.—Abraham, 1967: 10–16, figs. 1–25.—Monod, 1971: 174.

Mothocya species.—Bowman and Tareen, 1983: 25, fig. 19.

Mothocya renardi.—Bruce, 1986: 1169–1177, figs. 49–52, 55.—Williams and Williams, 1986: 215.—Yu and Li, 2003: 230–232, fig. 6.—Jones, Miller, Grutter and Cribb, 2008: 477–491.—Hadfield, Bruce and Smit, 2015: 150.—Aneesh, Sudha, Helna, Arshad, Anilkumar and Trilles, 2013: 1–9.—Trilles, Ravichandran and Rameshkumar, 2011: 446–459.—Rameshkumar, Ravichandran and Allayie, 2013a: 127–132.—Aneesh, Kappalli, Kottarathil and Gopinathan, 2016: 583–599.

Lironeca puhi.—Ravichandran, 2007: 87–93, fig. 3 [misidentification].

Non *Lironeca Renardi*.—Miers, 1880: 465–466.

Non *Irona renardii*.—Lanzing and O'Connor, 1975: 355–361, fig. 1 c–d [*Mothocya halei*].

Syntypes: Two presumed syntypes ♀♀ (19.5–21.5 mm TL) from Jakarta Bay ('mer de Batavia'), Java, Indonesia, collected by P. Bleeker (RMNH 4611) (Bruce, 1986). Not examined; redescribed by Bruce (1986).

Type locality: Jakarta Bay, Java. Indonesia.

Type host: Unknown.

5.5.1 Material examined

Eight specimens were examined, collected from Lamu archipelago, Kenya, during January 2009 by David Modry. Donated by Roman Kuchta. The host was identified as the banded needlefish *Strongylura leiura* (Bleeker, 1850), from photos by the collector, using Smith and Heemstra (1986). One ♀ and one ♂ were illustrated: *Mothocya renardi* ♀ (ovigerous, 17.0 mm TL, 8.0 mm W); *Mothocya renardi* ♂ (15.0 mm TL, 6.0 mm W). Additional material consisted of three ovigerous ♀♀ (18.0 mm TL, 9.0 mm W; 19.0 mm TL, 9.0 mm W; 18.0 mm TL, 8.0 mm W); and three ♂♂ (11.0 mm TL, 4.0 mm W; 12.0 mm TL, 5.0 mm W; 13.0 mm TL, 5.0 mm W).

5.5.2 Descriptions

Mothocya renardi ♀

Figs. 5.1–5.4

Body elongate ovoid, slightly twisted to the left, 2.1 times as long as greatest width, body dorsal surfaces smooth and polished in appearance, widest at pereonite 3, most narrow at pereonite 7, pereonite lateral margins mostly posteriorly ovate, medially indented. *Cephalon* ovate, 0.8 times longer than wide, visible from dorsal view, immersed in pereonite 1. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.2

times width of cephalon, 0.3 times length of cephalon. *Pereonite 1* smooth, anterior border deeply recessed, evenly concave, anterolateral angle wide, with inwardly produced point. Pereonite 1 anterolateral margins extend past the medial region of eyes. Posterior margins of pereonites smooth and straight; pereonite 7 medially recessed. Coxae 2–3 wide, with posteroventral angles rounded; coxae 4–7 rounded, not extending past pereonite margins. Pereonites 4–7 becoming progressively narrower, pereonites 2–3 subequal. Pleon 0.3 times as long as total body length, with pleonite 1 completely concealed by pereonite 7, not visible in dorsal view; pleonites posterior margin smooth, mostly concave. Pleonite 2 largely overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonite 5 widest, free, not overlapped by lateral margins of pleonite 4, posterior margin straight. *Pleotelson* 0.9 times as long as anterior width; dorsal surface smooth. Pleotelson lateral margins weakly convex, posterior margin evenly rounded.

Antennula approximately the same width as antenna. Antennula consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 1.1 times as long as article 1; article 3 1.6 times as long as wide, 0.6 times as long as combined lengths of articles 1 and 2. Antennula flagellum with 5 articles, extending to middle of eye, with tufts of simple setae on articles 4–8; with tufts of plumose setae on articles 1–3. Antenna consists of 9 articles; peduncle article 3 as long as article 2; article 4 1.4 times as long as wide, as long as article 3; article 5 1.4 times as long as wide, 0.9 times as long as article 4. Antenna flagellum with 3 articles, terminal article terminating in 1–5 short simple setae; extending to middle of the eye. Mandibular molar process present, ending in an acute incisor, without setae; mandible palp article 2–3 without setae. Maxillula simple, with 4 terminal robust setae. Maxilla mesial lobe partly fused to lateral lobe; lateral lobe with 2 recurved robust setae; mesial lobe with 2 large recurved robust setae. Maxilliped consists of 3 articles; article 3 with 4 recurved robust setae.

Pereopod 1 basis 1.4 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.4 times as long as wide; dactylus slender, as long as propodus, 2.5 times as long as basal width. Pereopod 3 similar to pereopod 2, gradually increasing in size towards posterior, without robust or simple setae. *Pereopod 7* basis without carina, basis 1.8 times as long as greatest width; ischium 0.9 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 1.6 times as long as wide, 0.5 times as long as ischium; carpus 0.8 times as long as wide, 0.4 times as long as ischium; propodus 1.5 times as long as wide, 0.8 times as long as ischium; dactylus slender, 1.2 times as long as propodus, 2.5 times as long as basal width.

Pleopods simple*,* without setae. Pleopod 1 exopod 1.4 times as long as wide, lateral margin distally convex, distally broadly rounded, mesial margin weakly convex.

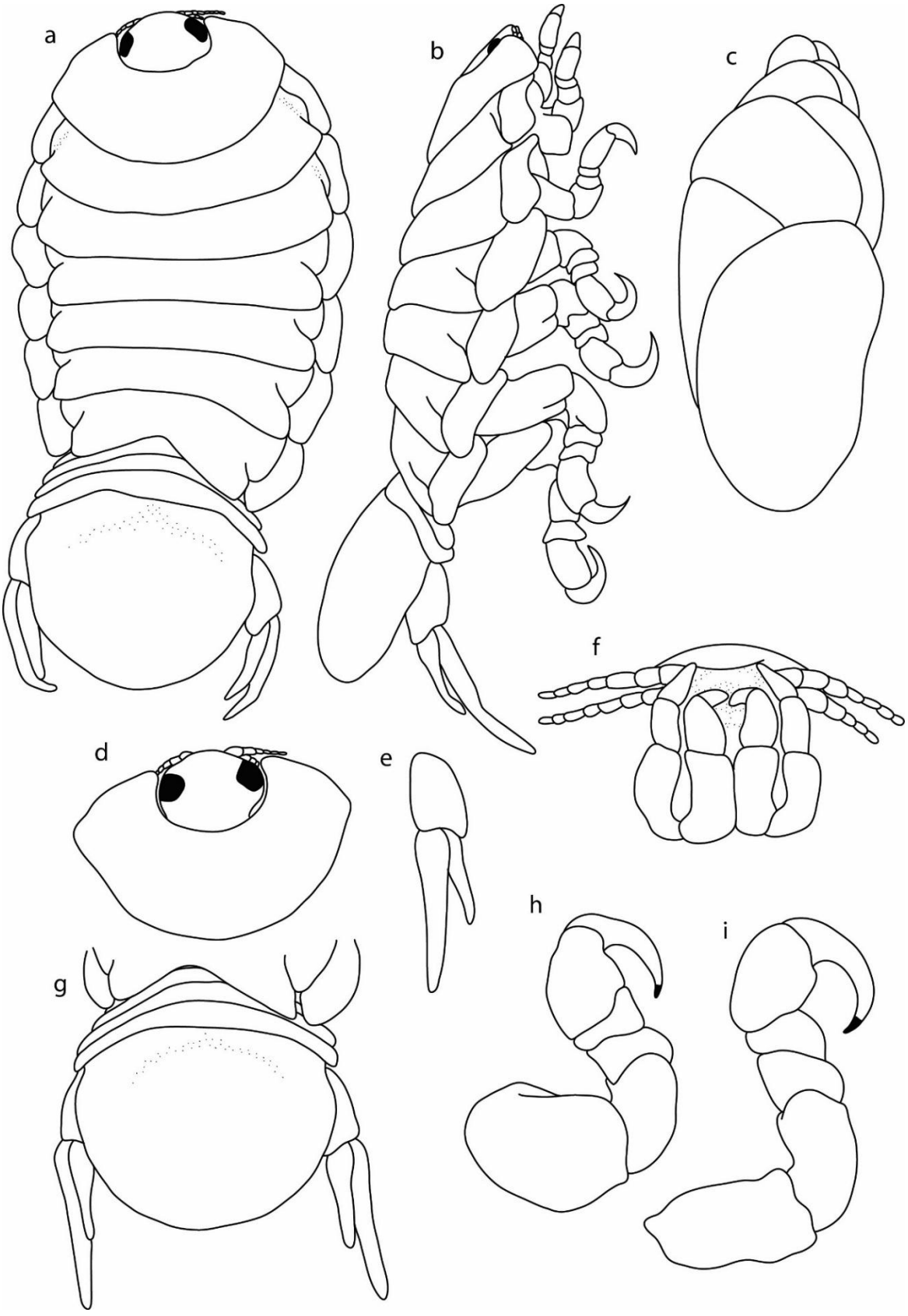

Figure 5.11: *Mothocya renardi* (Bleeker, 1857) ♀ (ovigerous, 20.0 mm TL, 8.0 mm W) from Lamu archipelago, Kenya. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon and pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Dorsal view of pleon. **h** Pereopod 1. **i** pereopod 7.

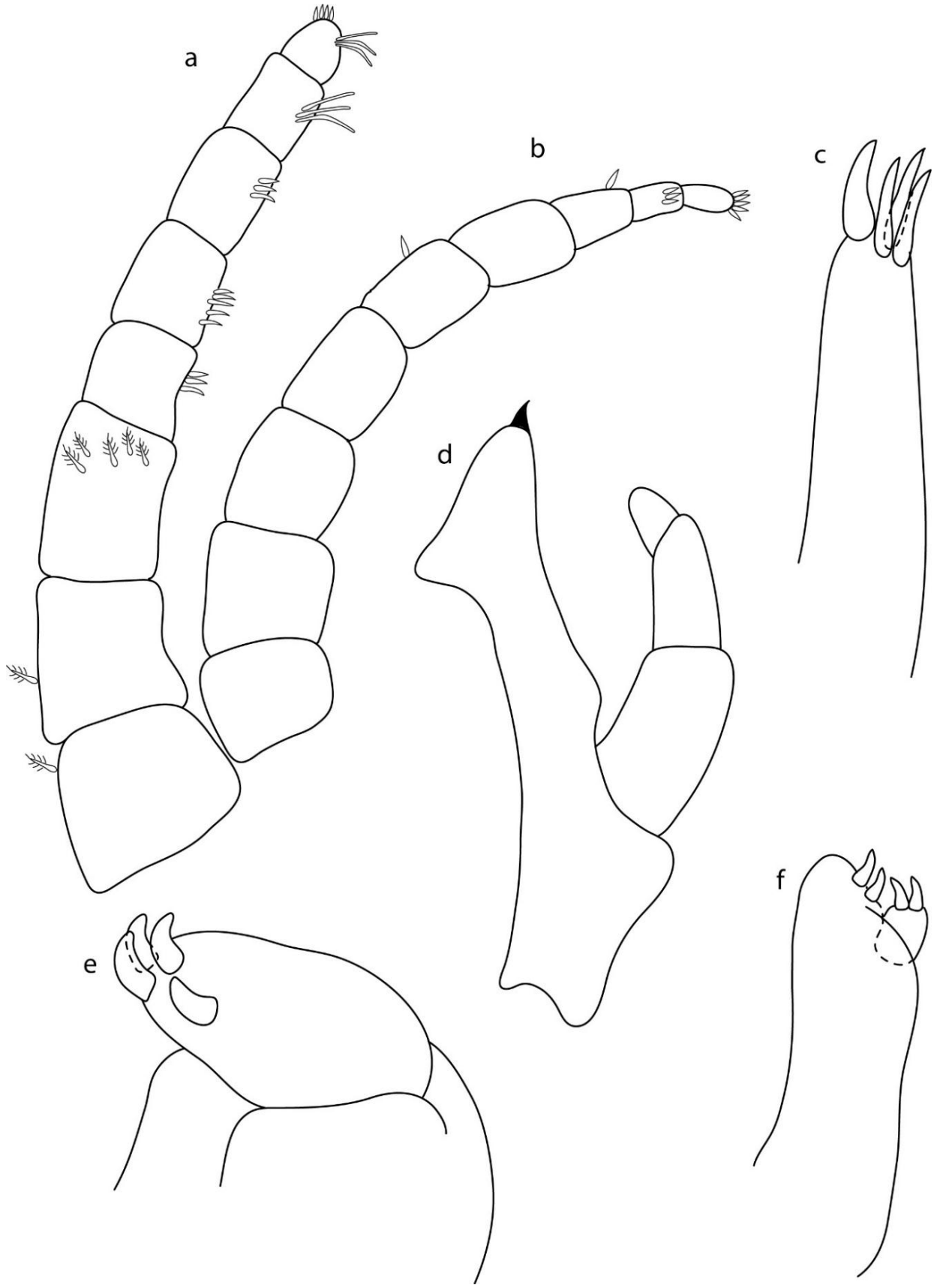

Figure 5.2: *Mothocya renardi* (Bleeker, 1857) ♀ (ovigerous, 20.0 mm TL, 8.0 mm W) from Lamu archipelago, Kenya. **a** Antennula. **b** Antenna. **c** Tip of maxillula. **d** Mandible. **e** Maxilliped article 3. **f** Maxilla.

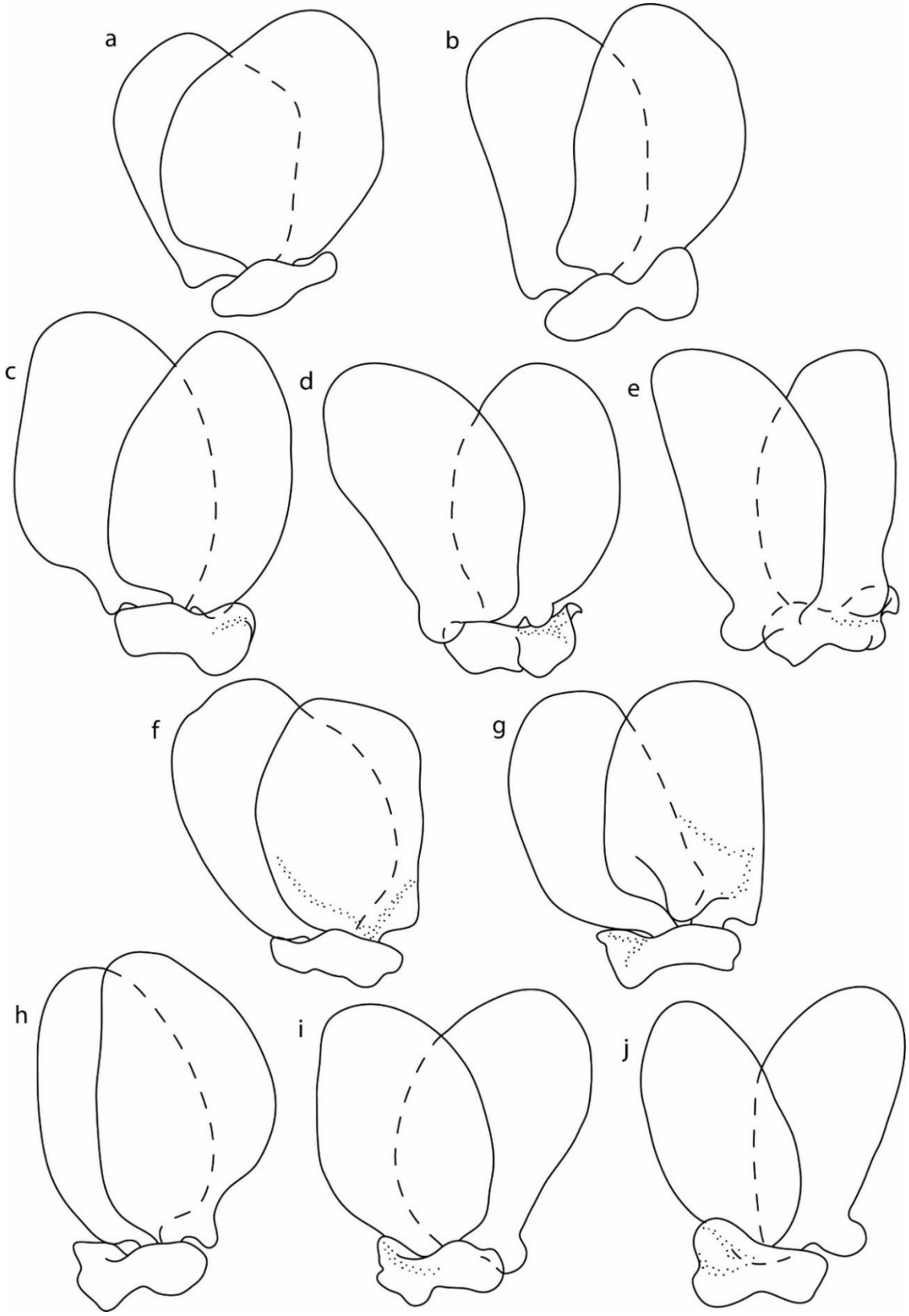

Figure 5.3: *Mothocya renardi* (Bleeker, 1857) ♀ (ovigerous, 20.0 mm TL, 8.0 mm W) from Lamu archipelago, Kenya. **a** Pleopod 1 dorsal view. **b** Pleopod 2 dorsal view. **c** Pleopod 3 dorsal view. **d** Pleopod 4 dorsal view. **e** Pleopod 5 dorsal view. **f** Pleopod 1 ventral view. **g** Pleopod 2 ventral view. **h** Pleopod 3 ventral view. **i** Pleopod 4 ventral view. **j** Pleopod 5 ventral view.

Endopod 1.4 times as long as wide, lateral margin convex, distally broadly rounded, mesial margin straight, peduncle 2.9 times as wide as long. Pleopods 1–5 proximomedial lobes increasing in size, with proximomedial lobes. Pleopods 2–5 similar to pleopod 1. Peduncle lobes increasing in size from pleopod 2–5.

Uropod extending beyond pleotelson, peduncle 0.5 times longer than rami, peduncle lateral margin without setae; marginal setae absent. *Endopod* apices narrowly rounded, 3.7 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae. *Exopod* extending beyond the end of endopod, 5.1 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae.

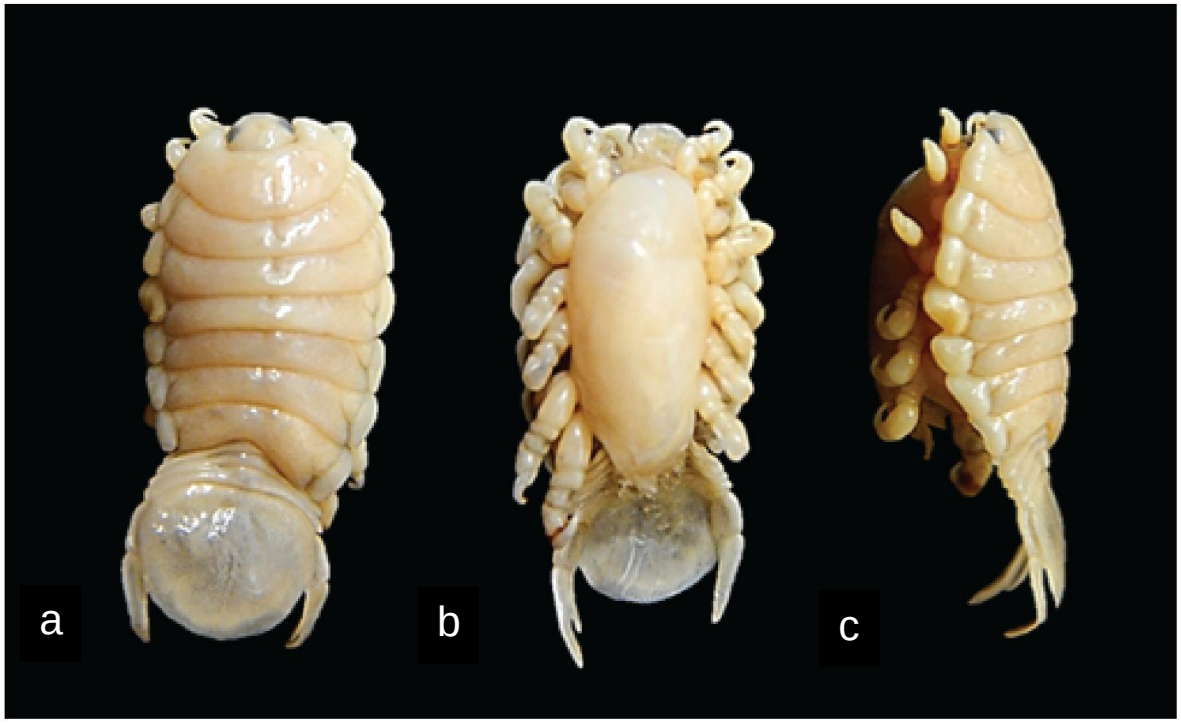

Figure 5.4: Photos of *Mothocya renardi* (Bleeker, 1857) ♀ (ovigerous, 20.0 mm TL, 8.0 mm W) from Lamu archipelago, Kenya. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

Mothocya renardi ♂

Figs. 5.5–5.8

Body rectangular; straight, not twisted, 2.5 times as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 3, most narrow at pereonite 7, pereonite lateral margins subparallel, mostly posteriorly ovate. *Cephalon* 0.4 times longer than wide, visible from dorsal view, triangular, immersed in pereonite 1. *Frontal margin* thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.3 times width of cephalon, 0.4 times length of cephalon. *Pereonite 1* smooth, anterior border medially straight, curved laterally, anterolateral angle rounded, anteriorly projected; extending to the

medial region of eyes. Posterior margins of pereonites smooth and straight, pereonite 7 medially recessed. Coxae 2–3 wide; with posteroventral angles rounded; coxae 4–7 rounded; not extending past pereonite margin. Pereonites 1–3 increasing in length and width; 4–7 decreasing in length and width and becoming progressively narrower. *Pleon* 0.24. times as long as total body length, with pleonite 1 largely concealed by pereonite 7; pleonites posterior margin smooth, mostly concave. Pleonite 2 partially overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 widest, free, not overlapped by lateral margins of pleonite 4, posterior margin straight, curved laterally. *Pleotelson* as long as anterior width, dorsal surface smooth. Pleotelson lateral margins weakly convex, posterior margin converging to caudomedial point, slightly damaged.

Antennula approximately the same width as antenna. Antennula consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 0.7 times as long as article 1; article 3 1.5 times as long as wide, 0.6 times as long as combined lengths of articles 1 and 2; antennula flagellum with 5 articles, extending to middle of eye, with tufts of simple setae on articles 4–8. *Antenna* consists of 7 articles, peduncle article 3 1.2 times as long as article 2; article 4 1.1 times as long as wide, 0.7 times as long as article 3; article 5 1.5 times as long as wide, 0.9 times as long as article 4. Antenna flagellum with 3 articles, terminal article terminating in 1–5 short simple setae, extending to middle of the eye. *Mandible palp* article 2– 3 without setae. *Maxillula* simple with 4 terminal robust setae. *Maxilla* lateral lobe with 2 recurved robust setae; mesial lobe with 2 large recurved robust setae. *Maxilliped* consists of 3 articles, palp article 2 without setae; article 3 with 4 recurved robust setae.

Pereopod 1 basis twice as long as greatest width; ischium 0.4 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.8 times as long as wide; dactylus slender, 1.1 times as long as propodus, 3.3 times as long as basal width. *Pereopod* 3 similar to pereopod 2, gradually increasing in size towards posterior, without setae. *Pereopod 7* basis without carina, 1.7 times as long as greatest width; ischium as long as basis, without protrusions; merus proximal margin without bulbous protrusion, as long as wide, 0.4 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium; propodus 1.6 times as long as wide,

Pleopods simple. Pleopod 1 exopod 1.7 times as long as wide, lateral margin straight, distally broadly rounded, mesial margin straight; endopod 1.5 times as long as wide, lateral margin weakly concave, distally broadly rounded, mesial margin slightly convex, peduncle 1.4 times as wide as long.

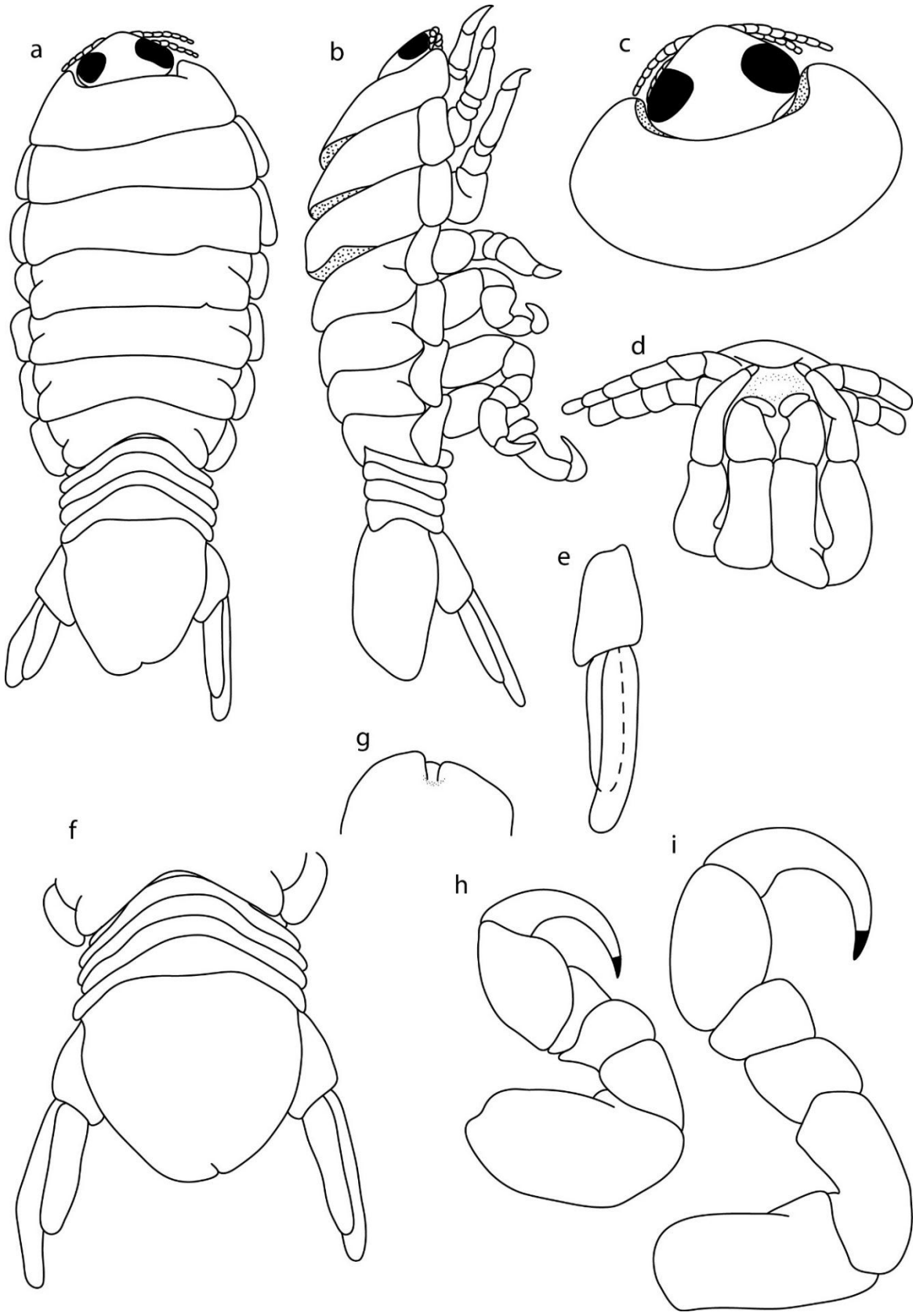

Figure 5.12: *Mothocya renardi* (Bleeker, 1857) ♂ (15.0 mm TL, 6.0 mm W) from Lamu archipelago, Kenya. **a** Dorsal body. **b** Lateral body. **c** Dorsal view of cephalon with pereonite 1. **d** Ventral cephalon. **e** Uropod. **f** Dorsal view of pleon. **g** Penes. **h** Pereopod 1. **i** Pereopod 7.

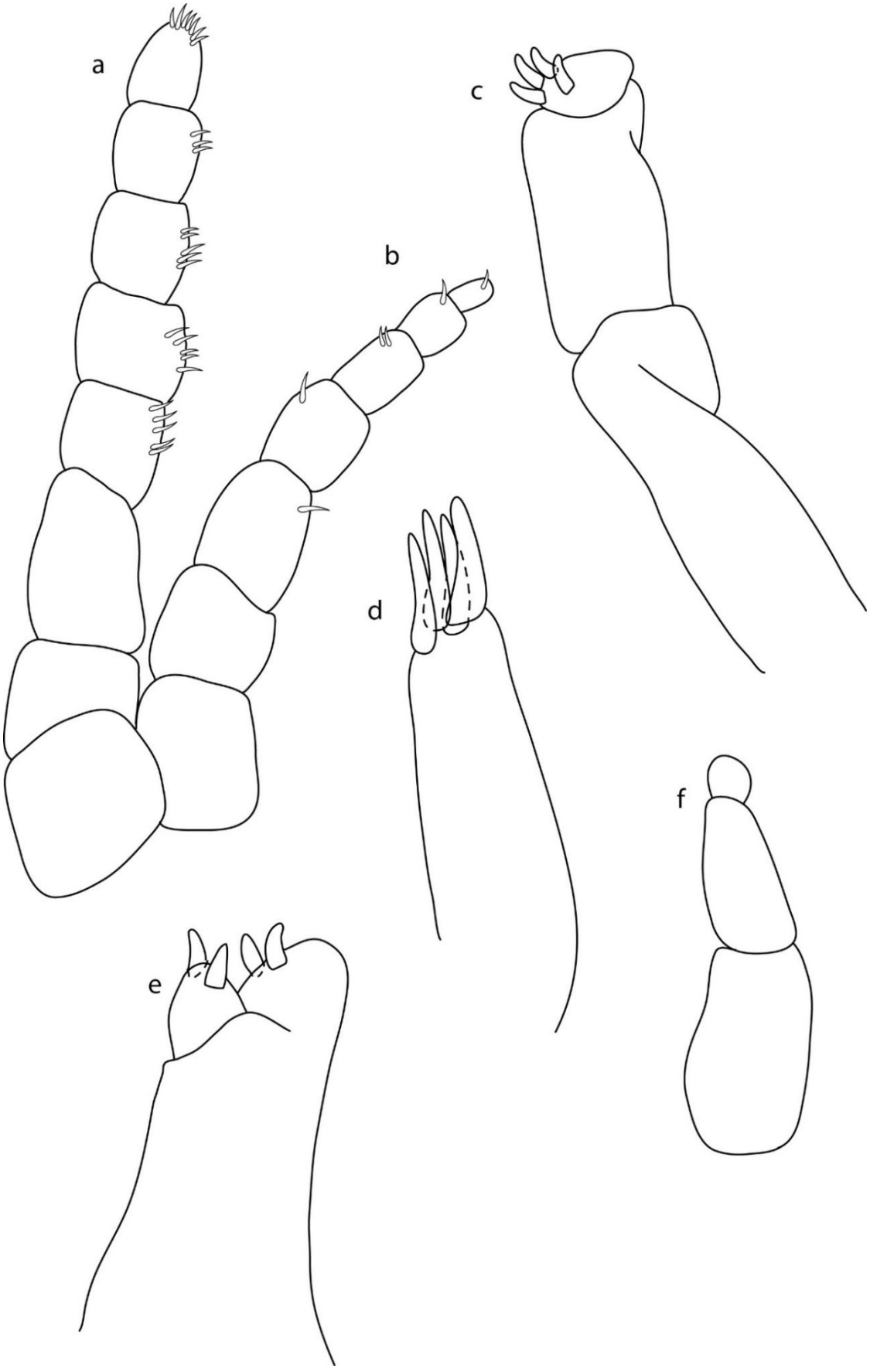

Figure 5.6: *Mothocya renardi* (Bleeker, 1857) ♂ (15.0 mm TL, 6.0 mm W) from Lamu archipelago, Kenya. **a** Antennula. **b** Antenna. **c** Maxilliped. **d** Tip of maxillula. **e** Maxilla. **f** Mandible palp.

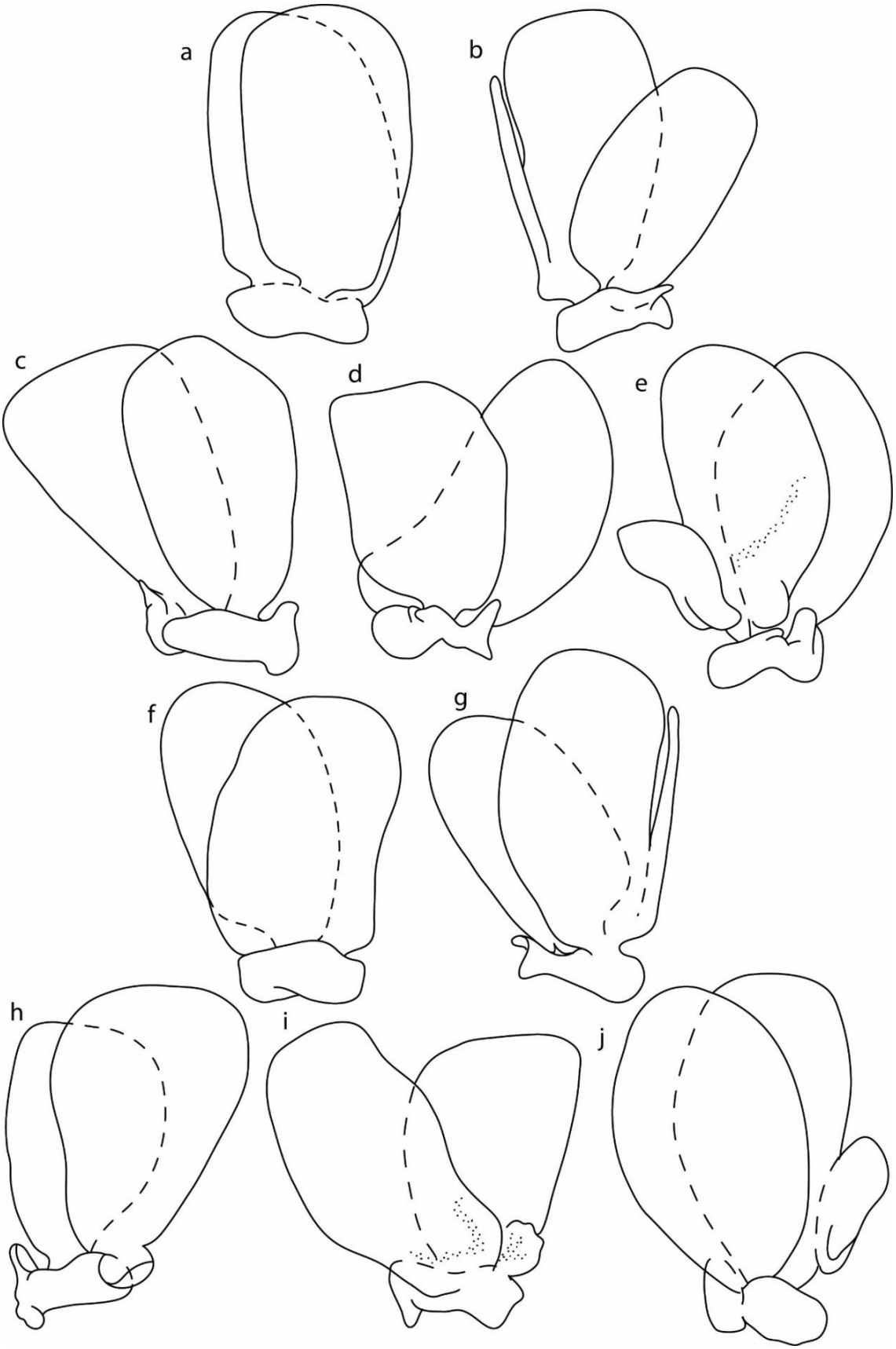

Figure 5.13: *Mothocya renardi* (Bleeker, 1857) ♂ (15.0 mm TL, 6.0 mm W) from Lamu archipelago, Kenya. **a** Pleopod 1 dorsal view. **b** Pleopod 2 dorsal view. **c** Pleopod 3 dorsal view. **d** Pleopod 4 dorsal view. **e** Pleopod 5 dorsal view. **f** Pleopod 1 ventral view. **g** Pleopod 2 ventral view. **h** Pleopod 3 ventral view. **i** Pleopod 4 ventral view. **j** Pleopod 5 ventral view.

Pleopod 2 appendix masculina with parallel margins, 0.8 times as long as endopod, distally bluntly rounded. Pleopods 1–5 proximomedial lobes increasing in size, with proximomedial lobes. Peduncle lobes increasing in size from pleopod 2 to 5.

Uropod extending beyond pleotelson, peduncle 0.5 times longer than rami, peduncle lateral margin without setae; rami extending beyond pleotelson, marginal setae absent,

Endopod apices broadly rounded, 3.9 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae. *Exopod* extending beyond end of endopod, 5.3 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae.

Penes low tubercles, penial process 0.4 times as long as basal width.

Figure 5.14: Photos of *Mothocya renardi* (Bleeker, 1857) ♂ (15.0 mm TL, 6.0 mm W) from Lamu archipelago, Kenya. **a** Dorsal view; **b** Ventral view ; **c** Lateral view.

5.5.3 Distribution

Africa: St Lucia, South Africa (Hadfield et al., 2015); Kenya (Bruce, 1986); Mozambique (Bruce, 1986), Antsirane, Madagascar (Trilles, 1976); Zambezi River (Barnard, 1914). **Middle east:** Kuwait (Bowman and Tareen, 1983). **India:** India (Nair 1950); Cochin, Palk Bay, Pondichery (Bruce, 1986; Trilles et al., 2011); Malabar Coast, (Aneesh et al., 2013) Parangipettai (Rameshkumar et al., 2013c). **Western Pacific Ocean:** Songkhla Fish Market, Gulf of Thailand (Williams and Williams, 1986); Japan (Bruce, 1986); China (Yu and Li, 2003). Jakarta Bay, Java, Indonesia (Bleeker, 1857); Philippines (Bruce, 1986) and Manila (Schioedte and Meinert, 1884); Papua New Guinea (Bruce, 1986). **Australia**: Georges

River, New South Wales (Hale, 1926); Queensland, Townsville (Hale, 1926); Western Australia, Fremantle (Hale, 1926); Western and Eastern Australia (Bruce, 1986). **Eastern Indian Ocean** (Nierstrasz, 1915).

5.5.4 Hosts

Mothocya renardi is considered to have a high host specificity, having only been recorded from a single fish host family, the Belonidae Bonaparte, 1835. Known hosts include: *Strongylura leiura* (Bleeker, 1850) (see Aneesh et al., 2013; 2016; Hadfield et al., 2015); *Strongylura incisa* (Valenciennes, 1846) and *Strongylura anastomella* (Valenciennes, 1846) (see Bruce, 1986). *Tylosurus crocodilus crocodilus* (Péron & Lesueur, 1821) (see Hale, 1926) and *Strongylura strongylura* (van Hasselt, 1823) (see Bowman and Tareen, 1983) are still unconfirmed (Bruce 1986; Aneesh et al., 2016). *Tylosurus choram* (Rüppell, 1837) (see Barnard, 1914).

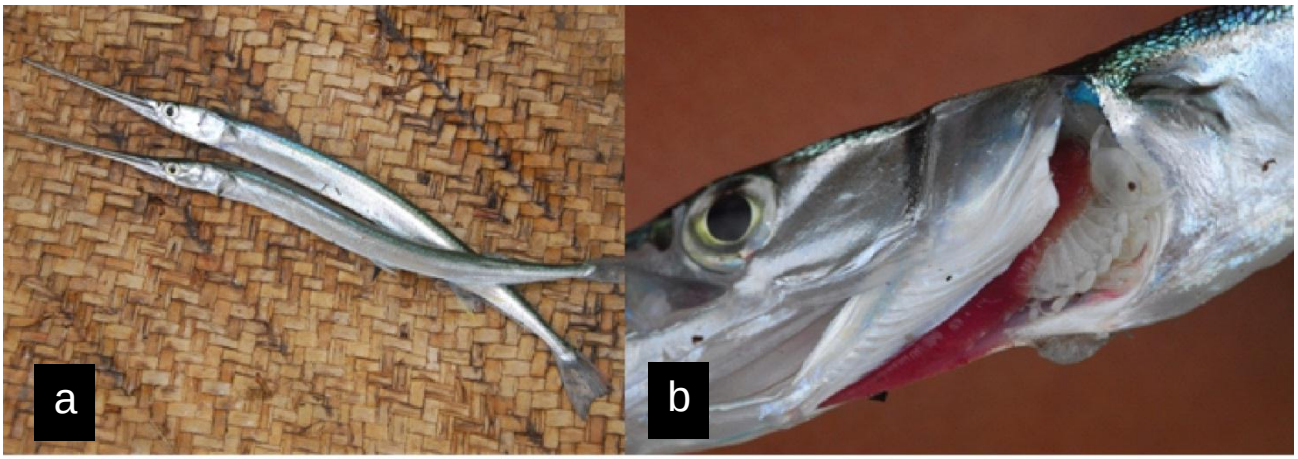

Figure 5.9: a *Mothocya renardi* (Bleeker, 1857) host, *Strongylura leiura* (Bleeker, 1850) from Kenya. **b** Position of *M. renardi* ovigerous female in host branchial cavity, photos by Roman Kuchta.

5.5.5 Remarks

Mothocya renardi was originally described as *Livoneca renardi* (Bleeker, 1850) and later transferred to *Mothocya* (Bruce, 1986). Descriptions of this species are well documented (Bowman and Tareen, 1983; Bruce, 1986; Hadfield et al., 2015; Aneesh et al., 2016). Bruce (1986), Hadfield et al. (2015) and Aneesh et al. (2016) provided diagnostic characteristics for *M. renardi.* The most prominent features are its large size (ranging between 24.0–36.0 mm

TL); a narrow pleon; and long, narrow uropodal rami extending considerably past the pleotelson posterior margin. It can further be identified by the twist in the planes of the pleon in relation to the pereon.

The position of attachment of *M. renardi* to the host branchial cavity is with the brood pouch to the ventral surface of the cavity and with the cephalon towards the anterior end of the host (Williams and Williams, 1986; Aneesh et al., 2013). This position is also visible in Figure 6.9 for the collected *M. renardi* specimen from Kenya. Aneesh et al. (2016) stated that *M. renardi* can successfully be used for host-parasite studies, as they have a very high prevalence and site-specificity within its most common host, *Strongylura leiura*.

5.6 *Mothocya* sp. 1

5.6.1 Material examined

Five specimens were examined from Andoni Creek of Niger Delta, Nigeria, during August 2015 by B Olaosebikan from the African moony, *Monodactylus sebae* (Cuvier, 1829). Two ♀♀ and one ♂ were illustrated: holotype ♀ (ovigerous, 15.0 mm TL, 8.0 mm W); paratype ♀ (ovigerous, 18.0 mm TL, 9.0 mm W); ♂ (12.0 mm TL, 5.0 mm W). Additional material consisted of two paratype ♂♂ (12.0 mm TL, 5.0 mm W; 7.0 mm TL, 4.0 mm W).

5.6.2 Descriptions

***Mothocya* sp. 1 holotype ♀**

Fig. 5.10, 5.13

Body elongated ovoid, slightly twisted to the right, 1.9 times as long as greatest width. Body dorsal surfaces smooth, polished in appearance, widest at pereonite 4, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate. *Cephalon* sub-triangular, 0.8 times longer than wide, visible from dorsal view, with rounded anterior point. *Frontal margin* slightly ventrally folded. *Eyes* irregular in outline; one eye 0.2 times width of cephalon, 0.4 times length of cephalon. Pereonite 1 smooth, anterior border anteriorly expanded with connective tissue, slightly convex, anterolateral angle broadly rounded, extending to posterior margin of eyes. Posterior margins of pereonites smooth and straight; pereonite 7 medially recessed. Coxae 2–3 wide; with posteroventral angles rounded; coxae 4–7 rounded. Coxae 1–5 not extending past pereonite posterior margin, 6–7 extending slightly past pereonite posterior margin. Pereonites 1–4 increasing in length and width; 5–7

decreasing in length and width; 4–7 becoming progressively narrower, 2–3 subequal. *Pleon* 0.2 times as long as total body length, with pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin smooth and curved, mostly concave. Pleonite 2 partially overlapped by pereonite 7. Pleonite 5 widest, posterior margin straight. *Pleotelson* 0.6 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins convex, posterior margin slightly damaged, broadly rounded.

Antennula more stout than antenna, consists of 6 articles; peduncle articles 1 and 2 distinct and articulated; article 2 0.6 times as long as article 1; article 3 1.4 times as long as wide, 0.2 times as long as combined lengths of articles 1 and 2; antennule flagellum with 4 articles, extending to posterior margin of eye, without setae. *Antenna* consists of 7 articles; peduncle article 3 1.1 times as long as article 2; article 4 1.6 times as long as wide, 1.1 times as long as article 3; article 5 1.6 times as long as wide, 1.1 times as long as article 4. Antenna flagellum with 5 articles, terminal article terminating without setae. *Mandibular molar process* present, ending in an acute incisor; mandible palp article 2–3 without setae. *Maxillula* simple with 4 terminal robust setae; lateral lobe with 2–3 recurved robust setae; mesial lobe with 2 large recurved robust setae. *Maxilliped* consists of 3 articles, article 3 with 4 recurved robust setae.

Pereopod 1 basis 1.9 times as long as greatest width; ischium 0.6 times as long as basis; carpus with rounded proximal margin; propodus 1.4 times as long as wide; dactylus slender, 1.8 times as long as propodus, 3.9 times as long as basal width. *Pereopod 7* basis without carina, 3.8 times as long as greatest width; ischium 0.7 times as long as basis, with slight bulbous protrusion; merus proximal margin without bulbous protrusion, 0.7 times as long as wide, 0.3 times as long as ischium; carpus 0.9 times as long as wide, 0.5 times as long as ischium, without bulbous protrusion; propodus twice as long as wide, as long as ischium; dactylus slender, as long as propodus, 3.3 times as long as basal width.

Pleopods simple, without setae. *Pleopod 1* exopod 1.1 times as long as wide, lateral margin strongly convex, distally broadly rounded, mesial margin strongly convex. *Endopod* 1.6 times as long as wide, lateral margin straight, distally broadly rounded, mesial margin slightly convex, peduncle 2.7 times as wide as long. Pleopods 1–5 proximomedial lobes increasing in size, with proximomedial lobes. Pleopods 2–5 similar to pleopod 1. Peduncle lobes increasing in size from pleopod 2–5.

Uropod same length or slightly longer than the pleotelson, peduncle 0.6 times longer than rami, peduncle lateral margin without setae; rami extending beyond pleotelson, marginal setae absent. *Endopod* narrowly rounded; 3.6 times as long as greatest width, lateral margin straight, mesial margin weakly convex, terminating without setae. *Exopod* extending beyond end of endopod, 4.6 times as long as greatest width, lateral margin weakly convex, mesial margin straight, terminating without setae.

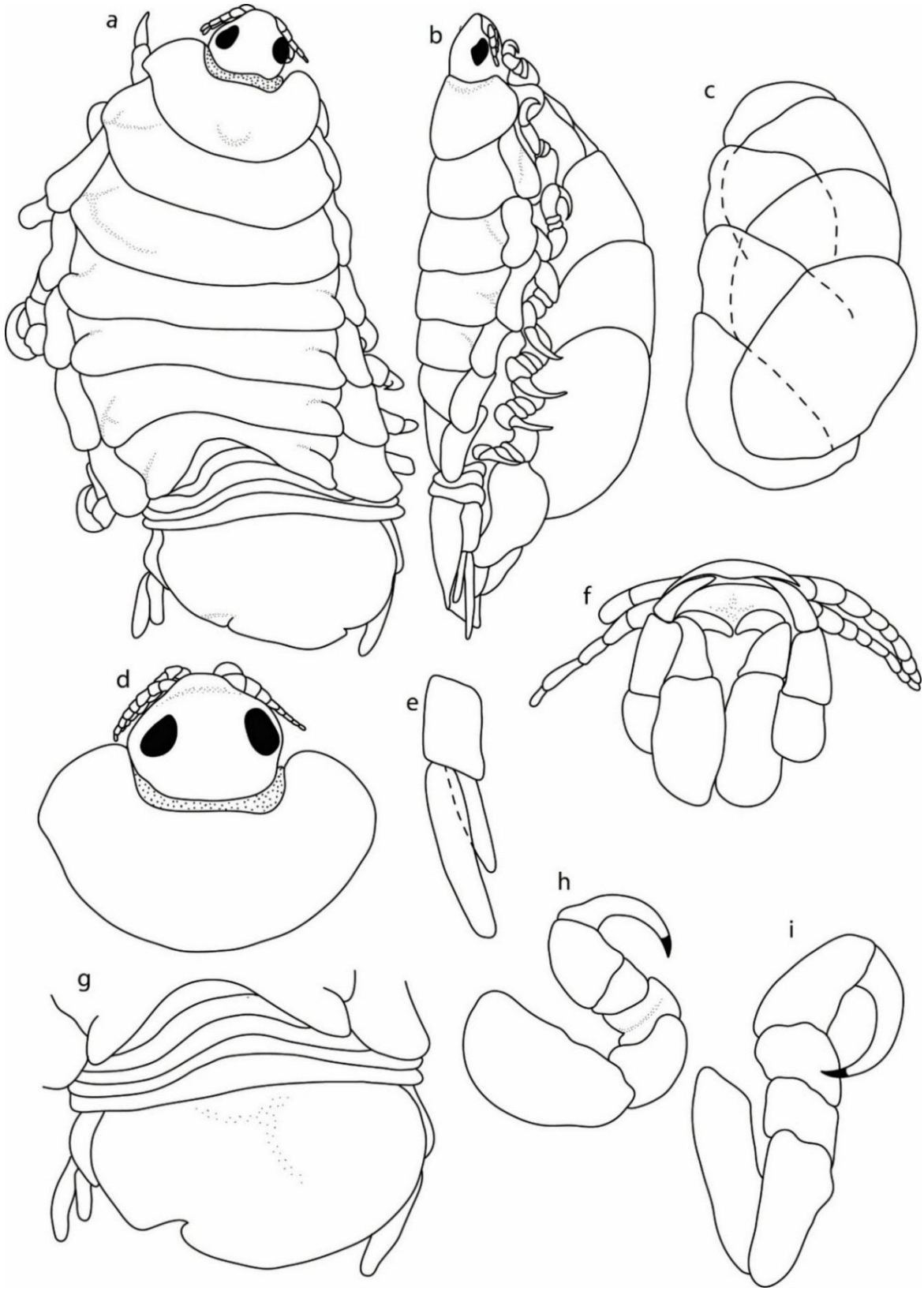

Figure 5.10: *Mothocya* sp.1 holotype ♀ (ovigerous, 15.0 mm TL, 8.0 mm W) from Andoni Creek, Nigeria. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon and pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Dorsal view of pleon. **h** Pereopod 1. **i** Pereopod 7.

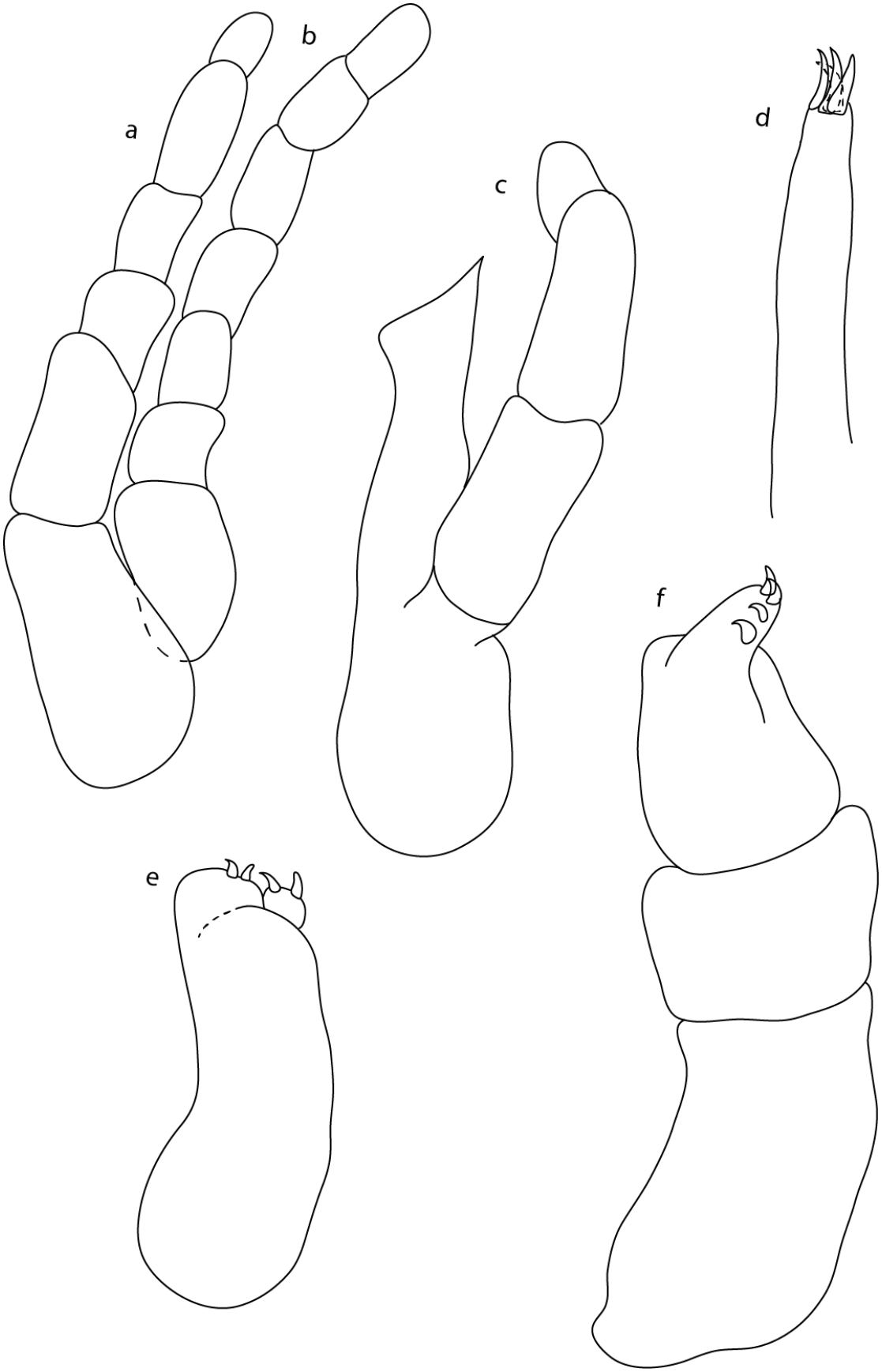

Figure 5.11: *Mothocya* sp. 1 paratype ♀ (non-ovigerous, 18.0 mm TL, 9.0 mm W) from Andoni Creek, Nigeria. **a** Antennula. **b** Antenna. **c** Mandible. **d** Maxillula. **e** Maxilla. **f** Maxilliped.

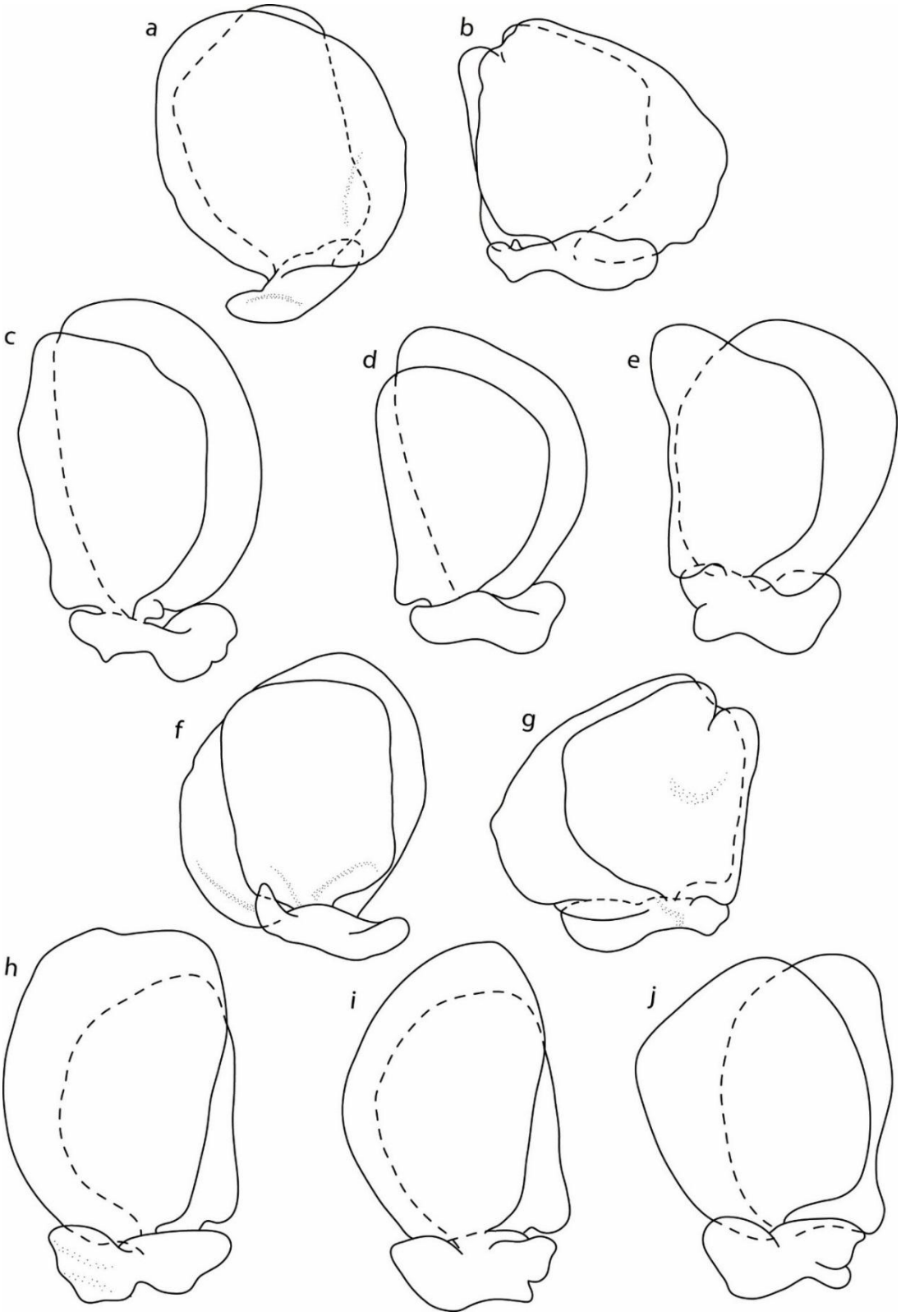

Figure 5.12: *Mothocya* sp. 1 paratype ♀ (non-ovigerous, 18.0 mm TL, 9.0 mm W) from Andoni Creek, Nigeria. **a** Pleopod 1 ventral view. **b** Pleopod 2 ventral view. **c** Pleopod 3 ventral view. **d** Pleopod 4 ventral view. **e** Pleopod 5 ventral view. **f** Pleopod 1 dorsal view. **g** Pleopod 2 dorsal view. **h** Pleopod 3 dorsal view. **i** Pleopod 4 dorsal view. **j** Pleopod 5 dorsal view.

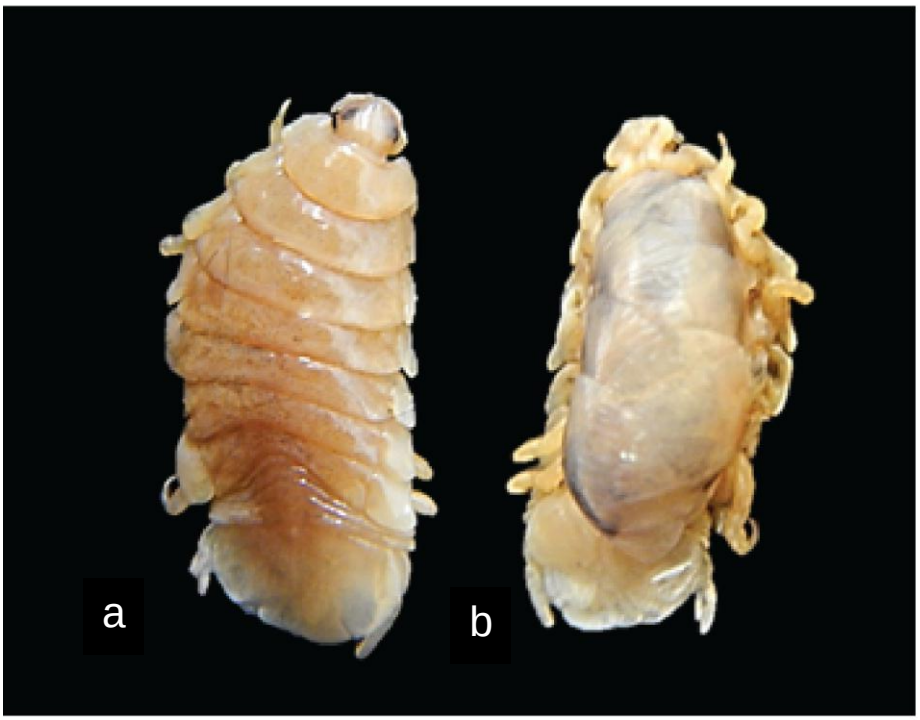

Figure 5.13: Photos of *Mothocya* sp. 1 holotype ♀ (ovigerous, 15.0 mm TL, 8.0 mm W) from Andoni Creek, Nigeria. **a** Dorsal view. **b** Ventral view.

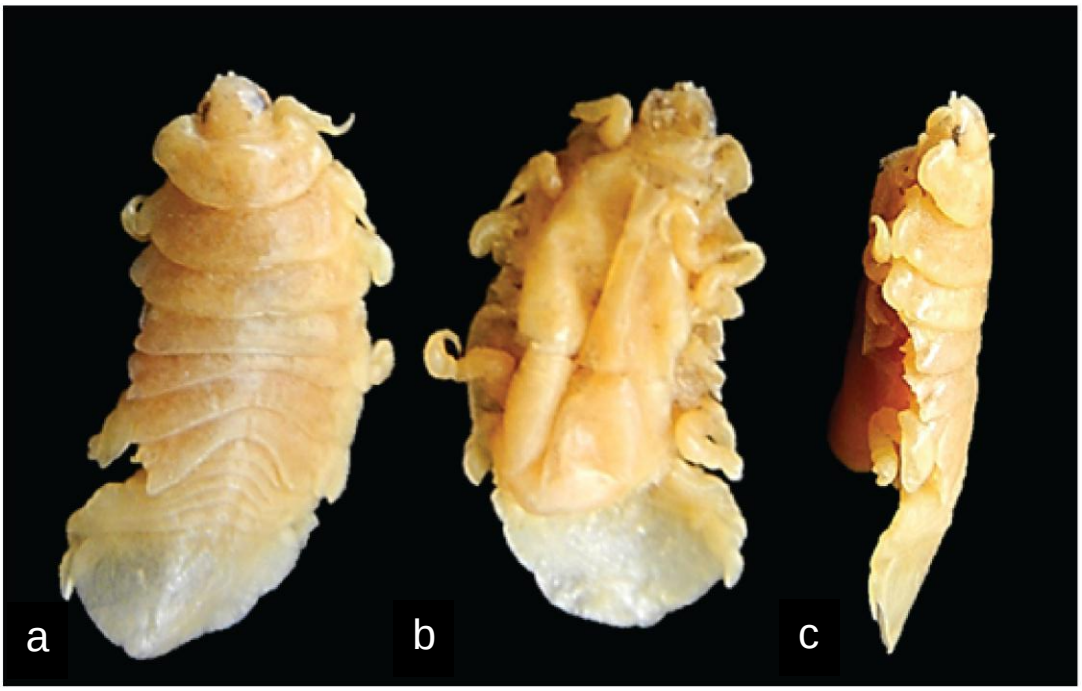

Figure 5.14: Photos of *Mothocya* sp. 1 paratype ♀ (non-ovigerous, 18.0 mm TL, 9.0 mm W) from Andoni Creek, Nigeria. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

***Mothocya* sp. 1 ♂**

Figs. 5.15–5.18

Body rectangular, 2.4 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 3, most narrow at pereonite 7, pereonite lateral margins mostly posteriorly ovate. *Cephalon* sub-triangular, 0.3 times longer than wide; visible from dorsal view, with blunt point. *Eyes* oval with distinct margins, one eye 0.2 times width of cephalon, 0.3 times length of cephalon. *Pereonite 1* smooth, anterior border medially straight, curved laterally, anteriorly expanded, anterolateral angle wide, with inwardly produced point, extend to posterior margin of eyes. Posterior margins of pereonites smooth and slightly curved laterally, pereonite 7 medially recessed. Coxae 2–3 wide; with posteroventral angles rounded; coxae 4–7 rounded. Coxae 6–7 extending slightly past pereonite posterior margin. Pereonites 1–3 increasing in length and width; 4–7 decreasing in length and width; 4–7 becoming progressively narrower, 2–3 subequal. *Pleon* with pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin smooth, straight. Pleonite 2 partially overlapped by pereonite 7, posterior margin straight. *Pleotelson* 0.7 times as long as anterior width, dorsal surface smooth. Pleotelson lateral margins weakly convex, posterior margin converging to slight caudomedial point, evenly rounded.

Antennula more stout than antenna, consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 1.6 times as long as article 1; article 3 1.6 times as long as wide, 0.6 times as long as combined lengths of articles 1 and 2; antennule flagellum with 4 articles, without setae. *Antenna* consists of 8 articles; peduncle article 3 1.9 times as long as article 2; article 4 1.8 times as long as wide. 0.9 times as long as article 3; article 5 1.2 times as long as wide, 0.7 times as long as article 4; terminal article terminating without setae. Mandible palp article 2 with 3 distolateral setae. *Maxillula* simple with 4 terminal robust setae; lateral lobe with 2 recurved robust setae; mesial lobe with 2 large recurved robust setae. *Maxilliped* consists of 3 articles, article 3 with 4 recurved robust setae.

Pereopod 1 basis 2.1 times as long as greatest width; ischium 0.5 times as long as basis; propodus 1.9 times as long as wide; dactylus slender, 1.2 times as long as propodus, 3.8 times as long as basal width. Pereopod 7 basis without carina. *Pereopod 7* basis 2.4 times as long as greatest width; ischium 0.6 times as long as basis, without protrusions; merus 0.9 times as long as wide, 0.6 times as long as ischium; carpus 0.9 times as long as wide, 0.6 times as long as ischium; as long as ischium; dactylus slender, 1.3 times as long as propodus, 3.4 times as long as basal width.

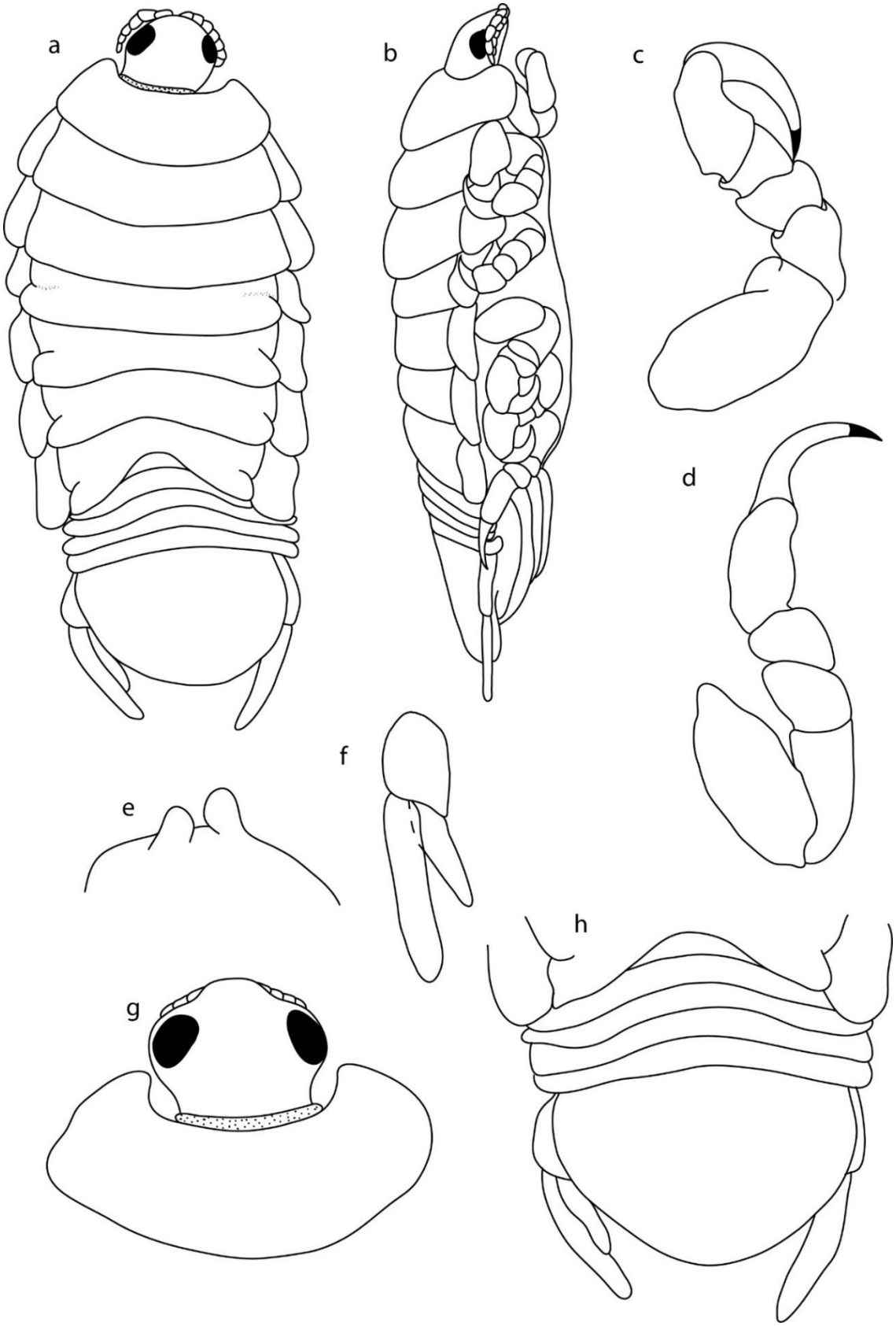

Figure 5.15: *Mothocya* sp. 1 ♂ (12.0 mm TL, 5.0 mm W) from Andoni Creek, Nigeria. **a** Dorsal body. **b** Lateral body. **c** Pereopod 1. **d** Pereopod 7. **e** Penes. **f** Uropod. **g** Dorsal view of cephalon with pereonite 1. **h** Dorsal view of pleon.

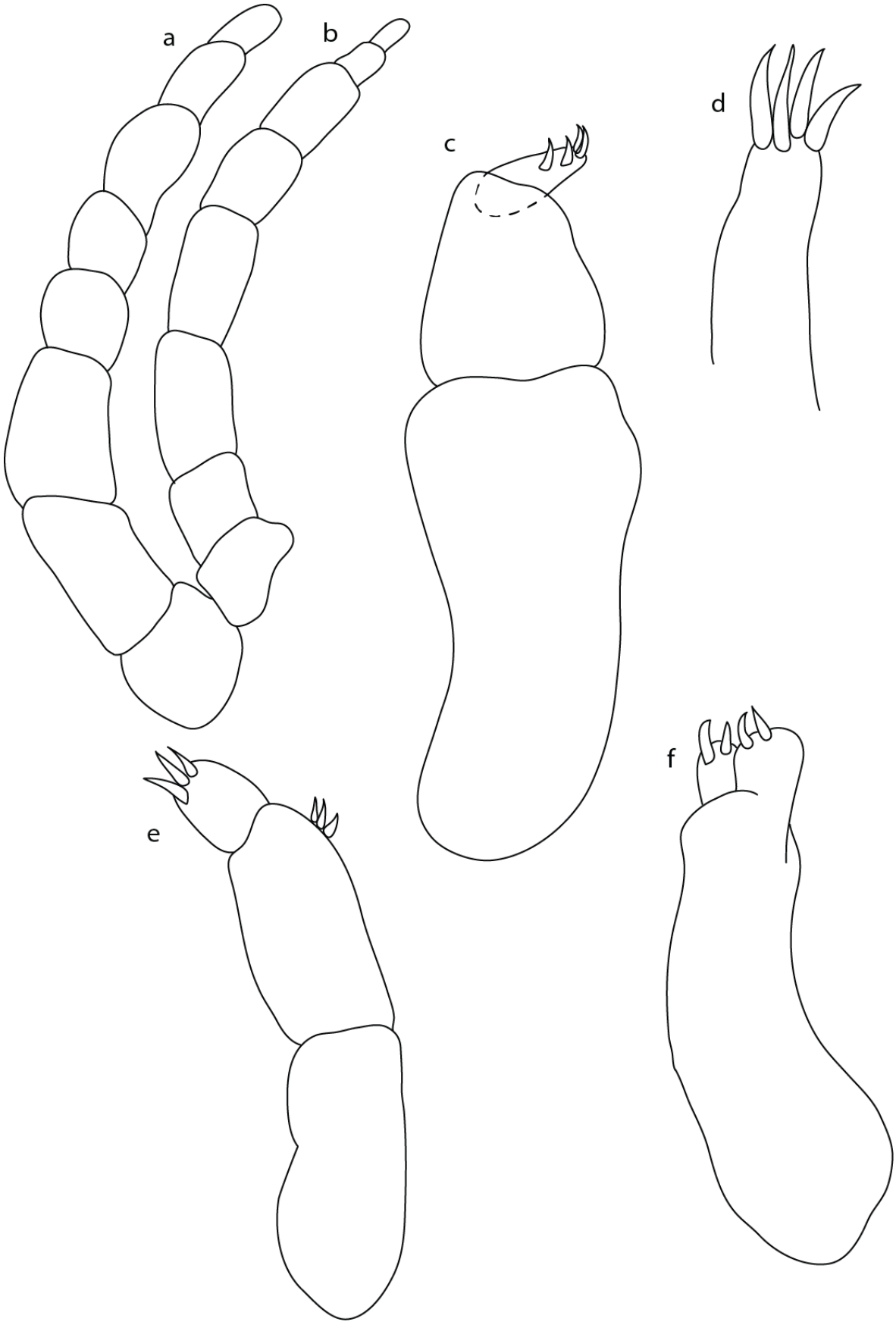

Figure 5.16: *Mothocya* sp. 1 ♂ (12.0 mm TL, 5.0 mm W) from Andoni Creek, Nigeria. **a** Antennula. **b** Antenna. **c** Maxilliped. **d** Tip of maxillula. **e** Mandible palp. **f** Maxilla.

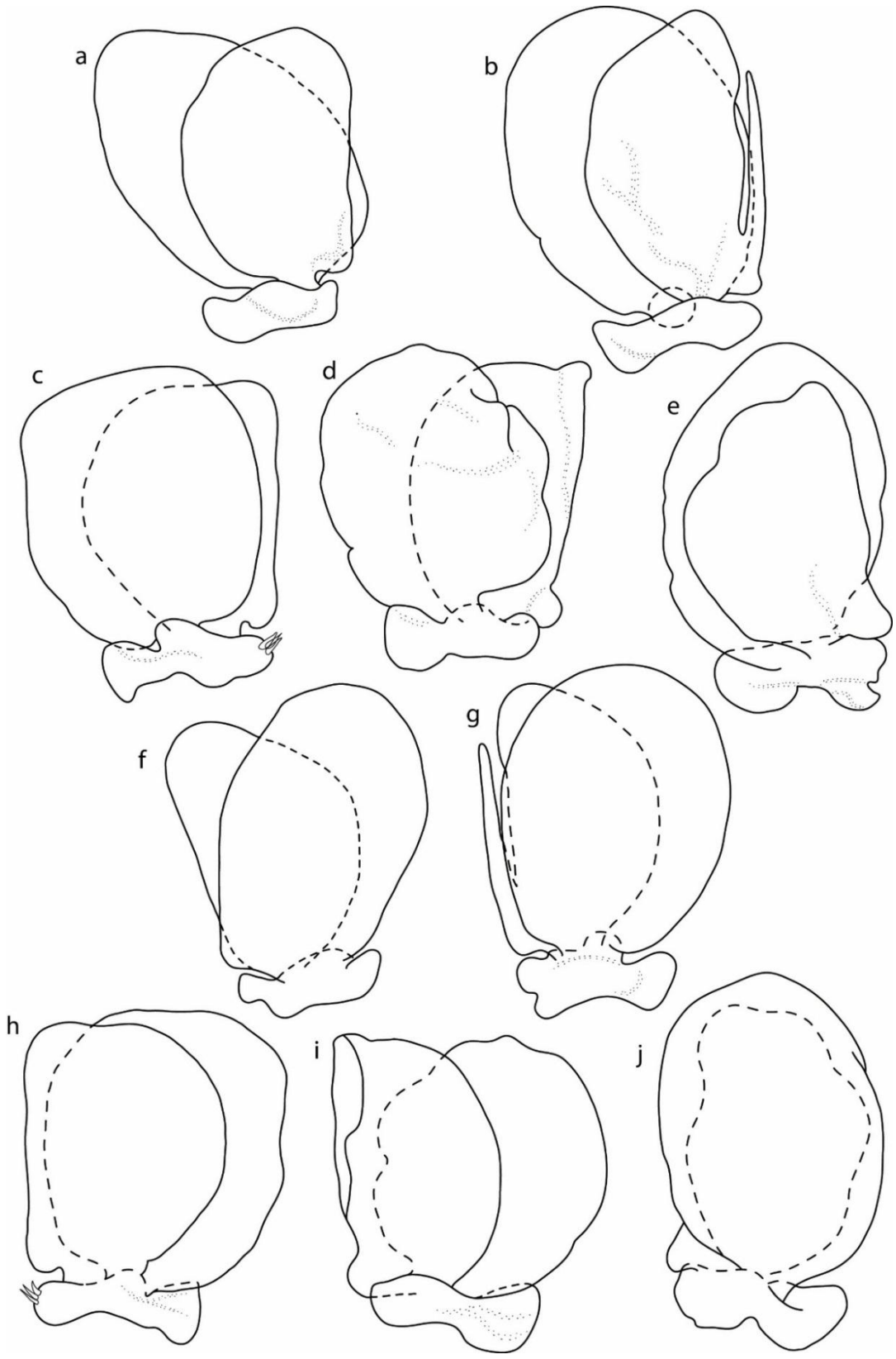

Figure 5.17: *Mothocya* sp. 1 ♂ (12.0 mm TL, 5.0 mm W) from Andoni Creek, Nigeria. **a** Pleopod 1 ventral view. **b** Pleopod 2 ventral view. **c** Pleopod 3 ventral view. **d** Pleopod 4 ventral view. **e** Pleopod 5 ventral view. **f** Pleopod 1 dorsal view. **g** Pleopod 2 dorsal view. **h** Pleopod 3 dorsal view. **i** Pleopod 4 dorsal view. **j** Pleopod 5 dorsal view.

Pleopods simple, with setae on peduncle of pleopod 3; exopod larger than endopod. *Pleopod 1* exopod 1.4 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin weakly convex. *Endopod* 1.6 times as long as wide, lateral margin weakly convex, distally broadly rounded, mesial margin straight, peduncle 2.8 times as wide as long. Pleopod 2 appendix masculina with parallel margins, 0.8 times as long as endopod, distally narrowly rounded. Pleopods 1–5 proximomedial lobes increasing in size, with proximomedial lobes. Peduncle lobes increasing in size from pleopod 2 to 5.

Uropod longer than the pleotelson, peduncle 0.6 times longer than rami, peduncle lateral margin without setae; rami extending beyond pleotelson, marginal setae absent. *Endopod* 3.3 times as long as greatest width, lateral margin straight, mesial margin weakly convex, terminating without setae. *Exopod* extending beyond end of endopod, 5.4 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae.

Penes 1.2 times as long as basal width.

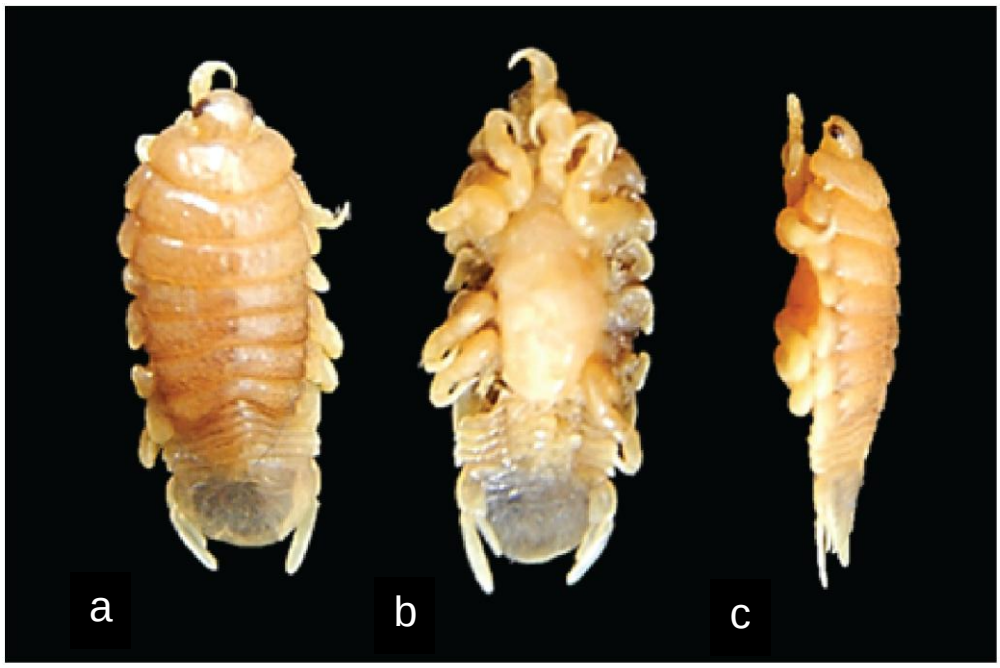

Figure 5.18: Photos of *Mothocya* sp. 1 ♂ (12.0 mm TL, 5.0 mm W) from Andoni Creek, Nigeria. **a** Dorsal view. **b** Ventral view. **c** Lateral view.

5.6.3 Remarks

No significant intra-specific variation was noted among the ovigerous and non-ovigerous females of *Mothocya* sp. 1, except for the obvious lack of brood pouch from the non-ovigerous female.

Mothocya sp. 1 can be characterised by particularly large and wide coxae 7; sub-triangular shaped cephalon; pereonite 1 anterior margin expanded with connective tissue and slightly produced medially, with anterolateral angles only reaching to the posterior margin of eyes; and pereopod 7 has a narrow basis without carina; mouthparts positioned near anterior margin of ventral cephalon. It was also noted that pleonite lateral margins expanded past lateral margins of coxae 7 and that the uropods on the side of the body twist are smaller than the opposite uropods. This may be due to the positioning of the species in the host branchial cavity, causing the uropod on one side to have growth restrictions. *Mothocya* sp. 1 paratype was fragile with damaged oostegites and uropods on the right side, which could not be illustrated.

Mothocya omidaptria Bruce, 1986 and *Mothocya longicopa* Bruce, 1986 closely resemble *Mothocya* sp. 1. *Mothocya omidaptria* is known from Rio de Janero, Brazil and Curaçao, West Indies from *Hyporhamphus unifasciatus* (Ranzani, 1841), a Beloniform host. It also has the expanded pereonite 1 anterior margin with visible connective tissue as well as a similar body and cephalon shape. *Mothocya* sp. 1 can be distinguished from *Mothocya omidaptria* in having wider coxae 7 with more rounded posterior margins; shorter uropodal rami; and different pleon morphology, especially regarding the shape of the pleotelson.

Mothocya longicopa is limited in distribution to western Africa, particularly from the Gulf of Guinea, but is specific to hosts *Ablennes hians* (Valenciennes, 1846) and *Tylosurus crocodilus crocodilus* (Péron & Lesueur, 1821), both from the order Beloniformes. *Mothocya* sp. 1 can be distinguished from *Mothocya longicopa* by the larger body length to width ratio (narrower body); expanded pereonite 1 anterior margin with cephalon more anteriorly produced; wider coxae 7; shorter uropodal rami; and male specimen without the characteristically large proxomedial lobe on pleonite 5 peduncle. No substantial variation was noted between the examined ovigerous female holotype and non-ovigerous female paratype other than the obvious large brood pouch of the ovigerous specimen.

5.7 *Mothocya* sp. 2

5.7.1 Material examined

A single ♀ (ovigerous, 7.0 mm TL, 5.0 mm W) collected at Birakiki, seaward end of Hughes Channel, Bonny, Nigeria during December, 1985. Collected and donated by CB Powell and B Olaosebikan. No host was recorded. This singular specimen was fragile and dried out at the time of examination, and was therefore not subjected to dissection.

5.7.2 Descriptions

***Mothocya* sp. 2 holotype ♀**

Figs. 5.19–5.20

Body ovoid, slightly twisted to the right, 1.4 times as long as greatest width. Body dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate, medially indented. Cephalon subtriangular, 0.7 times longer than wide, visible from dorsal view, immersed in pereonite 1. Frontal margin thickened, ventrally folded. *Eyes* oval with distinct margins; one eye 0.3 times width of cephalon, 0.3 times length of cephalon. *Pereonite 1* with slight indentations, anterior border straight, anterolateral angles broadly rounded, extend past the medial region of eyes. Posterior margins of pereonites mostly straight, smooth, pereonite 7 medially recessed. Coxae 2–3 wide; with posteroventral angles right-angled; 4–7 rounded, large and produced. Coxae not extending past pereonite posterior margins. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width and narrower; 3–5 subequal. Pleon 0.4 times as long as total body length, with pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view; pleonites posterior margin concave; pleonite 5 posterior margin straight. Pleonite 2 partially overlapped by pereonite 7. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 widest, lateral margins overlapped by pereonite 7, posterior margin straight. *Pleotelson* triangular, 0.7 times as long as anterior width, dorsal surface smooth, with anteromedial furrow. Pleotelson lateral margins straight, posterior margin converging to caudomedial point.

Antennula more stout than antenna, consisting of 5 articles; antennula peduncle articles 1 and 2 distinct and articulated; extending to middle of eye. Antenna consisting of 5 articles, extending to middle of the eye.

Pereopod 1 basis 1.8 times as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.4 times as long as wide.

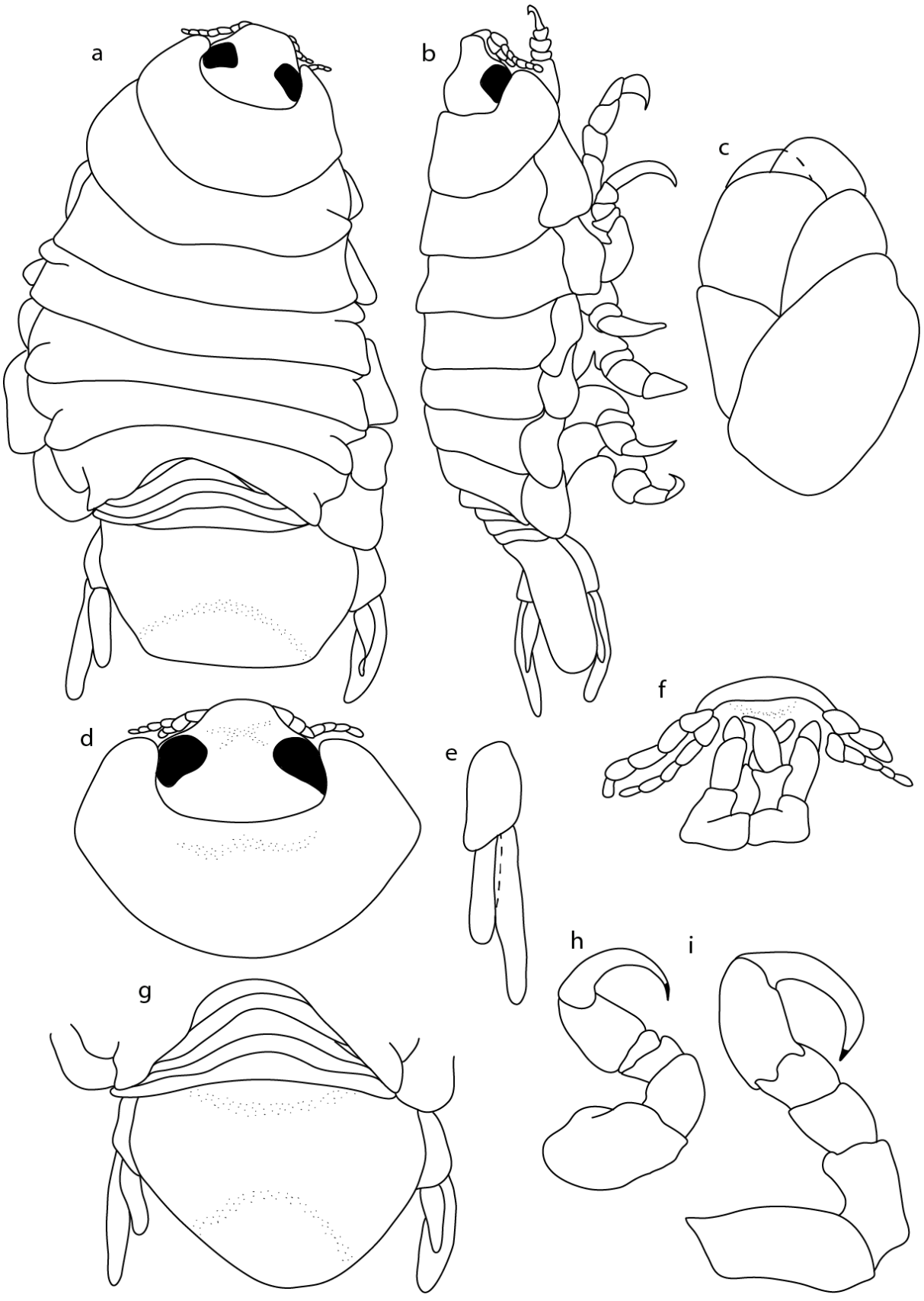

Figure 5.19: *Mothocya* sp. 2 holotype ♀ (ovigerous, 7.0 mm TL, 5.0 mm W) from Birakiki, Nigeria. **a** Dorsal body. **b** Lateral body. **c** Oostegites. **d** Dorsal view of cephalon with pereonite 1. **e** Uropod. **f** Ventral cephalon. **g** Dorsal view of pleon. **h** Pereopod 1. **i** Pereopod 7.

Dactylus slender, 1.7 times as long as propodus, 3.5 times as long as basal width. *Pereopod 7* basis without carina, basis 2.2 times as long as greatest width; ischium 0.7 times as long as basis, with slight bulbous protrusion; merus proximal margin without bulbous protrusion, 0.9 times as long as wide, 0.5 times as long as ischium; carpus 0.9 times as long as wide, 0.4 as long as ischium, without bulbous protrusion; propodus 2.1 times as long as wide, as long as ischium; dactylus slender, 1.2 times as long as propodus, 3 times as long as basal width.

Uropod same length as pleotelson, peduncle 0.6 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, apices broadly rounded. *Endopod* 3.6 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae. *Exopod* extending beyond end of endopod, 6.6 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae.

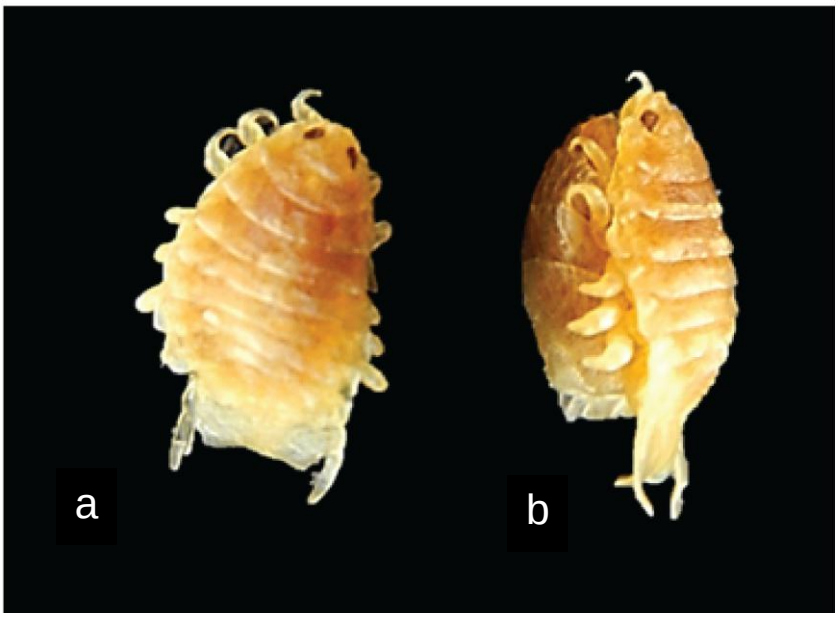

Figure 5.20: Photos of *Mothocya* sp. 2 holotype ♀ (ovigerous, 7.0 mm TL, 5.0 mm W) from Birakiki, Nigeria. **a** Dorsal view. **b** Lateral view.

5.7.3 Remarks

Mothocya sp. 2 can be recognised by its posteriorly ovoid body shape; blunt anterior margin of the cephalon; prominent triangular pleotelson; short pleon with all pleonite lateral margins overlapped by pereonite 7; carina on pereopod 7 ischium; and uropods reaching to the posterior margin of pleotelson.

This species can be distinguished from *Mothocya* sp. 1 by the notable difference in size; and mouthparts of *Mothocya* sp. 2 are more posteriorly positioned. *Mothocya* sp. 2 pereonite 1 is

not expanded with visible connective tissue and with a cephalon immersed in pereonite 1, reaching past medial region of eyes. *Mothocya* sp. 2 is posteriorly ovoid, with body widest at posterior end, in contrast to the medially ovoid *Mothocya* sp. 1. *Mothocya* sp. 2 pereonite 7 overlaps the lateral margins of all pleonites, whereas pereonite 7 of *Mothocya* sp. 1 only overlaps the lateral margins of pleonites 3–4. *Mothocya* sp. 2 has a triangular, evenly rounded pleotelson; and the uropod exopod is almost twice as long as the endopod.

5.8 Effect on host – Case study of *Mothocya affinis* Hadfield, Bruce & Smit, 2015 parasitising *Hyporamphus affinis* (Günther, 1866)

5.8.1 Introduction

Branchial cavity inhabiting cymothoids, such as *Norileca, Elthusa* and *Mothocya*, can cause severe damage to the gills, branchial arches and opercula cavity during infection. The degree of damage is often correlated to the size of the parasite and the duration of the infection (Romestand and Trilles 1977a). Parasitism by cymothoids has also been found to induce a decreasing fecundity of some adult fish (Adlard and Lester 1995; Fogelman et al. 2009). Aneesh et al. (2016) reported atrophied gills and deformed branchial cavities, with large depressions as a result of branchial inhabiting cymothoids. The effect that parasites have on their fish host can be quantified or determined by the condition factor; blood parameters such as haematocrit and white blood cell count; as well as the actual morphological damage to structures (Goede and Barton, 1990; Adams et al., 1993).

In 1902, Fulton proposed the use of a mathematical formula to quantify the condition of fish. The analysis of fish condition is a quantitative measure to determine the effects that parasites might have on their fish hosts, and has become a standard procedure in fisheries ecology. This analysis is typically based on the visual assessment of a fish, considering its general shape, length and weight, and its appearance. Using the fish condition index, it is generally accepted that heavier fish of the same given length, are in better condition than those that are lighter in weight (Bolger and Connolly, 1989). Thus, the higher the condition factor, the better the condition of the fish (Barnham and Baxter, 2003). Previous studies have shown that the condition factor of non-parasitised specimens was higher than that of the parasitised fishes (Horton and Okamura, 2001; Gomiero et al., 2012). This condition analysis can be used in association with the growth in body length and the concentration of condition and tissue energy to indicate the general and overall expression of the ‘well-being or fitness’ of a fish population (Jakob et al., 1996).

Many disadvantages and critique are connected with the use of Fulton's condition index and it is recommended that other indices are used in addition to the traditional condition factor. These indices include the hepatosomatic index, the body water content, visceral-somatic index, gut index, the protein to energy ration, RNA to DNA ratios of the liver and muscle tissue, calorific tissue values (protein or lipid portions) as well as partial condition factors (Bolger and Connolly, 1989). These indices are much more informative and extensive, but time consuming processes in comparison to the basic condition index.

Fish health assessments are more suitable, inexpensive and rapid methods that can be used to determine fish condition, even in the field (McHugh et al., 2015). The objective of a FHAI (Fish Health Assessment Index) method is to enable a researcher to conduct a quick and simple assessment, describing the overall health of a collected fish population (Adams et al., 1993). It is based on the observed level of damage and abnormalities on fish extremities and organs as well as the severity thereof. Thus, higher FHAI values are associated with a poorer health (Welicky et al., 2017). FHAI is conducted by considering the following aspects (Goede and Barton, 1990): 1) determining biometric indices, such as hepato-, spleno- and gonado-somatic indices, using measurements and weights; 2) blood constituents, including heamtocrit and leucocrit values; 3) damage to extremities, categorised from normal to abnormal/severe. For the purposes of this study, FHAI was selected as the appropriate method to study and determine the effects of parasitic cymothoid isopods on the overall health of a fish population.

Mothocya affinis Hadfield, Bruce and Smit, 2015 is the most recently described *Mothocya* species, recorded from the tropical halfbeak *Hyporamphus affinis* (Günther, 1866), at Sodwana Bay, South Africa. *Mothocya affinis* can be distinguished by its especially large and wide coxae, with coxae 7 extending over the pleon; uropods that do not extend to the posterior margin of the pleotelson; anterolateral margins of pereonite 1 that reach the anterior margin of eyes; a sub-triangular cephalon; as well as a twisted pleon and subtruncate pleotelson (Hadfield et al., 2015).

5.8.2 Results

A total of 26 *Hyporamphus affinis* (Günther, 1866) (12 female and 14 male), were collected at Sodwana Bay, South Africa, during August 2016. Of these 26 fish, 15 were infected with one or two *M. affinis* branchial cavity inhabiting cymothoids (Figure 5.21). Infected hosts consisted of 63% young females, whereas uninfected hosts consisted of 75% young females.

Tables 6.1 and 6.2 provide the analyses of infected and uninfected *H. affinis* respectively. From the results presented in these tables it is evident that infected fish had a lower mean total body weight and total length than uninfected hosts. The liver, spleen and gonad weights were used to calculate various somatic indices. One male's testis could not be measured as they were tangled and unable to process without damaging the organ.

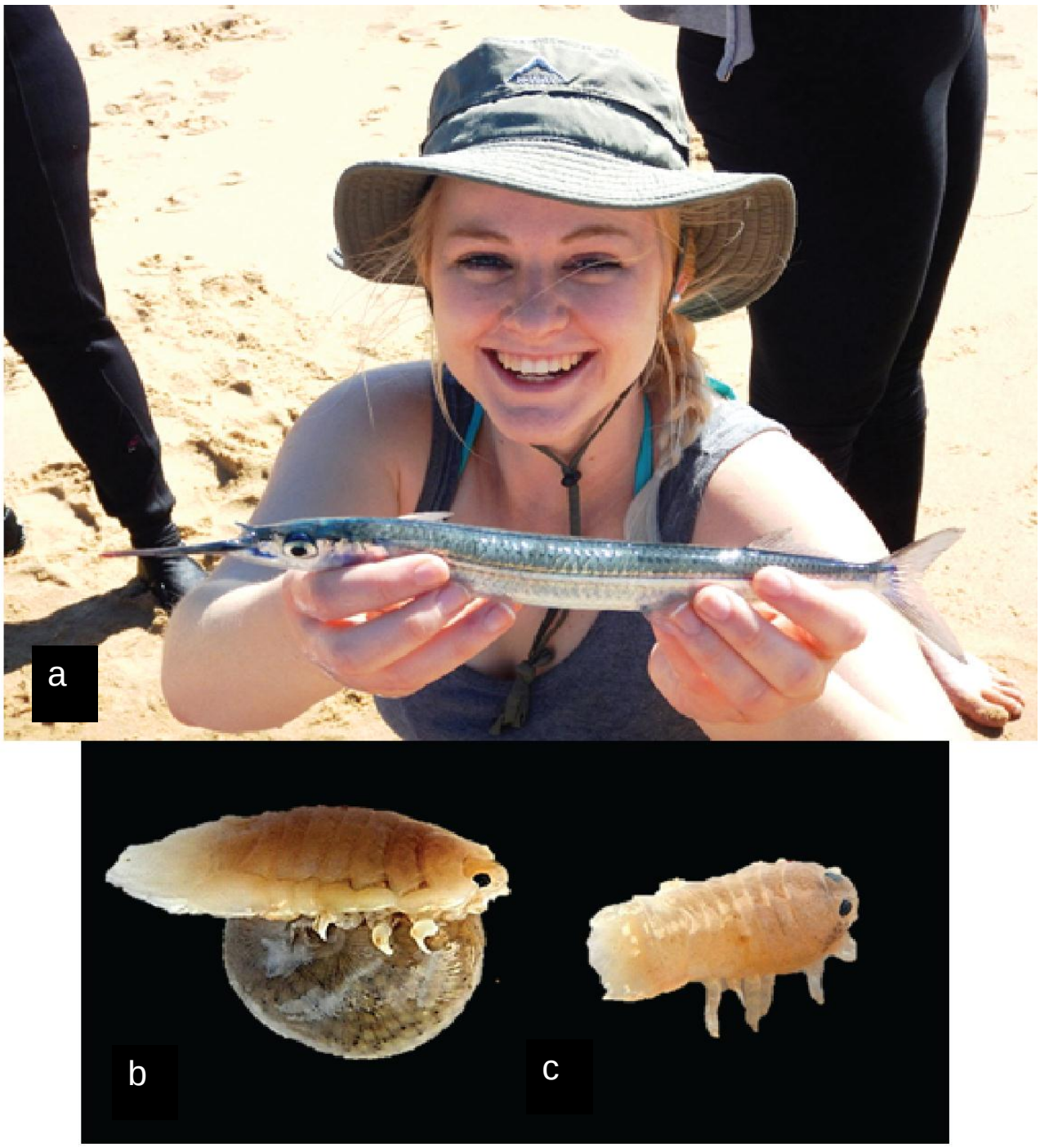

Figure 5.21: **a** Collection of fish host *Hyporamphus affinis* (Günther, 1866) at Sodwana Bay, South Africa. **b** *Mothocya affinis* Hadfield, Bruce & Smit, 2015 female (ovigerous, 13.0 mm TL, 7.0 mm W) collected from *Hyporamphus affinis*. **c** *Mothocya affinis* Hadfield, Bruce & Smit, 2015 male (8.0 mm TL, 4.0 mm W).

Table 5.3: Body mass, length and organ mass of infected tropical halfbeak *Hyporhamphus affinis* (Günther, 1866) collected at Sodwana Bay. Host sex (j = juvenile); body mass (g); body length (mm) for total length (TL), fork length (FL) and standard length (SL); liver mass (g); spleen mass (g); gonad mass (g) and testis length (mm) are provided. Mean values and standard deviations (STDEVA) are included.

Fish #	Sex	Body Mass (g)		Body lengths (mm)			Liver Mass (g)	Spleen Mass (g)	Gonad Mass (g)			Testis length (mm)	
		Total	Gutted	TL	FL	SL			Left	Right	Total	Left	Right
1	Male	78.42	67.10	315.00	299.00	294.00	0.73	0.11	0.37	0.42	0.79	65.00	70.00
3	Male	76.29	63.76	315.00	295.00	288.00	0.94	0.08	0.05	0.05	0.10	54.00	60.00
5	Male	73.81	63.86	310.00	285.00	275.00	0.93	0.09	0.10	0.11	0.21	Tangled	
7	Female (j)	34.61	30.29	257.00	240.00	234.00	0.59	0.03	0.00	0.01	0.01	N/A	
9	Male	71.99	62.04	301.00	28.00	272.00	1.23	0.10	0.19	0.20	0.39	60.00	55.00
10	Male	44.04	38.05	268.00	254.00	245.00	0.46	0.07	0.04	0.05	0.09	42.00	46.00
11	Female (j)	42.99	36.39	255.00	239.00	230.00	0.49	0.07	0.04	0.04	0.08	N/A	
13	Male	77.88	66.54	318.00	290.00	280.00	1.45	0.15	0.30	0.27	0.57	75.00	68.00
14	Female	76.30	70.61	318.00	290.00	280.00	1.57	0.10	0.29	0.27	0.56	N/A	
16	Female	71.05	64.70	310.00	290.00	280.00	1.08	0.09	0.07	0.07	0.14	N/A	
19	Female (j)	40.96	37.00	262.00	250.00	240.00	0.67	0.05	0.06	0.06	0.12	N/A	
20	Female (j)	49.30	45.54	291.00	254.00	250.00	0.81	0.07	0.09	0.07	0.16	N/A	
21	Male	87.17	81.25	320.00	300.00	290.00	1.46	0.10	0.21	0.24	0.45	92.00	89.00
22	Female (j)	49.12	44.75	261.00	242.00	250.00	0.84	0.05	0.02	0.02	0.04	N/A	
23	Female	43.53	41.06	260.00	250.00	240.00	1.03	0.08	0.09	0.08	0.17	N/A	
Average		61.16	54.19	290.73	253.73	263.20	0.95	0.08	0.13	0.13	0.26	64.67	64.67
STDEVA		17.77	15.72	26.67	66.73	22.45	0.35	0.03	0.12	0.12	0.23	35.37	14.80

Table 5.2: Body mass, length and organ mass of uninfected tropical halfbeak *Hyporhamphus affinis* (Günther, 1866) collected at Sodwana Bay. Host sex (j = juvenile); body mass (g); body length (mm) for total length (TL), fork length (FL) and standard length (SL); liver mass (g); spleen mass (g); gonad mass (g) and testis length (mm) are provided. Mean values and standard deviations (STDEVA) are included.

Fish #	Sex	Body Mass (g)		Body lengths (mm)			Liver Mass (g)	Spleen Mass (g)	Gonad Mass (g)			Testis length (mm)	
		Total	Gutted	Total	Fork	SL			Left	Right	Total	Left	Right
2	Male	99.30	84.05	330.00	298.00	310.00	2.01	0.11	0.36	0.31	0.67	85.00	78.00
4	Male	98.72	78.91	325.00	302.00	295.00	1.31	0.11	0.31	0.27	0.58	70.00	75.00
6	Female	92.22	74.93	317.00	310.00	300.00	1.47	0.12	0.63	0.63	1.26	N/A	
8	Female (j)	56.63	38.15	273.00	260.00	252.00	1.00	0.08	0.10	0.08	0.18	N/A	
12	Male	44.55	38.49	262.00	244.00	236.00	0.88	0.01	0.05	0.04	0.09	43.00	40.00
15	Female (j)	114.63	106.08	360.00	330.00	320.00	1.51	0.11	0.45	0.42	0.87	N/A	
17	Male	71.40	66.43	325.00	308.00	295.00	1.08	0.07	0.20	0.18	0.38	75.00	62.00
18	Male	91.85	84.61	325.00	308.00	300.00	1.26	0.10	0.15	0.15	0.30	68.00	66.00
24	Female (j)	39.79	37.77	255.00	245.00	230.00	0.94	0.04	0.08	0.08	0.16	N/A	
25	Male	53.01	49.98	285.00	270.00	258.00	0.90	0.07	0.07	0.09	0.16	40.00	38.00
26	Male	42.94	40.51	265.00	240.00	234.00	0.62	0.12	0.07	0.07	0.14	38.00	36.00
Average		73.19	63.63	302.00	283.18	275.45	1.18	0.08	0.23	0.21	0.44	59.86	56.43
STDEVA		27.01	23.87	35.03	32.07	33.68	0.39	0.04	0.19	0.18	0.37	32.85	18.07

5.8.3 Fish health assessment index (FHAI) and biometric indices

Results from the host external examination and fish health assessment index (FHAI) variables are summarised in Table 5.3. The values assigned to each variable from infected and uninfected hosts are presented in Appendix C, Tables C1–C2. The hindgut, kidney and skin examination columns were excluded as all of the hosts (infected and uninfected) portrayed normal values. Three host specimens showed signs of haemorrhaging in the eye, which was not a cause of host containment during transportation. Seven fish had moderately frayed fins and one had moderately / severe frayed fins. Less than normal gills were exhibited by 66.6% of infected hosts (frayed or discoloured / pale), while only 36.4% of uninfected fish had minor fraying or discolouration (Figure 5.22). The gills of three infected hosts were particularly abnormal, displaying signs of fraying as well as discolouration. After removing the cymothoid parasites, a pit-like depression was visible at the site where the brood pouch of an ovigerous female was positioned (Figure 5.23).

Most hosts had normal opercula, except in two cases where the opercula were shortened / damaged due to the positioning of *M. affinis* in the branchial cavity, taking up a large amount of space. This can be seen as a gap of approximately 0.5 mm between the posterior margin of the opercula and the host body, exposing the interior part of the branchial cavity. The bile colour varied between light and dark green, with most fish having less than 50% mesenteric fat. Three female fish had no mesenteric fat, whereas two males had 100% mesenteric fat visible. All of the fish hosts had a slightly discoloured liver, and one specimen's liver was particularly fatty. Spleens all appeared normal except one that was slightly discoloured (dark brown).

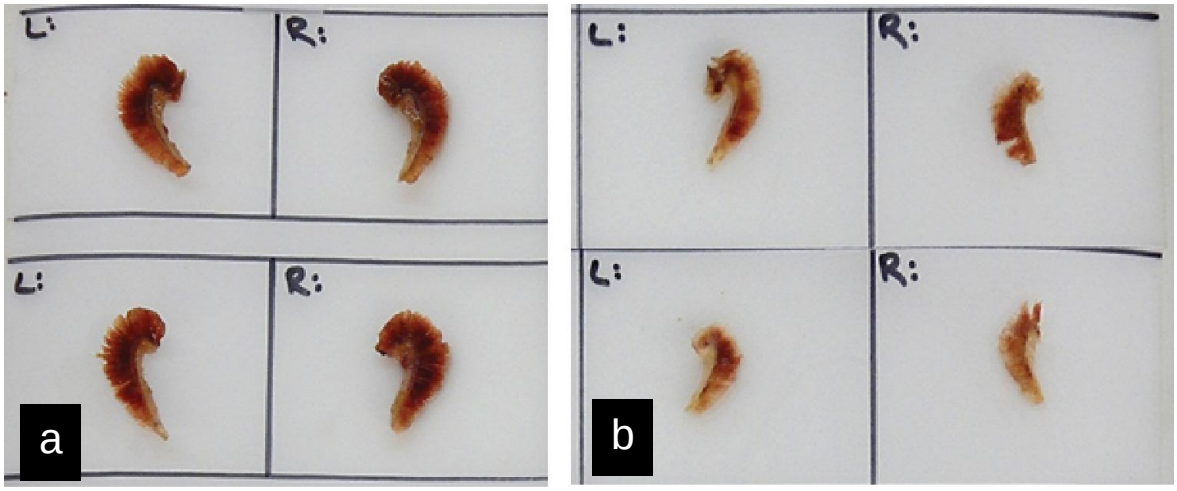

Figure 5.22 Examples of first and second gill arches from host fish *Hyporhamphus affinis* (Günther, 1866). **a** Normal, healthy gills from an uninfected host. **b** Frayed and discoloured gills from an infected host.

Table 5.3: Fish Health Assessment Index (FHAI) variables for the external examination of a population of *Hyporhamphus affinis* (Günther, 1866) at Sodwana Bay, South Africa. D= dark, L= light, j = juvenile.

Fish #	Sex	Eyes	Fins	Gills	Opercula	Bile	Mesenteric Fat	Liver	Spleen	*Mothocya affinis*	
										Left cavity	Right cavity
1	Male	Normal	Moderately frayed	Frayed	Normal	D Green	50%	Discoloured	Normal	Ovigerous female	Male
2	Male	Normal	Moderately frayed	Frayed	Normal	D Green	> 50%	Discoloured	Normal	Uninfected	
3	Male	Normal	Normal	Frayed/ discoloured	Normal	L Green	< 50%	Discoloured	Normal		Male
4	Male	Normal	Moderately frayed	Normal	Normal	L Green	> 50%	Discoloured / Fatty	Normal	Uninfected	
5	Male	Normal	Normal	Normal	Normal	L Green	< 50%	Discoloured	Normal	Ovigerous female	Male
6	Female	Normal	Normal	Normal	Normal	L Green	< 50%	Discoloured	Normal	Uninfected	
7	Female (j)	Normal	Normal	Frayed/ discoloured	Normal	L Green	< 50%	Discoloured	Normal	Male	Non-ovigerous ♀
8	Female (j)	Normal	Moderately / severely frayed	Normal	Normal	D Green	50%	Discoloured	Normal	Uninfected	
9	Male	Normal	Moderately frayed	Normal	Normal	L Green	< 50%	Discoloured	Normal		Non-ovigerous ♀
10	Male	Haemorrhaging	Moderately frayed	Frayed	Normal	D Green	< 50%	Discoloured	Normal	Ovigerous female	Male
11	Female (j)	Normal	Normal	Frayed	Normal	D Green	< 50%	Discoloured	Normal	Ovigerous female	Male
12	Male	Normal	Normal	Discoloured	Normal	D Green	< 50%	Discoloured	Normal	Uninfected	
13	Male	Normal	Moderately frayed	Discoloured	Damaged	L Green	< 50%	Discoloured	Normal	Ovigerous female	Male
14	Female	Normal	Normal	Normal	Normal	L Green	< 50%	Discoloured	Normal	Ovigerous female	Male
15	Female (j)	Normal	Normal	Discoloured	Normal	D Green	50%	Discoloured	Discolouration	Uninfected	
16	Female	Normal	Normal	Normal	Normal	L Green	> 50%	Discoloured	Normal	Non-ovigerous female	Male
17	Male	Normal	Moderately frayed	Normal	Normal	L Green	100%	Discoloured	Normal	Uninfected	
18	Male	Normal	Normal	Discoloured	Normal	D Green	100%	Discoloured	Normal	Uninfected	
19	Female (j)	Normal	Normal	Discoloured	Damaged	D Green	None	Discoloured	Normal	Male	Ovigerous female
20	Female (j)	Normal	Normal	Normal	Normal	L Green	None	Discoloured	Normal	Ovigerous female	Male
21	Male	Normal	Normal	Frayed	Normal	L Green	< 50%	Discoloured	Normal	Male	Ovigerous female
22	Female (j)	Normal	Normal	Frayed/ discoloured	Normal	D Green	< 50%	Discoloured	Normal	Male	Ovigerous female
23	Female	Normal	Normal	Discoloured	Normal	L Green	None	Discoloured	Normal	Ovigerous female	Male
24	Female (j)	Normal	Normal	Normal	Normal	L Green	< 50%	Discoloured	Normal	Uninfected	
25	Male	Haemorrhaging	Normal	Normal	Normal	L Green	50%	Discoloured	Normal	Uninfected	
26	Male	Haemorrhaging	Normal	Normal	Normal	L Green	< 50%	Discoloured	Normal	Uninfected	

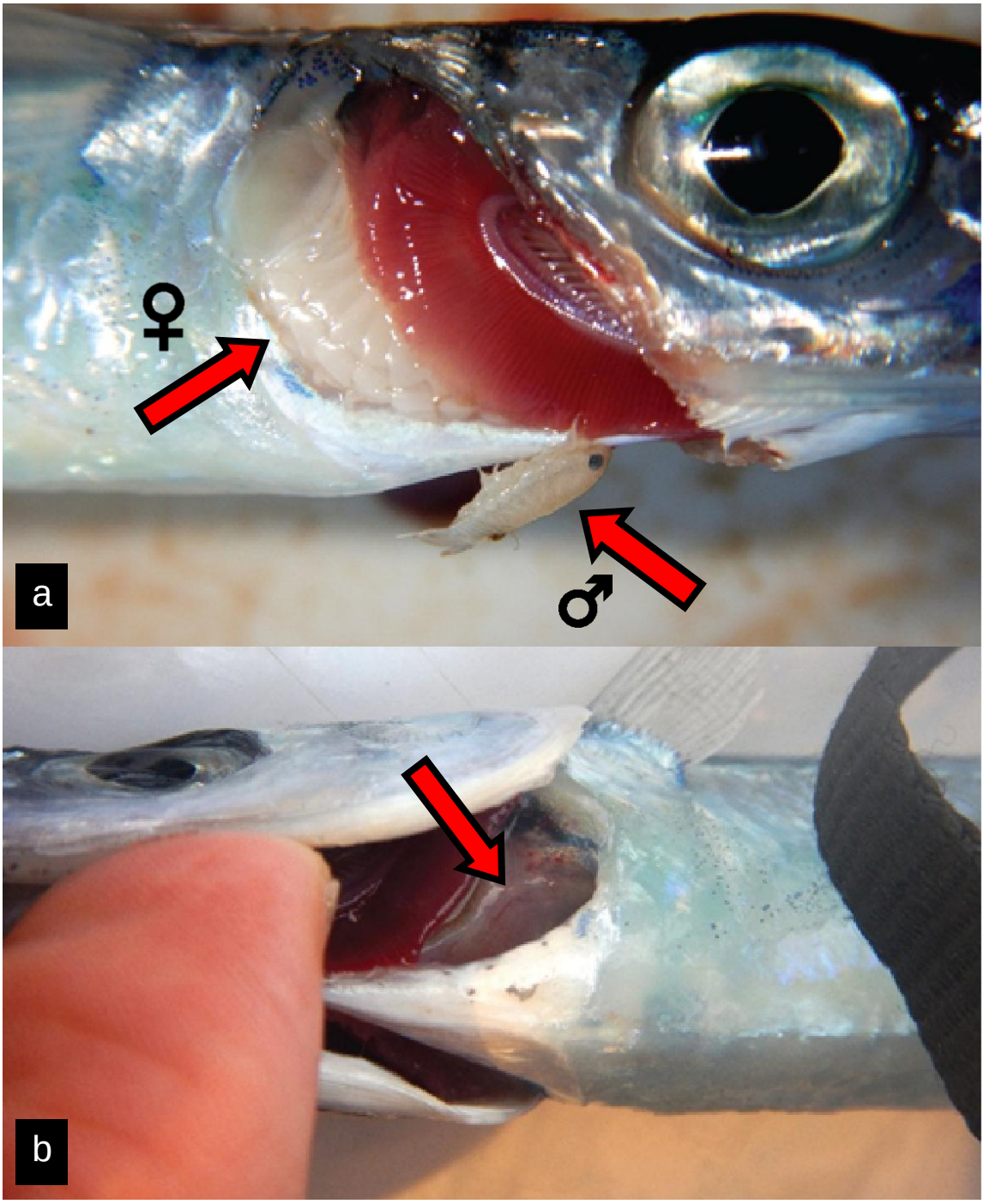

Figure 5.23: Fish host *Hyporhamphus affinis* (Günther, 1866) infected with *Mothocya affinis* Hadfield, Bruce & Smit, 2015. **a** Attachment position of *M. affinis* ovigerous female in host branchial cavity, with a male in the process of detaching and abandoning the host, visible on the host ventral surface. Arrows pointing toward male (♂) and female (♀) cymothoids. **b** Pit-like depression developed in the branchial cavity of the host due to cymothoid attachment.

The following tables represent a summary of the mean condition factors, biometric indices percentages and the determined FHAI values of infected versus uninfected fish hosts. For the raw data of these calculations, see Appendix C, Tables C3–C6.

Table 5.4: Mean body mass, total body length (TL), condition factor (CF), Hepatosomatic index (HSI), Splenosomatic index (SSI), Gonadosomatic index (GSI) and fish health assessment index (FHAI) from uninfected *Hyporhamphus affinis* (Günther, 1866) from Sodwana Bay, South Africa.

Host species	Mass (g)	TL (mm)	CF (kg m^{-3})	HSI (%)	SSI (%)	GSI (%)	FHAI
Hyporhamphus affinis (Günther, 1866) (n = 11)	73.19 ± 27.01	302.00 ± 35.03	2.55 ± 0.27	1.93 ± 0.44	0.14 ± 0.07	0.61 ± 0.40	29.09 ± 13.75

Table 5.4: Mean body mass, total body length (TL), condition factor (CF), Hepatosomatic index (HSI), Splenosomatic index (SSI), Gonadosomatic index (GSI) and fish health assessment index (FHAI) from infected *Hyporhamphus affinis* (Günther, 1866) from Sodwana Bay, South Africa.

Host species	Mass (g)	TL (mm)	CF (kg m^{-3})	HSI (%)	SSI (%)	GSI (%)	FHAI
Hyporhamphus affinis (Günther, 1866) (n = 15)	61.16 ± 17.77	290.73 ± 26.67	2.44 ± 0.22	1.76 ± 0.39	0.15 ± 0.04	0.42 ± 0.32	54.00 ± 15.02

5.8.4 Discussion

During this case study, 28 branchial cavity inhabiting *M. affinis* cymothoid parasites were collected. They consisted of 11 ovigerous females, 3 non-ovigerous females and 14 males. The cymothoids did not exhibit host sex preference, as 58% male hosts were infected, and 57% female hosts were infected. One infected host only had a male cymothoid, while all the rest carried a pair of cymothoids with the female and male in opposite branchial cavities. The cymothoids were all positioned with the brood pouch to the ventral side of the gill cavity and the dorsal surface against the operculum, facing to the anterior end of the host (Figure 5.23a).

Some of the obvious effects of infection with *M. affinis* included a shortened opercula cover, exposing the branchial cavity, which is also evident with the infection of *M. renardi* (see Figure 5.9b); the large depressions in the cavity caused by the positioning of the parasite (Figure 5.23b) and frayed, discoloured gill filaments. These branchial effects were similarly reported by Ravichandran et al. (2011) and Aneesh et al. (2016b).

The HSI and SSI values of infected and uninfected hosts in this study were similar, and had no significant difference (HSI: t= -1.06; df=24; p=0.299; SSI: t= +0.67; df=24; p=0.509), suggesting that these parasites do no influence liver or spleen condition. The FHAI values of infected hosts were higher than that of the uninfected hosts; although not significant, it indicates a poorer overall health of infected host fish. The calculated GSI of uninfected hosts was higher than that of infected hosts (see Tables 5.4–5.5), but this was also not significant (GSI: t= -1.35; df= 24; p=0.189). Separate calculations proved that male hosts (infected and uninfected), produced the same GSI.

Adult cymothoids, especially the buccal attaching genera, including Ce*ratothoa oestroides* (Risso, 1816) and *Cymothoa borbonica* Schioedte & Meinert, 1884, have proven to hinder or constrain the normal growth pattern and reproduction of fish hosts (Kroger and Guthrie, 1972; Horton and Okamura, 2001; Ravichandran et al., 2009; Parker and Booth, 2013). The average total body length and mass of uninfected fish from this study were greater than that of infected hosts, confirming that cymothoids might be the cause of constraint in growth. The total length and mass differences between infected and uninfected hosts in this study were not significant (TL: t= -0.93; df=24; p=0.361; Mass: t= -1.29; df=24; p=0.216). Landau et al. (1995) reported similar condition results, where there were not significant difference in the size of the bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) infected with the branchial attaching *Livoneca ovalis* (Say, 1818), and those that were uninfected. A non-significant condition factor between uninfected *Trachinotus botla* and those infected by male branchial attaching *C. borbonica,* was also reported by Parker and Booth (2013).

Infected fish from this study all had a lower condition factor than uninfected fish (Tables 5.4–5.5), indicating that *M. affinis* might have a negative impact on fish condition, however this difference was not statistically significant (t= -1.32; df=24; p=0.199). A study done by Bakenhaster et al. (2006) on the effects of the buccal and branchial attaching cymothoid isopod, *Glossobius hemiramphi* Williams & Williams, 1985, on the ballyhoo halfbeak *Hemiramphus brasiliensis* (Linnaeus, 1758), similarly did not provide any significant effect on the host in terms of length-weight condition. Leonardos and Trilles (2003) also reported no significant difference in the condition factor, gonadosomatic and hepatosomatic indices of *M. epimerica* infected and uninfected hosts.

The non-significant condition effect of branchial attaching cymothoids were also confirmed by Bello et al (1997), where *M. epimerica* infected and uninfected big-scale sand smelt, *Atherina boyeri* Risso, 1810, portrayed non-significant differences. The degree of impact on the host seems to correspond with the size and age of the host fish (Bakenhaster et al., 2006), where small or juvenile fish are prone to more severe negative effects of cymothoid

parasites than larger hosts and adults. Parker and Booth (2013) noted severe and significant growth effects and constraints limited to fish older than one year, whereas the growth of younger fish were unaffected.

Leonardos and Trilles (2003) mention that the lack of negative effects on host condition and health, might be due to the evolutionary development of host-parasite relationships, where the survival of the host is advantageous to the cymothoid parasite. Thus, the survival of the cymothoid parasite is maintained by the biological performance of its host. The negative results on effects from this study may support the view of Williams and Bunkley–Williams (2000), stating that the use of condition factors might not be a sufficient measure of the growth-impairment effects of cymothoids on their hosts. The sensitivity of condition factors may not be adequate to determine negative effects unless the infection is severe or intense. This interpretation may be valid with regards to previous studies on the condition of infected and uninfected hosts, where some provided evidence of significant effects and others of no significant effects (Marks et al., 1996; Colorni et al., 1997)

CHAPTER 6: CONCLUSION

6.1 Outcomes of this study

The main aim of this study was to determine the biodiversity and systematics of branchial cavity inhabiting cymothoids from the sub-Sahara African region. In addition, it aimed to provide information on the effects of these parasites on their fish hosts. The outcomes include the following:

This study presents the first detailed redescription of an ovigerous female *Norileca indica* (Milne Edwards, 1840) specimen. Ovigerous females display diagnostic characteristics and structures that may not be present or as well developed in non-ovigerous females and males. The first comprehensive description of a male *N. indica* specimen was provided as well as the first report of *N. indica* from Maputo Bay, Mozambique. The first molecular characterisation of *N. indica* by means of sequencing a fragment of the mitochondrial cytochrome oxidase I (COI) gene of *N. indica* was presented. This contributes to the limited pool of molecular information of the Cymothoidae (currently only 28 of the 385 known and accepted cymothoid species have been sequenced), while also providing a *Norileca* COI sequence that can be used for cymothoid studies as well as species identifications. An identification key for species of the genus *Norileca* was included.

Three sub-Sahara African *Elthusa* species were identified. One of these, *Elthusa raynaudii* (Milne Edwards, 1840), is a well-known and described species from this region. Descriptions were provided for the two new *Elthusa* species along with an identification key with diagnostic characteristics to distinguish between sub-Sahara African *Elthusa* species. Intra-specific variation was noted and recorded. A targeted part of the mitochondrial cytochrome oxidase I (COI) gene from each of the identified species were generated. These sequences, along with a maximum likelihood (ML) analysis, confirm the three distinct species of *Elthusa* and present their placement among other cymothoid genera. A comprehensive summary of all species from the genus *Elthusa* are provided, including host and location records of each.

The ML analysis present two major clades that form within genera from the family Cymothoidae. The first and basal clade contain the Elthusa, which forms a sister taxa to the remaining cymothoid genera. The second and larger clade contains both buccal and externally attaching genera, providing evidence that the externally attaching clade (or “Anilocra” clade) evolved from within this larger clade. With the intra-specific divergence of the Cymothoidae recognised at less than 1%, the genetic divergence in Elthusa COI sequences present inter-specific variation between the three identified Elthusa species. Elthusa sp. 2 and E. raynaudii are more closely related and represent the same species

according to COI analysis alone. Since the morphological provide a clear distinction between these two species, it might be advised to review the cymothoid divergence ranges or propose an alternative sequencing gene to COI.

Three *Mothocya* species have been identified, from which two are newly described for the sub-Sahara African region. The defining characteristics of these species are provided to aid in future identifications. A rapid fish health assessment of on the tropical halfbeak, *Hyporhamphus affinis* (Günther, 1866), revealed no significant health effect due to the attachment of the *Mothocya affinis* Hadfield, Bruce & Smit, 2015 branchial cavity inhabiting cymothoid. Fish health assessment index (FHAI), condition factor, and biometric indices showed no significant effect between the health of infected and uninfected fish hosts. Although the case study did not demonstrate any negative impact of the *Mothocya* on the health of the host, localised effects included frayed and discoloured gills, a shortened operculum and a pit-like depression at the position of attachment.

6.2 Recommendations and future studies

Throughout the duration of this dissertation, several challenges regarding cymothoid taxonomy and phylogenetics were noted. A number of cymothoid specimens, including museum material, were deposited without sufficient collection data such as details on host, location and date of collection. It is essential to record as much detail of the collected material as possible, labelling each collection container properly. All material should be preserved in 70% ethanol, which should regularly be checked and refilled. This is to ensure that the specimens do not degrade, allowing it to be re-examined at a later stage.

The limited number of available and accurate genetic sequences for cymothoids may cause uncertainty for future studies. Many species are yet to be sequenced, limiting the data available to compare and identify species on a molecular level. It was noted that some species, which have been subjected to molecular characterisation, were misidentified, causing confusion and erroneous phylogenetic data. Future research should focus on combining morphological and molecular data to ensure the correct identification of a species as well as to provide evidence of this data for re-examination. It is also advised to include descriptions of all the collected life stages, where possible, to aid future identifications where only certain life stages were available. In addition, future studies should consider alternative molecular markers to that of COI for cymothoid characterisation, in order to differentiate between closely related species and variation.

Future studies on the effects of branchial cavity inhabiting cymothoids and parasitic isopods in general should be recorded over a longer period of time, at the same location. This will allow for the assessment of fish hosts at an early stage of infection as well as long after. The health effects of the cymothoids increase with the duration of their attachment to the host (Romestand and Trilles, 1977a). Previous studies on effects of cymothoid attachment have shown that cymothoids do impact negatively on host health (see Chapter 1.6.6, 1.7). The result from the present study, showing no negative health effects on hosts, might indicate that the host population was recently infested, thereby only presenting mechanical damage due to attachment of the cymothoid. Another recommendation might include the review and conformation of different attachment site cymothoids as parasites by considering the symbiotic relationship between them and their hosts.

The first hypothesis of this study states that it is the lack of sampling and collection, rather than the lack of species that accounts for the low number of *Elthusa, Norileca* and *Mothocya* species from the sub-Sahara African region. Extensive research on cymothoids has been done in regions such as India, South America, Australia, New-Zealand, the Pacific Ocean, and increasingly from southern Africa. The results from this study confirm the first hypothesis, as four new branchial cavity inhabiting species have been described from the sub-Sahara African region. The second hypothesis, that branchial cavity inhabiting cymothoids would have a quantifiable or noticeable negative impact on the health of its host, was rejected. However, although the fish health assessment and condition factors provided no significant difference between the health of infected versus uninfected hosts, localised mechanical damage at the site of attachment were evident. The damage and deformation to branchial tissue was clearly observed, suggesting that branchial attaching cymothoids only affect the site of attachment and surrounding tissue of the host.

Studies on cymothoids are expanding in sub-Sahara Africa and globally. Traditional descriptions of new species are still very relevant, and are increasingly combined with modern techniques such as molecular characterisation. The combination of techniques will ensure that comprehensive, accurate data on cymothoid genera and species are made available. The future direction of cymothoid research should aim towards the establishment of parasite-host relationships for all genera including their role as parasitic symbiont in different stages of host development. Further studies should also focus on the phylogenetic history and ancestory of cymothoids and their attachment sites. Cymothoid taxonomy will continue to form the basis of any and all future studies that involve cymothoids.

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APPENDIX A

Sequence A1: ***Norelica indica*** **Milne-Edwards, 1884 ♀ (ovigerous, 30.0 mm TL, 17 mm W), 686 bp contig. Accession number: MF628258.**

ATCCGAACAGAACTAGGTCAACCTGGGAGATTTATCGGTAATGATCAAATCTACAATGCAATTGTCACCGCTCAT
GCTTTTATTATAATCTTTTTTATAGTTATACCAATTATAATTGGCGGTTTTGGAAACTGAATAGTTCCCCTTATAATT
GGMGCACCTGATATAGCTTTTCCCCGAATAAACAATATAAGATTCTGACTTTTACCTCCTGCATTAACTCTTCTTA
TTGCTAGAGCTTTTATTGAAAGAGGAGTAGGAACAGGATGAACGGTGTACCCTCCTTTATCTAGAAAAATTGCTC
ATAGTAGAGCTGCTGTAGATTTCTCAATCTTTTCTCTACACCTWGCGGGTGTATCTTCGATTTTAGGGGCAGTAA
ATTTTATTACCACTATTATTAATATACGACCTCTGTCTCTCTCTTTAACTCGRATATCATTATTTG

Sequence A2: ***Norelica indica*** **Milne-Edwards, 1884 ♀ (ovigerous, 26.0 mm TL, 13 mm W), 687 bp contig. Accession number: MF628259.**

ATCCGAACAGAACTAGGTCAACCTGGGAGATTTATCGGTAATGATCAAATCTACAATGCAATTGTCACCGCTCAT
GCTTTTATTATAATCTTTTTTATAGTTATACCAATTATAATTGGCGGWTTTGGAAACTGAATAGTTCCCCTTATAAT
TGGMGCACCTGATATAGCTTTTCCCCGAATAAACAATATAAGATTCTGACTTTTACCTCCTGCATTAACTCTTCTT
ATTGCTAGAGCTTTTATTGAAGGAGGAGTAGGAACAGGATGAACGGTGTACCCTCCTTTATCTAGAAAAATTGCT
CATAGTAGAGCTGCTGTAGATTTCTCAATCTTTTCTCTACACCTTGCGGGTGTATCTTCAATTTTAGGGGCAGTAA
ATTTTATTACCACTATTATTAATATACGACCTCTGTCTCTCTCTTTAACTCGGATATCATTATTTG

Sequence A3: ***Norelica indica*** **Milne-Edwards, 1884 ♀ (ovigerous, 26.0 mm TL, 13 mm W), 679 bp contig. Accession number: MF628260.**

ATCCGAACAGAACTAGGTCAACCTGGGAGATTTATCGGTAATGATCAAATCTACAATGCAATTGTCACCGCTCAT
GCTTTTATTATAATCTTTTTTATAGTTATACCAATTATAATTGGCGGTTTTGGAAACTGAATAGTTCCCCTTATAATT
GGCGCACCTGATATAGCTTTTCCCCGAATAAACAATATAAGATTCTGACTTTTACCTCCTGCATTAACTCTTCTTAT
TGCTAGAGCTTTTATTGAAGGAGGAGTAGGAACAGGATGAACGGTGTACCCTCCTTTATCTAGAAAAATTGCTCA
TAGTAGAGCTGCTGTAGATTTCTCAATCTTTTCTCTACACCTTGCGGGTGTATCTTCAATTTTAGGGGCAGTAAAT
TTTATTACCACTATTATTAATATACGACCTCTGTCTCTCTCTTTAACTCGGATATCATTATTTG

Sequence A4: ***Elthusa raynaudii*** **(Milne Edwards, 1840) ♀ nr. 1 (20 mm TL, 12 mm W), 694 bp contig**.

AAAGATATTGGAACACTCTATTTTATATTTGGGAGGTGGGCGGGCGCCATGGGGGTGACCTTAAGAATATTAATC
CGAACCGAATTGGGGCAGCCGGGGTCCTACTTAGGTGACGCCCAACTCTACAACACTATTACCACTGCCCACGC
CTTCGTTATAATCTTTTTTATAGTTATACCTATTATGATTGGGGGGTTTGGGAATTGATTAGTGCCCCTAATAATTG
GGGCCCCAGATATAGCTTTCCCTCGCATGAATAATATAAGATTTTGGCTCCTCCCTCCTTCTTTAACCTTGTTAAT
CATAAGAGGTCTGGTCGAAAAGGGTGCGGGCACAGGGTGAACAGTTTACCCCCCCCTCTCTTCACAAATCGCG
CACAGGGGAGCCTCCGTGGATCTTGCAATCTTCTCCCTCCACCTAGCGGGCGCCTCTTCAATCCTAGGTGCTAT
TAATTTTATTACAACAATTATTAACATACGCCCTACTACAATAACTATAACACGACTACCTCTACTCGTGTGATCTA
TCCTCATCACCGCCATCCTCCTCCTCCTCTCCCTCCCTGTCCTTGCTGGAGCAATTACCATATTATTAACAGACC
GGAATCTAAATACCTCTTTCTTCGACCCTAGTGGGGGGGGAGATCCCGTCCTATACCAACACCTATTTTGATTTT
TTGGTCACCCTGGAAGTT

Sequence A5: *Elthusa* sp.1 ♀ (ovigerous, 34 mm TL; 17 mm W), 671 bp contig.

CTCTATTTTATATTTGGGAGGTGGGCGGGCGCCATGGGAGTGACCTTAAGAATATTAATCCGAACCGAACTAGG
GCAGCCGGGGTCCTACTTAGGTGACGACCAACTCTACAACACTATCACCACTGCCCACGCCTTTGTTATAATCTT
TTTTATAGTTATACCTATTATGATTGGGGGTTTCGGTAATTGATTAGTACCCTTAATAATTGGGGCCCCAGATATA
GCTTTTCCCCGTATGAATAATATAAGATTTTGGCTCCTCCCCCCTTCTTTAACCTTATTAATCATAAGAGGTTTGGT
CGAAAAGGGGGCGGGCACAGGGTGGACAGTCTACCCCCCCCTCTCCTCACAAATCGCACACAGGGGGGCTTC
CGTGGATCTTGCAATCTTCTCCCTCCACCTAGCAGGTGCCTCTTCAATCCTAGGTGCTATTAATTTTATTACAACA
ATCATTAACATACGTCCTGCTATAATAACTATAACACGACTACCTTTACTTGTGTGATCTATCCTCATCACCGCTAT
CCTCCTCCTCCTCTCCCTCCCCGTCCTTGCCGGAGCAATTACTATATTATTAACAGATCGGAATCTAAATACCTCT
TTCTTCGAYCCTAGCGGGGGGGGARATCCTGTCCTATATCAGCACCTATTTTGATTTTTTGGTCACCC

Sequence A6: *Elthusa* sp. 2 ♀ (ovigerous, 29 mm TL; 17 mm W), 693 bp contig.

AAAGATATTGGAACACTCTATTTTATATTTGGGAGGTGGGCGGGCGCCATGGGGGTAACCTTAAGAATATTAATC
CGAACCGAATTGGGGCAGCCGGGGTCCTACTTAGGTGACGCCCAACTCTACAACACTATTACCACCGCCCACG
CCTTCGTTATAATCTTTTTTATAGTTATACCTATCATGATTGGGGGGTTTGGGAATTGATTAGTGCCCCTAATAATT
GGGGCCCCAGATATAGCTTTCCCTCGCATGAATAATATAAGATTTTGGCTCCTCCCCCCTTCTTTAACCTTGTTAA
TCATAAGAGGTCTGGTCGAAAAGGGTGCGGGCACAGGGTGAACAGTTTACCCCCCCCTCTCTTCACAAATCGCG
CACAGGGGAGCCTCCGTGGATCTTGCAATCTTCTCCCTTCACCTAGCGGGCGCCTCTTCAATCCTAGGTGCTAT
TAATTTTATTACAACAATTATTAACATACGCCCTACTACAATAACTATAACACGACTACCTTTACTCGTGTGATCTA
TCCTCATCACCGCCATCCTCCTCCTCCTCTCCCTCCCTGTCCTTGCTGGAGCAATTACCATATTATTAACAGACC
GGAATCTAAATACCTCCTTCTTCGACCCTAGTGGGGGGGGARATCCCGTCCTATACCAACACCTATTTTGATTTT
TTGGTCACCCTGAAGTT

APPENDIX B

Sequence B1: ***Anilocra chromis Williams*** **& Williams, 1981, 696 bp contig - unpublished sequence**

Sequence B2: ***Anilocra haemuli*** **Williams & Williams, 1981, (KY562758.1), 625 bp contig.**

AGGTGTTGTAGGGGTAGCATTTAGAATATTAATTCGTGCAGAACTTTCTCAACCAGGAAATCTTTTAGGAAGAGAT
CAACTTTATAATGCCATTGTAACGGCTCATGCTTTTATCATAATCTTTTTTATAGTAATACCAATTATGATTGGAGG
CTTTGGAAATTGATTAGTTCCTTTGATAATTGGTGCTCCAGATATAGCATTCCCACGAATAAATAATATAAGATTTT
GACTTCTTCCTCCTGCACTTTCATTATTAATTGTAGGCGCTATAATTGAAGAAGGAGCAGGTACAGGGTGAACTG
TTTACCCTCCATTATCAAGTAAGATTGCACATAGAGGAACTTCTGTAGATTTTTCTATCTTTTCATTACATTTAGCA
GGAATTTCTTCTATTTTAGGGGCAGTAAATTTTATTACAACAATTATTAATATACGGCCTAATTTTATACCCTTCTCT
CAAATGCCTTTATTTGTATGAGCTATTCTAATTACAGCTGTTCTTTTATTATTATCATTACCTGTTTTAGCAGGTGC
AATTACCATGTTATTAACAGATCGAAACTTAAATACATCTTTCTTTGATCCTAGAGGAGGTGGAGACCCTATTTTAT
TTCAACATCTG

Sequence B3: ***Ceratothoa africanae*** **Hadfield, Bruce & Smit, 2014, 707 bp contig - unpublished sequence**

Sequence B4: ***Ceratothoa carinata*** **(Bianconi, 1869), 685 bp contig - unpublished sequence**

Sequence B5: ***Ceratothoa retusa*** **(Schioedte & Meinert, 1883), 685 bp contig - unpublished sequence**

Sequence B6: ***Cinusa tetrodontis*** **Schioedte & Meinert, 1884, 712 bp contig - unpublished sequence**

Sequence B7: ***Cymothoa eremita*** **(Brunnich, 1783), 671 bp contig - unpublished sequence**

Sequence B8: ***Cymothoa sodwana*** **Hadfield, Bruce & Smit, 2013, 715 bp contig - unpublished sequence**

Sequence B9: ***Mothocya affinis*** **Hadfield, Bruce and Smit, 2015, 700 bp contig - unpublished sequence**

Sequence B10: ***Mothocya bertlucy*** **Hadfield, Sikkel & Smit, 2014, 700 bp contig - unpublished sequence**

Sequence B11: ***Mothocya plagulophora*** **(Haller, 188), 682 bp contig - unpublished sequence**

Sequence B12: ***Mothocya renardi*** **(Bleeker, 1857), 705 bp contig - unpublished sequence**

Sequence B13: ***Mothocya xenobranchia*** **Bruce, 1986, 700 bp contig - unpublished sequence**

Sequence B14: ***Norileca indica*** **(Milne Edwards, 1840), 688 bp contig - unpublished sequence**

APPENDIX C

Table C1: The assigned values of uninfected *Hyporhamphus affinis* (Günther, 1866) organs to determine the fish health assessment index (FHAI). Values are assigned according to the degree of damage or abnormalities from 0 (normal) to 30 (severely damaged/ abnormal). The presence of cymothoid parasite *Mothocya affinis* Hadfield, Bruce & Smit, 2015 was scored 30.

Fish #	Eyes	Skin	Fins	Gills	Liver	Spleen	Hindgut	Kidney	*M. affinis*	FHAI
2	0	0	20	10	10	0	0	0	0	40
4	0	0	20	0	20	0	0	0	0	40
6	0	0	0	0	10	0	0	0	0	10
8	0	0	30	0	10	0	0	0	0	40
12	0	0	0	10	10	0	0	0	0	20
15	0	0	0	10	30	0	0	0	0	40
17	0	0	20	0	10	0	0	0	0	30
18	0	0	0	10	0	0	0	0	0	10
24	0	0	0	0	10	0	0	0	0	10
25	30	0	0	0	10	0	0	0	0	40
26	30	0	0	0	10	0	0	0	0	40
Average										**29.09**
STDEVA										**13.75**

Table C2: The assigned values of infected *Hyporhamphus affinis* (Günther, 1866) organs to determine the fish health assessment index (FHAI). Values are assigned according to the degree of damage or abnormalities from 0 (normal) to 30 (severely damaged/ abnormal). The presence of cymothoid parasite *Mothocya affinis* Hadfield, Bruce & Smit, 2015 was scored 30.

Fish #	Eyes	Skin	Fins	Gills	Liver	Spleen	Hindgut	Kidney	*M. affinis*	FHAI
1	0	0	20	20	10	0	0	0	30	80
3	0	0	0	30	10	0	0	0	30	70
5	0	0	0	0	10	0	0	0	30	40
7	0	0	0	30	10	0	0	0	30	70
9	0	0	10	0	0	0	0	0	30	40
10	30	0	10	10	0	0	0	0	30	80
11	0	0	0	10	10	0	0	0	30	50
13	0	0	20	10	0	0	0	0	30	60
14	0	0	0	0	10	0	0	0	30	40
16	0	0	0	0	10	0	0	0	30	40
19	0	0	0	10	0	0	0	0	30	40
20	0	0	0	0	10	0	0	0	30	40
21	0	0	0	10	10	0	0	0	30	50
22	0	0	0	30	0	0	0	0	30	60
23	0	0	0	10	10	0	0	0	30	50
Average										**54.00**
STDEVA										**15.02**

Table C3: Condition factor (CF) and gutted condition factor (GCF) calculations of uninfected *Hyporhamphus affinis* (Günther, 1866). Total length (TL) and mass (g) values are provided.

Condition factor			
Fish #	**TL (mm)**	**Mass (g)**	**CF**
2	330	99.30	2.76
4	325	98.72	2.88
6	317	92.22	2.89
8	273	56.63	2.78
12	262	44.55	2.48
15	360	114.63	2.46
17	325	71.40	2.08
18	325	91.85	2.68
24	255	39.79	2.40
25	285	53.01	2.29
26	265	42.94	2.31
Average			**2.55**
STDEVA			**0.27**

Gutted Condition factor			
Fish #	**TL (mm)**	**Gutted Mass (g)**	**GCF**
2	330	84.05	2.34
4	325	78.91	2.30
6	317	74.93	2.35
8	273	38.15	1.87
12	262	38.49	2.14
15	360	106.08	2.27
17	325	66.43	1.94
18	325	84.61	2.46
24	255	37.77	2.28
25	285	49.98	2.16
26	265	40.51	2.18
Average			**2.21**
STDEVA			**0.18**

Table C4: Biometric indices calculated for uninfected *Hyporhamphus affinis* (Günther, 1866). Hepatosomatic Index (HSI) determined using Gutted mass (g) of host and liver mass (g); Splenosomatic Index (SSI) determined using Gutted mass (g) of host and spleen mass (g); Gonadosomatic Index (GSI) determined using Gutted mass (g) of host and gonad mass (g).

Hepatosomatic Index			
Fish #	**Liver (g)**	**Gutted Mass (g)**	**HSI**
2	2.01	84.05	2.39
4	1.31	78.91	1.66
6	1.47	74.93	1.97
8	1.00	38.15	2.61
12	0.88	38.49	2.27
15	1.51	106.08	1.42
17	1.08	66.43	1.62
18	1.26	84.61	1.49
24	0.94	37.77	2.48
25	0.90	49.98	1.80
26	0.618	40.51	1.53
Average			**1.93**
STDEVA			**0.44**

Splenosomatic Index			
Fish #	**Spleen (g)**	**Gutted Mass (g)**	**SSI**
2	0.11	84.05	0.13
4	0.11	78.91	0.14
6	0.12	74.93	0.16
8	0.08	38.15	0.21
12	0.01	38.49	0.02
15	0.11	106.08	0.10
17	0.07	66.43	0.10
18	0.10	84.61	0.12
24	0.04	37.77	0.10
25	0.07	49.98	0.14
26	0.12	40.51	0.29
Average			**0.14**
STDEVA			**0.07**

Gonadosomatic Index			
Fish #	**Gonads (g)**	**Gutted Mass (g)**	**GSI**
2	0.67	84.05	0.80
4	0.58	78.91	0.73
6	1.25	74.93	1.67
8	0.18	38.15	0.48
12	0.10	38.49	0.25
15	0.87	106.08	0.82
17	0.38	66.43	0.58
18	0.30	84.61	0.35
24	0.16	37.77	0.42
25	0.16	49.98	0.32
26	0.14	40.51	0.33
Average			**0.61**
STDEVA			**0.40**

Table C5: Condition factor (CF) and gutted condition factor (GCF) calculations of infected *Hyporhamphus affinis* (Günther, 1866). Total length (TL) and mass (g) values are provided.

Condition factor			
Fish #	**TL (mm)**	**Mass (g)**	**CF**
1	315	78.42	2.51
3	315	76.29	2.44
5	310	73.81	2.48
7	257	34.61	2.04
9	301	71.99	2.64
10	268	44.04	2.29
11	255	42.99	2.59
13	318	77.88	2.42
14	318	76.30	2.37
16	310	71.05	2.38
19	262	40.96	2.28
20	291	49.30	2.00
21	320	87.17	2.66
22	261	49.12	2.76
23	260	43.53	2.48
Average			**2.44**
STDEVA			**0.22**

Gutted Condition factor			
Fish #	**TL (mm)**	**Gutted Mass (g)**	**GCF**
1	315	67.10	2.15
3	315	63.76	2.04
5	310	63.86	2.14
7	257	30.29	1.78
9	301	62.04	2.27
10	268	38.05	1.98
11	255	36.39	2.19
13	318	66.54	2.07
14	318	70.61	2.20
16	310	64.70	2.17
19	262	37.00	2.06
20	291	45.54	1.85
21	320	81.25	2.48
22	261	44.75	2.52
23	260	41.06	2.34
Average			**2.19**
STDEVA			**0.20**

Table C6: Biometric indices calculated for *Hyporhamphus affinis* (Günther, 1866) infected with *Mothocya affinis* Hadfield, Bruce & Smit, 2015. Hepatosomatic Index (HSI) determined using Gutted mass (g) of host and liver mass (g); Splenosomatic Index (SSI) determined using Gutted mass (g) of host and spleen mass (g); Gonadosomatic Index (G SI) determined using Gutted mass (g) of host and gonad mass (g).

Hepatosomatic Index			
Fish #	Liver (g)	Gutted Mass (g)	HSI
1	0.73	67.10	1.09
3	0.94	63.76	1.47
5	0.93	63.86	1.46
7	0.59	30.29	1.94
9	1.23	62.04	1.98
10	0.46	38.05	1.22
11	0.49	36.39	1.34
13	1.45	66.54	2.18
14	1.57	70.61	2.23
16	1.08	64.70	1.67
19	0.67	37.00	1.81
20	0.81	45.54	1.79
21	1.46	81.25	1.80
22	0.84	44.75	1.87
23	1.03	41.06	2.52
Average			**1.76**
STDEVA			**0.39**

Splenosomatic Index			
Fish #	Spleen (g)	Gutted Mass (g)	SSI
1	0.11	67.10	0.16
3	0.08	63.76	0.12
5	0.09	63.86	0.14
7	0.03	30.29	0.10
9	0.10	62.04	0.16
10	0.07	38.05	0.18
11	0.07	36.39	0.20
13	0.15	66.54	0.23
14	0.10	70.61	0.14
16	0.09	64.70	0.14
19	0.05	37.00	0.12
20	0.07	45.54	0.16
21	0.10	81.25	0.12
22	0.05	44.75	0.11
23	0.08	41.06	0.19
Average			**0.15**
STDEVA			**0.04**

Gonadosomatic Index			
Fish #	Gonads (g)	Gutted Mass (g)	GSI
1	0.78	67.10	1.16
3	0.10	63.76	0.15
5	0.21	63.86	0.33
7	0.01	30.29	0.03
9	0.39	62.04	0.63
10	0.09	38.05	0.23
11	0.08	36.39	0.21
13	0.57	66.54	0.86
14	0.56	70.61	0.80
16	0.14	64.70	0.22
19	0.12	37.00	0.31
20	0.17	45.54	0.36
21	0.45	81.25	0.55
22	0.04	44.75	0.09
23	0.17	41.06	0.41
Average			**0.42**
STDEVA			**0.32**