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Morphological and molecular characterisation of *Alella igillimpethu* n. sp. (Copepoda: Siphonostomatoida: Lernaeopodidae) parasitising the southern African endemic intertidal klipfish, *Clinus superciliosus*

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Abstract Lernaeopodidae Milne Edwards, 1840, is an ecological and economically important fish parasite family of copepods (Crustacea: Copepoda), consisting of 48 genera with 334 valid species. To date, approximately 17 genera have been documented from both teleost and elasmobranch hosts from South African marine waters. As part of parasitological surveys targeting parasites of the endemic intertidal klipfish, *Clinus superciliosus* (Linnaeus) (Clinidae) along the South African coast, a species of Lernaeopodidae was discovered on the gills of this host. *Alella igillimpethu* **n. sp.** is described from *Clinus superciliosus* from Langebaan marina on the west coast of South Africa, with a prevalence of 42.1% and mean intensity of 2.9 (ranging from 1–13). Morphological identification was done using light and scanning electron microscopy and the species description was generated with the aid of DEscription Language for TAxonomy (DELTA) software. The mitochondrial cytochrome *c* oxidase subunit 1 (COI) region, as well as partial 18S and 28S ribosomal RNA genes confirmed the classification within the family Lernaeopodidae. This is the first report of Lernaeopodidae from *C. superciliosus*, or in fact any member of Clinidae, as well as the first molecular characterisation of any marine lernaeopodid infecting teleost fish from South Africa. This study contributes valuable genetic and morphological data for this copepod family, as well as new host and distribution records.

Introduction

Copepods of the highly morphologically modified family Lernaeopodidae Milne Edwards 1840 are parasitic on predominantly marine elasmobranchs and teleosts, as well as some freshwater fishes (Kabata, 1979; Dippenaar, 2016a). These parasites are occasionally referred to as "gill maggots", derived from the most common site of infection within the gills of fish and the movement of the parasite's bodies when irritated (Ho, 1977). Approximately 48 genera and 334 valid species are known globally from this family, and many genera and species have been synonymised or transferred in the past (Piasecki et al., 2010; Lebepe & Dippenaar, 2016; Boxshall & Hayes 2019; Montes et al., 2022). The taxonomy of the lernaeopodid-group has been under contention from as early as the 19th

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century, impacting on its taxonomic arrangement and exacerbated by deficient information of its morphology and molecular phylogenetics (Kabata, 1979; Ohtsuka et al., 2020). Piasecki et al. (2010) and Montes et al. (2017) reported that most species of lernaeopodids exhibit narrow host specificity, as well as specific attachment sites on their fish hosts. Only female lernaeopodids are adapted to a parasitic lifestyle, using a chitinous attachment organ (bulla), while the much smaller male lives as a temporary associate on the body of the female.

To date, 17 of the 48 known Lernaeopodidae genera have been recorded from South Africa, the majority of which infect chondrichthyans (Dippenaar, 2004; 2016a; 2016b; 2020; Dippenaar & Sebone 2021) (see Schaeffner & Smit, (2019) for the full list of chondrichthyan lernaeopodids from South Africa). In comparison, little is known about the lernaeopodid parasites of actinopterygians (ray-finned fishes). One genus known from teleost fishes is *Alella* Leigh-Sharpe 1925. Originally *Alella* consisted of eight species, however, according to the World Register of Marine Species (Walter & Boxshall, 2021) only a single species is currently considered as valid: *Alella pagelli* (Krøyer, 1863). Five of the eight species were placed into synonymy with *A. pagelli* by Dippenaar (2016b) [*Alella canthari* (Heller, 1865) (see Kabata, 1964), *A. macrotrachelus* (Brian, 1906) (see Kawatow et al., 1980), *A. ditrematis* (Yamaguti, 1939) (see Ho, 1983), *A. pterobrachiata* (Kabata, 1968), and *A. gibbosa* Van Niekerk & Olivier, 1995]; while *A. tarakihi* Hewitt & Blackwell, 1987 was synonymised with *Clavellotis tarakihi* (Hewitt & Blackwell, 1987), and *A. berecynthia* Leigh-Sharpe, 1936 synonymised with *Clavellomimus berecynthia* (Leigh-Sharpe, 1936).

As part of a study on the parasite diversity of the endemic intertidal clinid, *Clinus superciliosus* (Linnaeus), lernaeopodids were collected from the gills of specimens sampled in the Langebaan marina, Saldanha Bay, along the west coast of South Africa. Based on morphological and molecular techniques, these specimens were identified as belonging to the genus *Alella* Leigh-Sharpe, 1925, and described as new to science. This study also provides the first DNA sequences for the genus *Alella*.

Materials and methods

Specimen collection

Over a three-year period (2018–2020), a total of 111 *Clinus superciliosus* were sampled along the South African coast [Langebaan marina (n = 19); Cape Town harbour (n = 16); Simons Town marina (n = 12); Hermanus (n = 20); Tsitsikamma (n = 20); Chintsa (n = 20)] (Fig. 1). Fish were examined for any parasitic infections according to standard protocols (Klimpel et al., 2019) and the site of infection and number of parasites were noted per fish. Only the Langebaan marina specimens were infested with lernaeopodids on the gills. Copepod specimens were fixed and preserved in 70% ethanol until further examination in the laboratory. All of the parasite species authority references are provided in the reference list. Fish were identified using Smith Sea Fishes (Smith & Heemstra, 2012), with fish taxonomy and host nomenclature following FishBase (Froese & Pauly, 2021) and Eschmeyer's Catalog of Fishes (Fricke et al., 2022). Host authorities are not included in the text or references. Research permits were obtained prior to sampling from the Department of Agriculture, Forestry and Fisheries (DAFF) (RES2018/35, RES2019/103 and RES2020/29) and South African National Parks (SANParks) (MALH-K/2016-005a), as well as ethical clearance through the North-West University AnimCare (NWU-00440-16-A5 and NWU-0051-19-A5).

Morphological methods. A dissection microscope (Zeiss Stemi 305 compact stereo microscope) with an AxioCam camera and ZEN lite imaging software (Zeiss, Oberkochen, Germany) was used to study the macro-morphology. Bright-field and phase-contrast microscopy (Nikon Eclipse N*i*, Nikon Instruments, Tokyo, Japan) were used to further study the specimens in the laboratory, where the z-dimensional stacking (Z-stack) function was applied for differential interference contrast (DIC) micrographs of different structures. Preserved specimens were cleared in lactic acid, stained with lignin pink, and appendages were dissected off to aid in morphological identification.

Six specimens were prepared for scanning electron microscopy (SEM) by dehydrating each in a graded ethanol series followed by a hexamethyldisilane (HMDS) series, placed on aluminium stubs using

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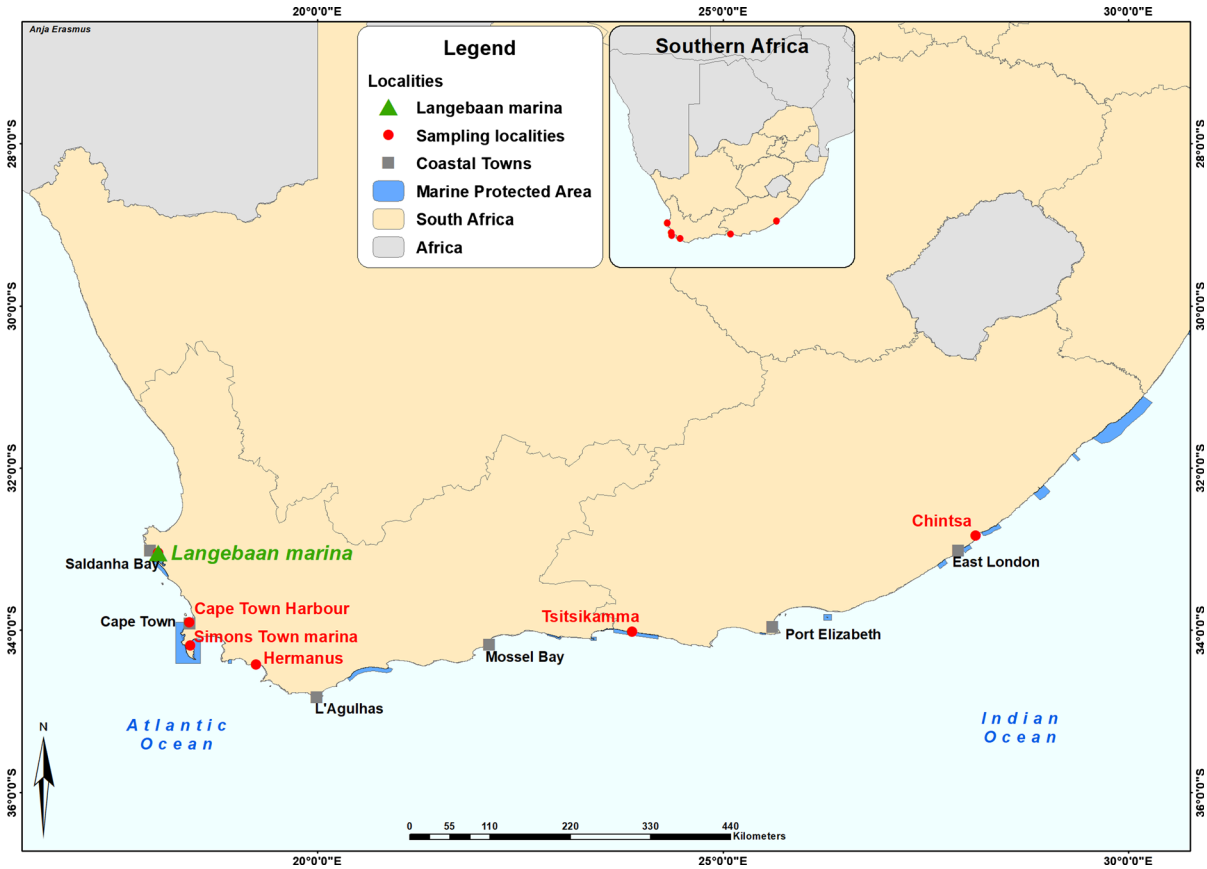

Fig. 1 Map indicating the localities in South Africa sampled for parasites infecting *Clinus superciliosus* in red, and the single locality where lernaeopodids were collected is indicated in green

carbon tape, and sputter coated in gold/palladium (SPI-module, SPI Supplies, Pennsylvania, USA). Specimens were examined and photographed using a Phenom Pro Desktop SEM (ThermoScientific, Waltham, Massachusetts, USA).

Illustrations were made with the aid of z-stack micrographs, Adobe Illustrator CC v25.4.1, Adobe Photoshop CC v25.0 and a WACOM Intuos Pro v6.3.44-1 (Saitama, Japan) drawing tablet with a grip pen. The species description was prepared using a software programme called DELTA (DEscriptive Language for TAxonomy) (available at https://www.delta-intkey.com/), which was used for encoding specific taxonomic characters to develop a comparative Lernaeopodidae character set. Measurements (TL – total length; L – length; W – width), given in millimetre (mm) as range and mean in parenthesis unless otherwise noted, and ratios were done using ImageJ software v1.53j (Schindelin et al., 2012) (available at https://imagej.net/software/fiji). All measurements were based on the maximum values of each measured structure, while all proportional values were rounded to one decimal place. Terminology for morphological features follow Kabata (1979), Huys & Boxshall (1991) and Boxshall & Halsey (2004).

PCR amplification and DNA extraction. DNA extraction of an excised piece of the egg sacs from the lernaeopodid specimens (n = 3) was done using a modified protocol for the PCRBIO Rapid Extract PCR Kit (PCRBiosystems, Analytical Solutions, Randburg, South Africa), where 10 µl of lysis buffer and 5 µl of protease buffer was added to the sample and diluted with 450 µl molecular grade water. The barcode region of the mitochondrial DNA cytochrome *c* oxidase subunit 1 (mtDNA COI) complex was amplified using universal primers LCO4190 (forward) (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (reverse) (5'-TAA ACT TCA GGG TGA

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CCA AAA AAT CA-3') (Hayes et al., 2021), with the following PCR conditions: 5 min initial denature at 95 °C, followed by 35 cycles of amplification of 30 s at 95 °C, 30 s at 47 °C and 1 min at 72 °C, and final 7 min extension at 72 °C. The ribosomal genes 28S rRNA and 18S rRNA were amplified with the primers 28SF (forward) (5'-ACA ACT GTG ATG CCC TTA G-3') and 28SR (reverse) (5'-TGG TCC GTG TTT CAA GAC G-3') as in Santacruz et al. (2020), as well as 18S-E (forward) (5'-CCG AAT TCG TCG ACA ACC TGG TTG ATC CTG CCA GT-3') and NEM18SR (reverse) (5'-GGG CGG TAT CTG ATC GCC-3') as in Neethling and Avenant-Oldewage (2020), respectively. The PCR conditions for 28S rRNA were as follows: 2 min initial denature at 94 °C, followed by 35 cycles of amplification of 30 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and final 7 min extension at 72 °C. For 18S rRNA the PCR conditions varied from 28S: 5 min initial denature at 94 °C, followed by 30 cycles of amplification of 30 s at 94 °C, 30 s at 56 °C and 2 min at 72 °C, and final 10 min extension at 72 °C. The amplified PCR products were sent for cleaning, purification and sequencing to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). Forward and reverse sequences were assembled, aligned and edited with Geneious 11.1.4 (Biomatters, Auckland, New Zealand).

DNA sequence alignment and phylogenetic reconstruction. In total, nine novel sequences were generated from three lernaeopodid specimens (three sequences per gene for COI, 28S and 18S). The consensus sequences were compared to available sequences on BLAST tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm that the specimens belong to the family Lernaeopodidae. Only sequences associated with peer-reviewed publications with per ident > 70% from the BLAST results were used for the phylogenetic comparison (Supplementary data Table 2). The selected outgroups (*Ergasilus* spp. von Nordmann, 1832) for the three alignments were based on the *Parabrachiella* Wilson, 1915 phylogenetic results from Montes et al. (2017). Due to the limited comparable sequences available for the 18S and 28S gene regions, a phylogenetic tree was only constructed for the COI region. The COI alignment was constructed using MUSCLE with the default parameters as in Geneious (Edgar, 2004), whereafter the extremes were trimmed to an alignment of 654 bp. A model test was performed using jModelTest 2.1.4 to determine the best nucleotide substitution model, based on the Akaike information criterion (AIC) (Guindon & Gascuel, 2003). The best AIC substitution model for COI mtDNA was GTR+G. A Bayesian Inference (BI) tree was constructed using MrBayes parameters in CIPRES Science Gateway v3.3 (available at https://www.phylo.org/) and a maximum likelihood (ML) tree was constructed using PhyML v3.0 (available at http://www.atgc-montpellier.fr/phyml/) (Miller et al., 2010). Nodal support for ML analyses was estimated at 100 bootstrap repetitions. The mtDNA COI phylogenetic tree was edited and visualised in FigTree v1.4.3 software (Rambaut, 2012). Pairwise genetic distance matrices (percentage differences and pairwise distances) were determined using Geneious and MEGA7 (Tamura et al., 2021).

Results

Genus: ***Alella*** Leigh-Sharpe, 1925

Type species: Anchorella pagelli Krøyer, 1863 (now known as *Alella pagelli* (Krøyer, 1863))

Remarks: *Alella*

Leigh-Sharpe 1925 has a cylindrical cephalothorax longer than the trunk, with buccal apparatus and antenna near the apex, and the maxilla moved to the base of the cephalothorax (Kabata, 1979). This morphological configuration was referred to by Kabata (1979) as a Type C body plan. Members of *Alella* are also known to have a short, fused maxilla with aliform expansions at the base of the cephalothorax (Kabata, 1979). Based on the keys in Kabata (1979) and Boxshall & Halsey (2004), the current specimens conformed to the generic characteristics of *Alella*.

Alella igillimpethu n. sp.

Type Host: Clinus superciliosus (Linnaeus)
Type Locality: Langebaan marina, Saldanha Bay, Western Cape South Africa (33° 2' 44.4582" S; 18° 2' 19.0602" E), collected in August 2019.
Type Material: Holotype 1 female (ovigerous, larger) (NMB P-902) with 1 male paratype (NMB P-903)

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attached to genital process ventral to egg sacs (smaller), from Langebaan marina, Saldanha Bay, Western Cape, South Africa (33° 2' 44.4582" S; 18° 2' 19.0602" E) collected in August 2019, from endemic intertidal klipfish, *Clinus superciliosus* (Linnaeus). Paratypes: 5 females (ovigerous, three dissected), 2 males dissected, 3 females (2 ovigerous; 1 non-ovigerous) and 3 males used for SEM, same information as the holotype (NMB P-904), deposited in the collections of the National Museum, Bloemfontein (NMB), South Africa.

Other material used: 3 females (2 ovigerous; 1 non-ovigerous) (NMB P-905) as hologenophores used for DNA, same information as the holotype.

Site of infection in host: Gills.

Prevalence: 42.1%

ZooBank registration number: The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub: 954DBB03-BF55-43F4-BE76-46DE5A217A05. The LSID for the new name *Alella igillimpethu* n. sp. is urn:lsid:zoobank.org:act:C6062338-0AD6-42D2-A9A5-CD3FAE630A90.

GenBank accession numbers: (18S). 957–978 bp long sequences of three hologenophores: OP548628-OP548630; *(28S).* 618–648 bp long sequences of three hologenophores: OP548634-OP548636; *(COI).* 654 bp long sequences of three hologenophores: OP548137-OP548139

Etymology: The specific name of this lernaeopodid was derived from the isiXhosa words for gill (igill) and maggot or worm (impethu) referring to the common name of "gill maggot" occasionally used for lernaeopodids (isiXhosa is an indigenous language to the Eastern and Western Cape of South Africa). The species specific name is a noun in apposition.

Description

Adult female (n = 5) 4.5–7.8 (6.2) mm total length (Figs. 2–5). *Cephalothorax* 1.8–2.4 (2.1) mm in length and 0.4–0.4 (0.4) mm in width (Figs. 2–4) cylindrical, longer than trunk, arising from anterior end of trunk. *Dorsal shield* (Figs. 2b, 4a) with indistinct posterior margin. *Aliform expansions* (Figs. 2, 3a, 4h, 5b) curving in a rounded shape. *Excretory ducts* (Figs. 3a, 3f, 4h, 5b) lateral to maxilla, posterior to aliform expansions. *Trunk* 1.1–1.7 (1.4) mm in length and 1–1.3 (1.1) mm in width (Figs. 2, 3a, 4i) unsegmented, oval; anal slit indistinct. *Genital process* (Figs. 2a, 3a; 4i; 5c) present, ventral to egg sacs, conical. *Posterior processes and caudal rami* absent. *Egg* sacs 1.6–3.8 (2.5) mm long and 0.5–0.6 (0.5) mm wide (Figs. 2; 3a) longer than trunk, length variable; eggs spherical, multiseriate, approximately 90–100 eggs.

Antennule (Figs. 3c, 4e) indistinctly segmented, tapered (basal part wider); armed with a whip posteroventrally, second segment with solus (stout protrusion) between whip and apical elements, apical armature with 5 elements (as per Kabata 1979): tubercle 1 (T1) on medial side; followed by digitiform seta 4 (S4); flagelliform seta 6 (S6) on lateral side; elongated setae 5 (S5) in centre; tubercle 2 (T2) between S5 and S6. *Antenna* (Fig. 3d) biramous; exopod 1-segmented, bulbous with rounded tip and sensory pit, prominent, stout agglomerated denticles on outer margin, armed with stout protrusion on lateral aspect near base; endopod indistinctly segmented, basal segment with small pectinate-like setae (Fig. 4c), terminal end with prominent denticles, 3–4 elements.

Mandible (Fig. 5b) with 6 teeth; anteriorly with 2 teeth of similar size, middle tooth largest, followed by 3 smaller teeth posteriorly. *Maxillule* (Figs. 3e, 4d) inner lobe with 2 terminal papillae armed with setae, 1 small seta at base of inner papillae; outer lobe smaller than endite, with 2 setae reaching half the length of papilla. *Maxilla* (Figs. 3f, 4h, 5b) short, not completely fused, bulbous collar bilobed anteriorly. *Bulla* (Figs. 3f, 4h, 5b) sub-spherical cup, not separated into manubrium and anchor. *Maxilliped* (Figs. 3g, 4f) meeting opposite at the base; broad corpus and myxa with short seta; corpus with short seta posterolaterally. *Subchela* (Figs. 3h, 4f, 4g) with denticles at the base, barb slender, reaching to half the length of the claw, slightly curved with pointed tip.

Male (n = 3) 489–645.9 (567.5) µm in length exhibits complete sexual dimorphism (Figs. 5–7). *Body* (Figs. 5c, 5d, 7g) not divisible into cephalothorax and trunk, with rounded posterior extremity. *Uropods* absent. *Posterior* process absent. *Genital process* (Figs. 6e, 7e, 7g) located posterior to maxilla. Male attached by maxilla to ventral surface of female. Illustrations of specimens from frontal view, with dissected appendages.

Antennule (Figs. 6a, 7a) indistinctly segmented, tapered; basal segment armed with narrow setae; second segment unarmoured, apical armature with 5 elements: digitiform seta on medial side with short tubercle, two setae part of element on lateral side, and

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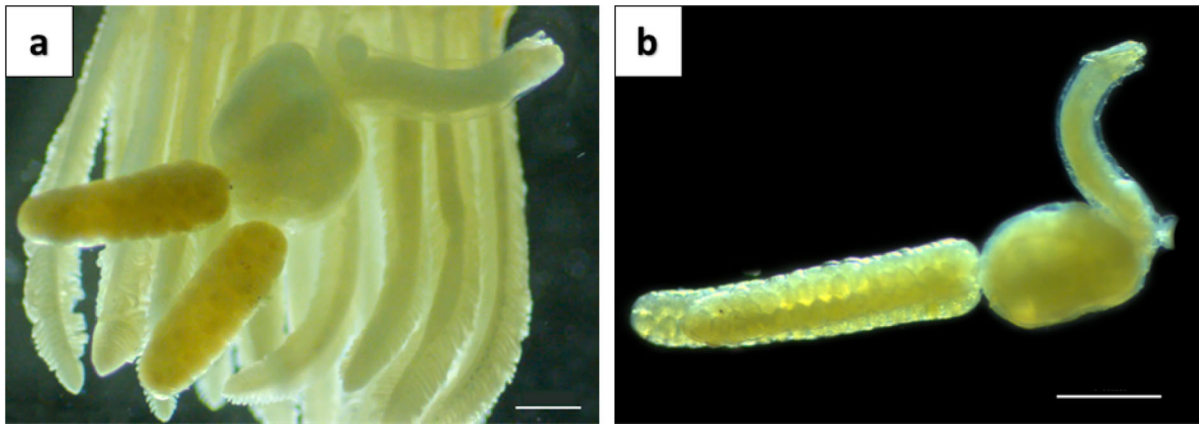

Fig. 2 Photographs of *Alella igillimpethu* **n. sp.** a) dorsal view of the lernaeopodid attached to the gills; b) lateral view of the lernaeopodid with cephalothorax, trunk and egg sacs visible. Scale bars: a, 0.5 mm; and b, 1 mm

elongated seta in centre. *Antenna* (Figs. 6b, 6c, 7b, 7c) biramous, exopod shorter than endopod; exopod 1-segmented, bulbous with rounded tip, agglomerated spines resembling pectinate scales unlike female denticles on outer margin, with one stout protrusion on lateral aspect; endopod 2-segmented, two sets of pectinate-like setae located one third from the base and at the terminal end of basal segment, terminal end with 4 elements, 3 spiniform setae and one prominent tubercle, with pectinate-like seta on medial side. *Mandible* not observed in dissected specimens. *Maxillule* (Fig. 7d) similar to female, but more distinctly segmented. *Maxilla* (Fig. 6d, 7e, 7g) medially fused at the base, corpus robust, armed with small seta, subchela slightly curved. *Genital process* (Figs. 6e, 7e, 7g) present, ventral, posterior to maxilla, bulge-like structure. *Maxilliped* (Figs. 6e, 7f, 7g) not fused at the base, distinctly two appendages, more elongated than maxilla; corpus with small myxa with at least two rows of four comb-like teeth, subchela sharply curved towards corpus forming a distinct claw at terminal end.

Remarks: *Alella igillimpethu* **n. sp.** is distinguished from other species of Lernaeopodidae by the combination of characters present: the shape and arrangement of prominent and stout agglomerated denticles on the female antenna, the presence of a solus, as well as the arrangement of the apical armature on the antennule, the bulbous bilobed collar of the maxilla, the shape and arrangement of six teeth of the mandible, and the small size and sub-spherical cup shape of the bulla. The shape and position of excretory ducts in relation to aliform expansions also differ from other reported species.

This female species of *Alella* is morphologically distinguishable from congeners through a combination of the following characteristics: i) antennule; ii) antenna; iii) mandible; iv) maxillule; v) maxilla; vi) bulla. *Alella igillimpethu* **n. sp.** can be discerned based on the solus not observed before on the antennule located closer to the whip than the apical armature, and the unique prominent and stout agglomerated denticles around the outer margin of the antenna exopod. The apical armature of the antennule are all elements relatively equal in size compared to the distinct size differences described in Kabata (1979). Based on the antennule description in Kabata (1979), the apical armature conforms to the *Charopinus*-branch that includes the structural type C body plan for lernaeopodids such as the genus *Alella*. *Alella pagelli*, a species described from sparid fish from South Africa, is morphologically the most similar species, however, based on the recent redescription of this species by Dippenaar (2016b), as well as comparative SEM photographs from Van Niekerk & Olivier (1995) of the now synonymised *Alella gibbosa* among others, it differs as follows: the distinctly prominent stout agglomerated denticles on the antenna; the arrangement of the apical armature and solus on the antennule; the mandible of *A. pagelli* has eight teeth, compared to six teeth in *Alella igillimpethu* **n. sp.**; the maxillule of the latter has additional setae on the inner papillae and a seta on the outer papillae; there is no mention of excretory ducts at the base of the maxilla

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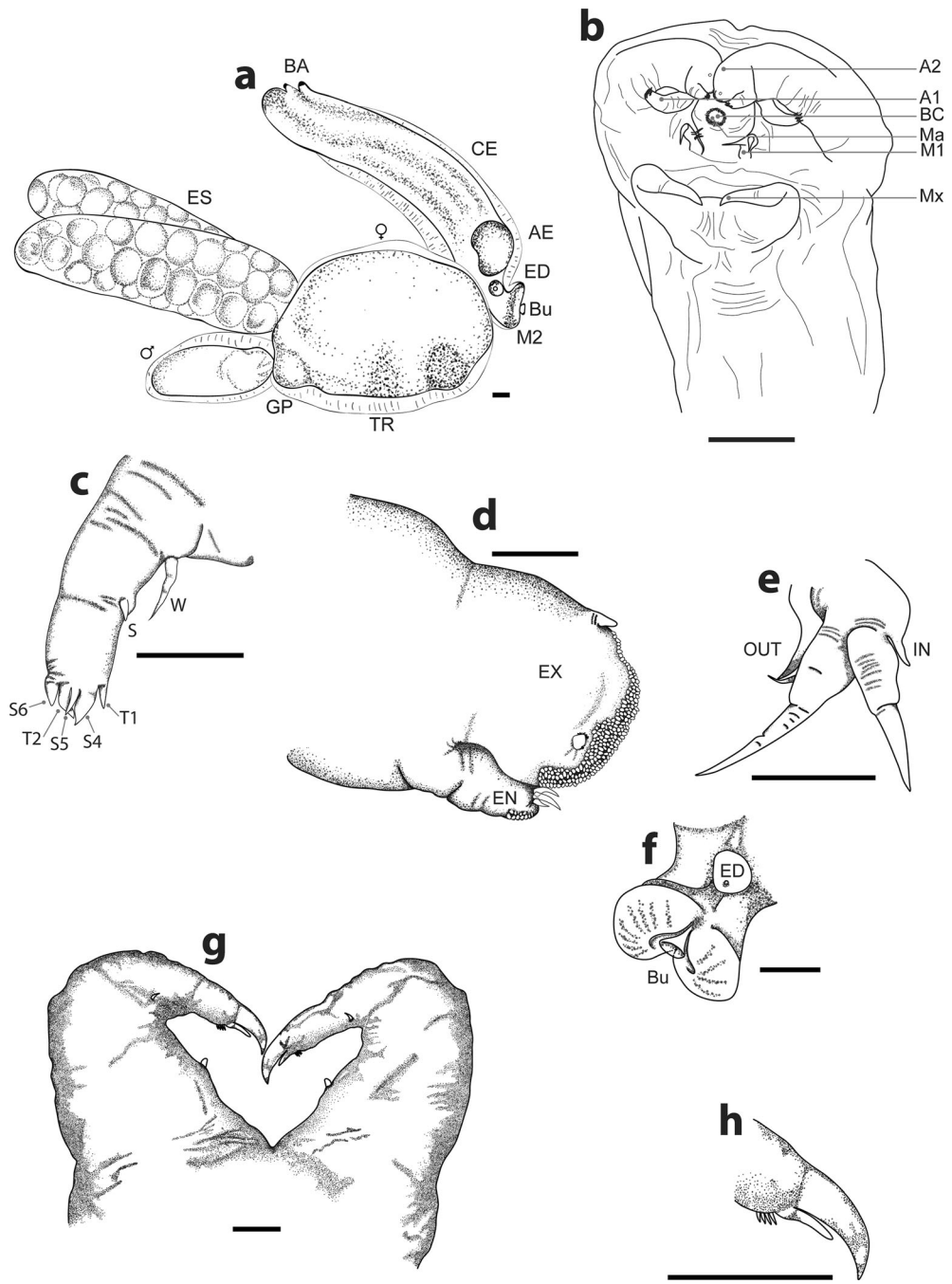

Fig. 3 *Alella igillimpethu* **n. sp.**, holotype adult female (large specimen; NMB P-902) with male (NMB P-903) attached (small specimen) to genital process ventral to egg sacs, ex *Clinus superciliosus* (a); dissected paratype female (NMB P-904) (b – h). a) habitus, lateral view; b) buccal apparatus with mouth parts, anteriorly; c) antennule with armature; d) antenna; e) maxillule; f) maxilla with bulla and excretory duct; g) maxillipeds; h) distal part of subchela and claw. Abbreviations: A1 – antennule; A2 – antenna; AE – aliform expansions; BA – buccal apparatus; BC – buccal cone; Bu – bulla; CE – cephalothorax; ED – excretory ducts; EN – endopod; ES – egg sacs; EX – exopod; GP – genital process; IN – Inner lobe (endite); M1 – maxillule; M2 – maxilla; Ma – mandibles; Mx – maxillipeds; OUT – outer lobe (palp); S – solus; S4 – digitiform seta 4; S5 – intermediate seta 5; S6 – flagelliform seta 6; T1 – tubercle 1; T2 – tubercle 2; TR – trunk; W – whip. Scale bars: a, b, f, 100 μm; c, d, e, g, h, 20 μm

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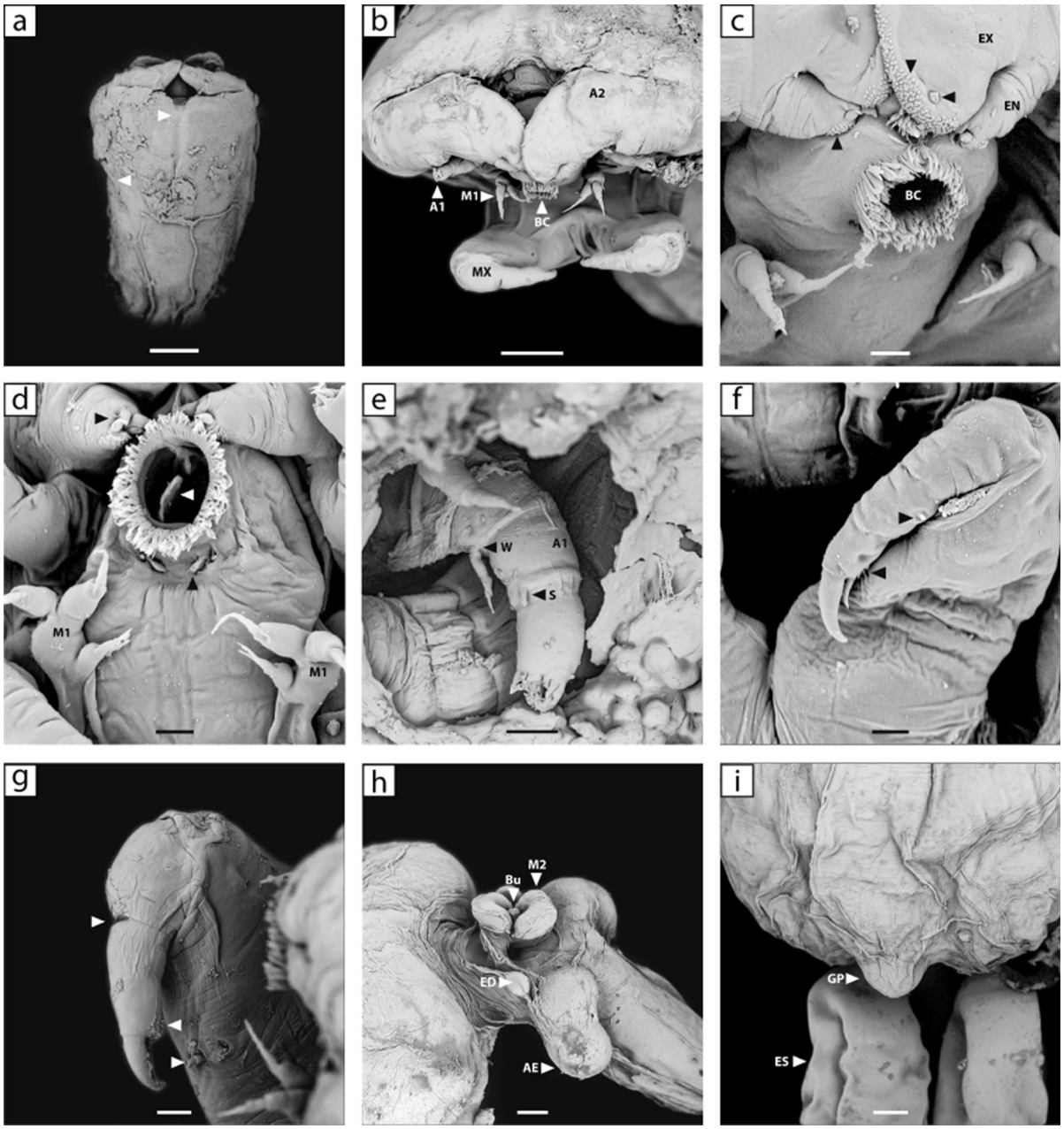

Fig. 4 Scanning electron photomicrographs of characteristic structures of the paratype female of *Alella igillimpethu* **n. sp.** (NMB P-904): a) Anterodorsal view of anterior end of cephalothorax, arrows indicating dorsal shield; b) anterior view of cephalothorax; c) endo- and exopod of antenna showing sensory pit and setae, buccal cone and maxillule, arrows indicating denticles; d) ventral view of mouthparts and buccal cone, white arrow indicating mandible and black arrows indicating setae; e) antennule; f) maxilliped with denticles on inner margin of subchela, arrows indicating setae; g) dorsal view of maxillipeds, top arrow indicating segment and bottom arrows indicating setae; h) lateral view of cephalothorax with maxilla, aliform expansions and excretory ducts; i) posterior end of trunk with genital process and egg sacs. Abbreviations: A1 – antennule; A2 – antenna; AE – aliform expansions (lateral swellings); BC – buccal cone; ED – excretory ducts; EN – endopod; ES – egg sacs; EX – exopod; GP – genital process; M1 – maxillule; M2 – maxilla; MX – maxillipeds; W – whip. Scale bars: a, h, I, 100 μm; b 50 μm; and c, d, e, f, g, 10 μm

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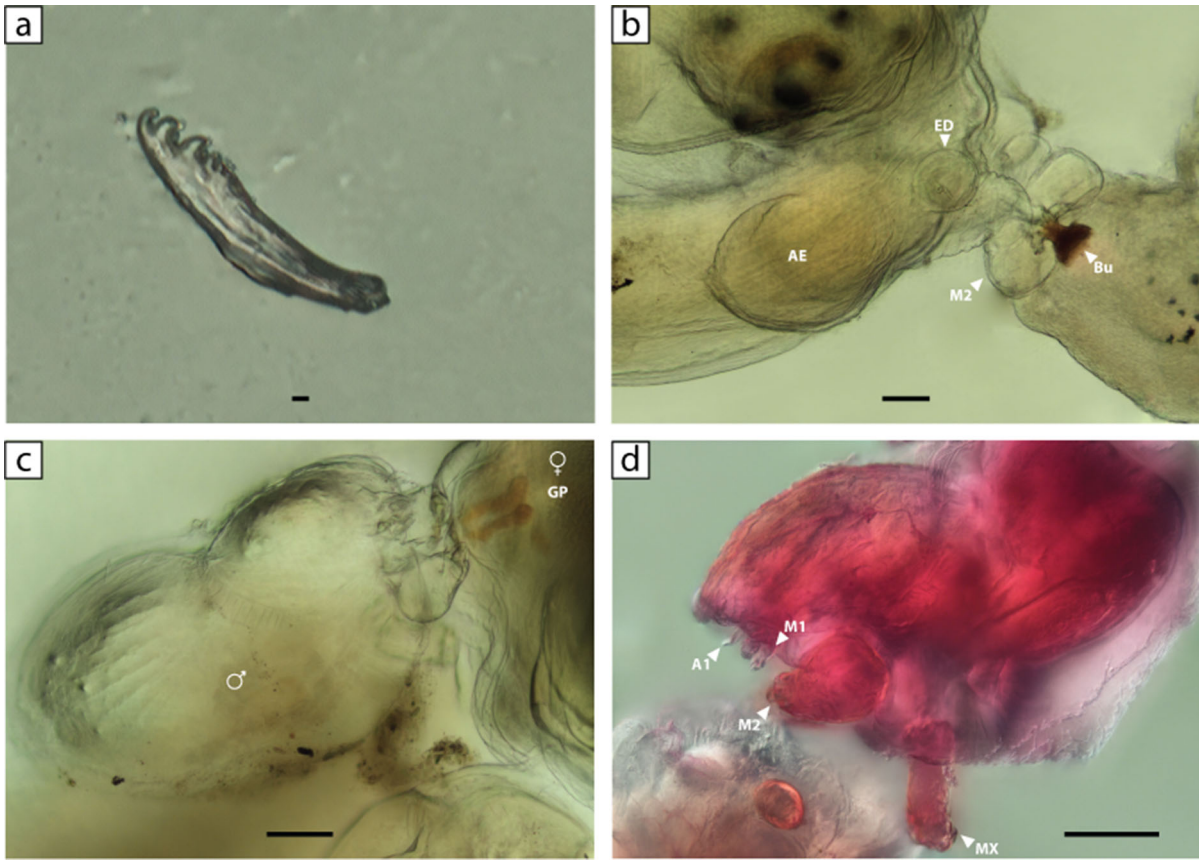

Fig. 5 Bright-field and phase-contrast photomicrographs with the z-dimensional stacking function applied to view *Alella igillimpethu* **n. sp.** dissected female paratype (a, b) and male paratype (c, d) (NMB P-904): a) mandible dissected from mouth cone; b) maxilla with bulla visible inside the gill filament; c) male as seen attached to female genital process; d) stained male with appendages visible. Abbreviations: ♀ – female; ♂ – male; A1 – antennule; AE – aliform expansions; Bu – bulla; ED – excretory ducts; GP – genital process; M1 – maxillule; M2 – maxilla; MX - maxilliped. Scale bars: a, c, d, e, f 100 μm; and b 10 μm

and aliform expansions; as well as the different host species, with only Sparidae as host to *A. pagelli*. The shape of the bulla of *Alella igillimpethu* **n. sp.** is significantly different compared to *A. pagelli,* as well as other species mentioned in Kabata & Cousens (1972).

The male of *Alella igillimpethu* **n. sp.** has similarities with *Clavellotis tarakihi* (Hewitt & Blackwell, 1987) considering the arrangement of the antennule and the maxillule, however, some major differences are noted with regards to the location of the genital process, the former attaches to the female by the maxilla, while the latter is reported to attach using the maxillipeds. Distinct differences are also noted in the armature of the antenna, as well as the shape of the maxilla and maxillipeds. The specimens from the present study also differ from the male of *A. pagelli* described by Dippenaar (2016b) in the arrangement of the terminal and basal armature on the antenna, apical armature of the antennule, number of setae on the maxillule, location of the genital process, and the fact that the maxillipeds are not fused medially and have different terminal armature.

Molecular analysis

Alignments used during the present study did not include any of the unpublished sequences, nor any sequences from retracted publications available on Genbank (Supplementary data Table 1). As a result of low support due to the limited number of published sequences, the results of the phylogenetic trees are not presented here (see Supplementary Fig. 1). Comparison of the partial 18S rRNA sequences from the

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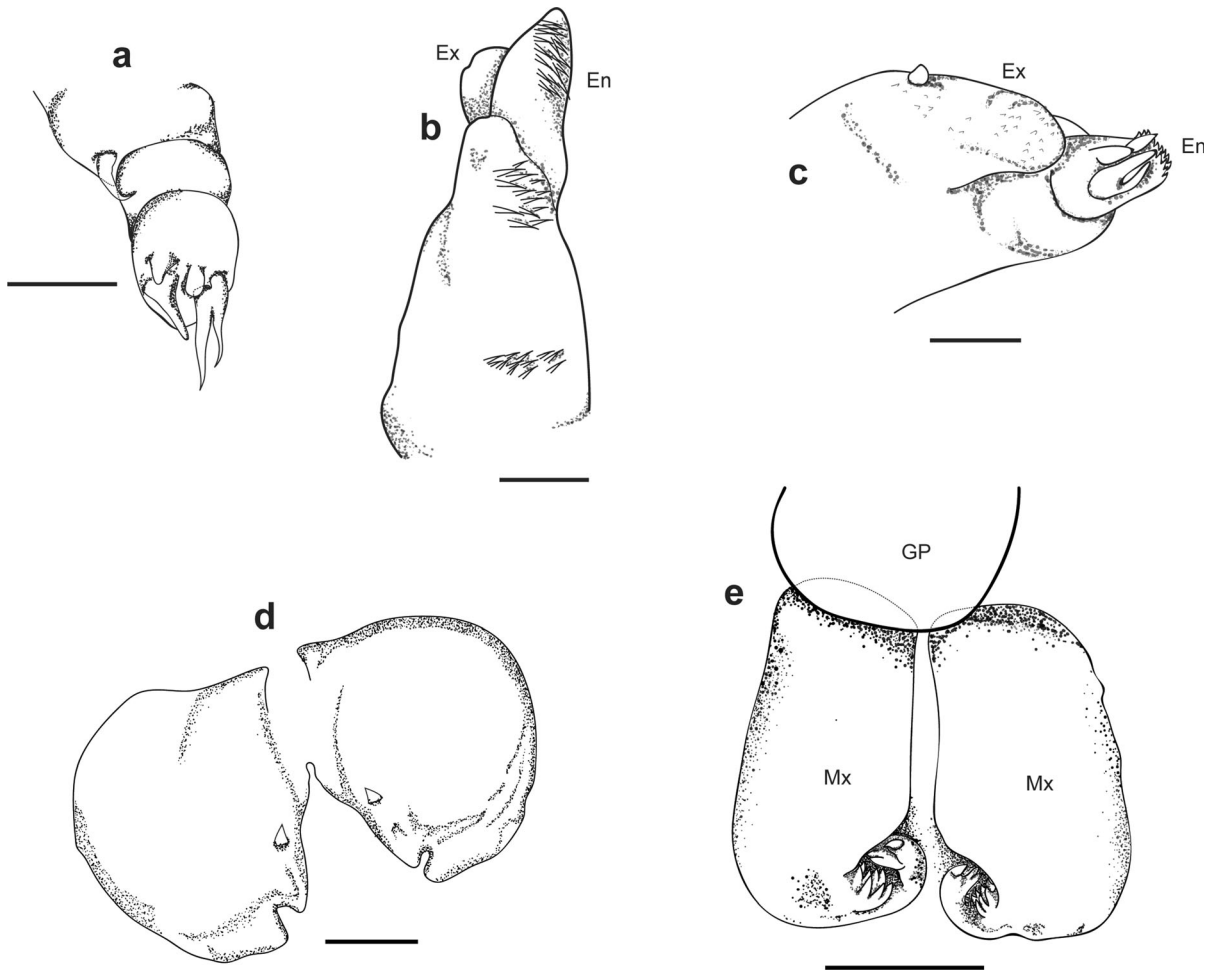

Fig. 6 *Alella igillimpethu* **n. sp.**, paratype male *ex Clinus superciliosus* (NMB P-904): a) antennule; b) antenna, ventral view; c) antenna, dorsal view; d) fused maxilla, with setae on corpus; e) maxillipeds with genital process visible. En – endopod; Ex – exopod; GP – genital process; Mx – maxilliped. Scale bars: a, b, c 10 μm; d, e 50 μm

present study (Supplementary data Table 2) to the limited GenBank sequences demonstrated genetic similarity of 98.2% (9 nt) to the closest relative (*Clavellisa emarginata* (Krøyer, 1837)), while for the partial 28S rRNA (Supplementary data Table 3) the highest similarity was found to be 73.9% (171 nt) for *Salmincola edwardsii* (Olsson, 1869), a freshwater copepod. The species similarity for COI (Supplementary data Table 4) between the novel sequences and the GenBank obtained sequences was 79.3% (130 nt), with sequences of *Parabrachiella kabatai* (Luque & Farfán, 1991) as the most closely related.

The phylogenetic analysis confirms the estimated phylogenetic classification of *Alella* as most closely related to *Clavella* Oken, 1815, with a bootstrap support value of 78%. The ingroup sequence divergence for COI was high, with an estimated 20.6% variation between *Alella igillimpethu* **n. sp**. and *Parabrachiella kabatai,* and 23.5% variation between the novel sequences and *Clavella perfida* Wilson, 1915. Monophyly of the genus *Alella* could not be determined since no other sequences from this genus were available for genetic comparison.

Discussion

Currently, *Alella* has been reported from Sparidae (Dippenaar, 2016b): *Alella pagelli* has been reported to infest *Rhabdosargus sarba* (Forsskål) (as reported

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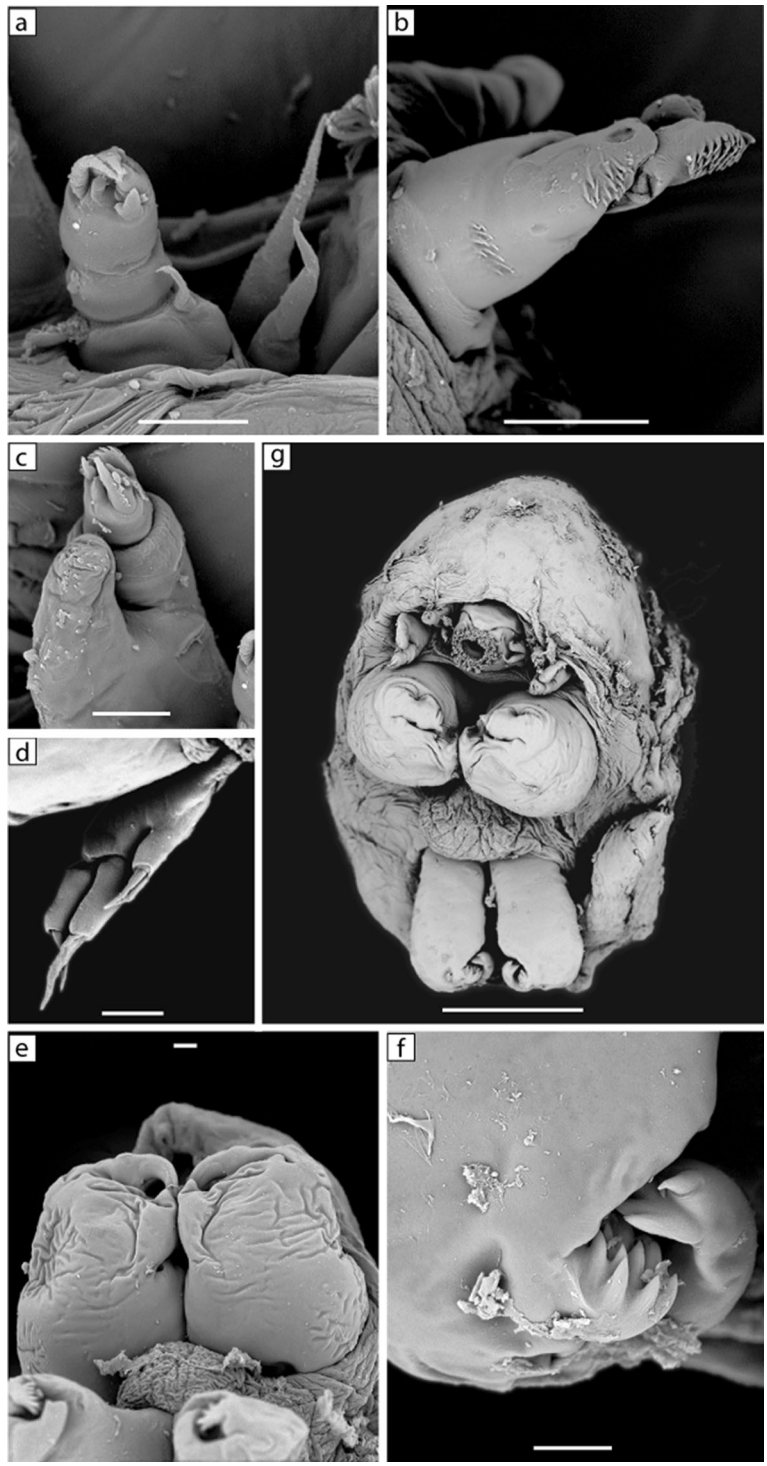

Fig. 7 Scanning electron photomicrographs of the paratype male *Alella igillimpethu* **n. sp**. *ex Clinus superciliosus* (NMB P-904): a) antennule; b) antenna, ventral view; c) antenna, dorsal view; d) maxillule; e) maxilla; f) terminal armature of right side maxillipeds; g) habitus. Scale bars: a, c, d, e, f, 10 μm; b, 20 μm; g, 100 μm

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for *A. gibbosa*), *R. holubi* (Steindachner), *R. globiceps* (Valenciennes) and *Acanthopagrus berda* (Forsskål), *Pachymetopon blochii* (Valenciennes) (as reported for *A. canthari*), *Ditrema temminckii* (Bleeker) (as reported for *A. ditrematis*), *Spicara melanurus* (Valenciennes) (see Kabata, 1979), *Acanthopagrus butcheri* (Munro), *A. berda, A. latus* (Houttuyn) (as in Byrnes, 1988), and *A. schlegeri* (Bleeker) (as in Kawatow et al., 1980; Ho, 1983) (as reported for *A. macrotrachelus*), *Epinephelus merra* (Bloch) (originally reported as *Clavellodes pterobrachiata* (Kabata, 1968)). These host species are distributed across the world's oceans, as well as within South Africa waters. The description of *Alella igillimpethu* **n. sp.** from clinid fishes is thus the first non-sparid host record for a species of *Alella* as well as the first record of clinids as hosts of Lernaeopodidae copepods. Although various localities along the South African coast were sampled, *Alella igillimpethu* **n. sp.** was only reported from the western most locality. The assumption can thus be made that the species is distributed along the west coast of South Africa.

Piasecki et al. (2016) state that basic morphological methods alone cannot be used to distinguish between host specific and closely related lernaeopodids, and suggests that molecular tools are needed in combination with the morphological data for a comprehensive revision of Lernaeopodidae. At this stage, however, too few sequences are available to make a definitive decision based on molecular differentiation. While the phylogenetic relationships of Lernaeopodidae are not well resolved it requires more representatives of the species and genera to provide a robust classification. Due to the limited number of published sequences, lower resolution on the deeper nodes were obtained for the phylogeny of the family, which again highlights the need for more representative sequences for the genera and the family. It is important to keep in mind that as more taxa are added into the phylogenetic tree, the relationships will change. To indicate distinct copepod genera, the authors accepted a range of 9–24% sequence divergence for COI (Bucklin et al., 1999; Bucklin et al., 2003; Mangena et al., 2014). Interspecific sequence divergence was reported as > 15% while intraspecific divergence was set at 0–4%, based on species reported from elasmobranch hosts species (Bucklin et al., 2003; Øines & Heuch, 2007; Dippenaar et al., 2010; Mangena et al., 2014).

The present study thus provides a description of *Alella igillimpethu* **n. sp.**, the first record of clinids as hosts of Lernaeopodidae copepods, and the first partial gene sequences of three different genes for the genus *Alella*. This genus is related to *Clavella*, forming a clade indicating that these two genera are morphologically closely related. Future studies on this copepod family, should involve an integrated approach to taxonomy, where species are described and compared based on multiple morphological techniques, as well as molecular data.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of AnimCare at the North-West University. All applicable international, national and/or institutional guidelines for the use and care of animals were followed. Research permits were obtained prior to sampling from the Department of Agriculture, Forestry and Fisheries (DAFF) (RES2018/35, RES2019/103 and RES2020/29) and South African National Parks

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(SANParks) (MALH-K/2016-005a). Ethical approval for collections were given to AE through the North-West University's AnimCare (NWU-00440-16-A5 and NWU-0051-19-A5).

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