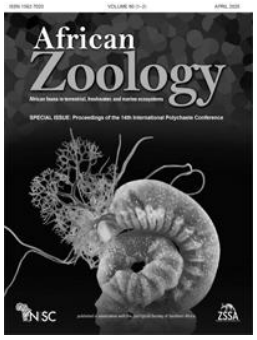

African Zoology

ISSN: 1562-7020 (Print) 2224-073X (Online) Journal homepage: www.tandfonline.com/journals/tafz20

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To cite this article: Adri van Niekerk, Jyothi Kara, Arturo Alvarez-Aguilar & Carol A Simon (2025) *Lumbrineris magalhaensis* Kinberg, 1865 (Annelida: Lumbrineridae) revealed as two indigenous species in South Africa, African Zoology, 60:1-2, 50-66, DOI: 10.1080/15627020.2024.2437168

To link to this article: https://doi.org/10.1080/15627020.2024.2437168

African Zoology 2025, 60(1–2): 50–66

AFRICAN ZOOLOGY
ISSN 1562-7020 EISSN 2224-073X
https://doi.org/10.1080/15627020.2024.2437168

Lumbrineris magalhaensis Kinberg, 1865 (Annelida: Lumbrineridae) revealed as two indigenous species in South Africa

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***Lumbrineris magalhaensis* Kinberg, 1865 (Annelida: Lumbrineridae) is a marine polychaete worm that was first described from the Magellan Strait, Chile; thereafter it was also reported in sub-Antarctic, temperate and tropical regions, where it was likely misidentified. In South African waters, *Lumbrineris magalhaensis* may have been erroneously synonymised with *Lumbriconereis pettigrewi* McIntosh, 1885, which was first described from off the Cape of Good Hope. Specimens collected from the Knysna Estuary on the south coast of South Africa were recently identified as *Lumbrineris magalhaensis* based on identification keys for local fauna. However, the specimens all differ from descriptions of the type material and previous descriptions of *Lumbrineris magalhaensis*, as well as from re-examined specimens of *Lumbriconereis pettigrewi* (three syntypes, collected off the Cape of Good Hope, South Africa), with regard to prostomium shape, numbers of teeth on maxillae III and IV, shape of maxilla II, size and colour of maxilla II connecting plates, arrangement of limbate chaetae and the habitat. The specimens from Knysna have therefore been designated as *Lumbrineris knysnaensis* van Niekerk, Kara, Alvarez Aguilar & Simon sp. nov. Furthermore, the synonymisation of *Lumbriconereis pettigrewi* and *Lumbrineris magalhaensis* has been reversed, owing to differences in prostomium shape, number of teeth on maxilla IV, depth of collection and habitat type; therefore, *Lumbrineris pettigrewi* comb. nov. reinst. is supported. Analysis of mtDNA cytochrome *c* oxidase subunit I (COI) indicated that the Knysna specimens represent a single species and do not match any other known species, corroborating the morphological data. Further morphological and genetic investigations of *Lumbrineris magalhaensis* from other regions are needed to resolve the taxonomy of this species.**

Keywords: COI sequences, Knysna Estuary, *Lumbrineris knysnaensis*, *Lumbrineris pettigrewi*, mtDNA, new species, phylogenetic tree, taxonomy

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The new species is registered in ZooBank under urn:lsid:zoobank.org:act:03727E90-9743-460F-ABCA-BEBF9FBB1CD2

Introduction

Since the late 1970s, taxonomists have shown that few of the polychaete species reported from wide global distributions are truly cosmopolitan (Hutchings and Kupriyanova 2018). Instead, several are morphologically identical (cryptic) or similar (pseudo-cryptic) species with distinct distributions but misidentified as single species (Nygren 2013; Hutchings and Kupriyanova 2018), or are alien species transported beyond their natural ranges (Çinar 2013). Consequently, Darling and Carlton (2018) recommended that widespread species that have not been revised taxonomically are unresolved cosmopolitan species, while alien species are neo-cosmopolitans. Recently, Simon et al. (2022) showed that more than half the species reported in Day (1967), the monograph on southern African polychaetes, are in fact unresolved cosmopolitan species. Furthermore, keys and descriptions in the monograph are not always detailed enough to distinguish between morphologically similar species, as evidenced by the detection of two morphologically distinct species of Lumbrineridae that were both identified as *Scoletoma tetraura* (Schmarda, 1861) in accordance with the key in Day (1967) (Simon et al. 2021). Thus, both the number of indigenous species and the total diversity of polychaetes in South Africa have been underestimated (Simon et al. 2022).

In South Africa, the family Lumbrineridae Schmarda, 1861 and the genus *Lumbrineris* Blainville, 1828 have both been prioritised for taxonomic revision, with each possibly containing more than 50% unresolved cosmopolitan species (Simon et al. 2022). *Lumbrineris* is the most-speciose genus in the family, and the latest estimates indicate that it contains about 140 species (Read and Fauchald 2022). However, *Lumbrineris* has been taxonomically problematic. Hartman (1948) divided *Lumbrineris* into three groups based on the types of chaetae; later, Fauchald (1977) added species with branchiae and fan-shaped pygidia to the genus, but neither feature is presently accepted as a diagnostic character for *Lumbrineris* (Carrera-Parra 2006a). More recently, some previously overlooked characters, such as wide connecting plates of the 2nd maxilla pair (MII) and dorsal cirri with

notoaciculae, were included as diagnostic for this genus (Orensanz 1973; Carrera-Parra 2006b). The available genetic data also suggest that the genus is polyphyletic (Borisova and Budeava 2022).

Lumbrineris magalhaensis Kinberg, 1865 is an unresolved cosmopolitan species that possesses a similarly complicated taxonomic history. This species was first collected from floating algae in the Magellan Strait, Chile, by Kinberg (1865), and again by Ehlers (1897) (Figure 1; Table 1). Thereafter, it was reported from a wide range of locations (Figure 1) and habitats. Monro (1930) collected *L. magalhaensis* from various substrates (mud, sand and rock), at a wide depth range (0–391 m) and from various locations, including South Georgia, the Falkland Islands, and the South Orkney and South Shetland islands. These collection localities are all in sub-Antarctic or Antarctic regions, 750–2 200 km from the type locality (Figure 1a), raising the question of whether all these samples really are *L. magalhaensis*. Furthermore, Branch (1994) later reported the species from sub-Antarctic waters at Marion Island, nearly 7 000 km from the type locality.

In 1959, Day collected specimens of *Lumbrineris* off the coast of Cape Town, South Africa (Day 1960), near the type locality of *Lumbriconereis pettigrewi* McIntosh, 1885 (Figure 1c). After examining that material and the type specimen of *L. pettigrewi*, and the descriptions of *L. magalhaensis* by Ehlers (1897), Monro (1930) and Hartman (1948, based on the type specimen), Day (1960) synonymised *L. magalhaensis* and *L. pettigrewi*. In this way, the distribution of *L. magalhaensis* was extended to temperate regions.

Lumbrineris magalhaensis has since been reported from a range of depths and various environments in both the Northern Hemisphere (e.g. at 3-m depth off the coast of Saudi Arabia: Lozano-Cortés et al. 2019) and Southern Hemisphere (e.g. at 14–130-m depth off New Zealand: Probert and Wilson 1984; at Fuerte Bulnes and Bahía Laredo, Chile: Ríos et al. 2007; in an intertidal soft-bottom area in the South Shetland Islands: Bick and Arlt 2013), which considerably broadens its depth and geographic distribution ranges (Figure 1). Temperature regimes at these locations range from sub-Antarctic/Antarctic (Probert and Wilson 1984; Ríos et al. 2007; Bick and Arlt 2013) to sub-tropical/tropical (Corrêa et al. 2014; Lozano-Cortés et al. 2019), providing further evidence that *L. magalhaensis* may comprise a species complex. *Lumbrineris magalhaensis* was recently reported from holdfasts of the brown alga *Himantothallus grandifolius* in Antarctic waters (Pabis and Sicinski 2010) and as an epibiont on the carapace of a hawksbill sea turtle *Eretmochelys imbricata* off the south coast of Brazil (Corrêa et al. 2014), suggesting the potential for natural, wide range dispersal. Nonetheless, the aforementioned ranges appear still too wide, and conditions too varied, for one species to occupy naturally.

Specimens identified as *L. magalhaensis* using the monograph on the Polychaeta of southern Africa by Day (1967) were recently collected from the Knysna Estuary on the south coast of South Africa. Therefore, the aims of this study were to investigate whether *L. magalhaensis* does occur in South African waters, through comparisons with the most-updated description of the nominal species according to Carrera-Parra (2006a) and other sources, including McIntosh (1885) and Day (1960, 1967), and to generate mtDNA cytochrome *c* oxidase subunit I (COI) sequences for future reference. We hypothesise that: (i) *Lumbrineris magalhaensis* sensu stricto and *Lumbrineris pettigrewi* comb. nov. reinst. are separate, valid species; and (ii) the specimens recently collected from the Knysna Estuary represent neither of those species, as the samples were collected at notably different depths and salinities (and latitudes in the case of *L. magalhaensis* sensu stricto).

Materials and methods

Sample specimens

The museum specimens examined included *L. magalhaensis* from the Iziko South African Museum (A20561, 1 specimen; A21131, 10 specimens), and *L. pettigrewi* from the Natural History Museum, London (1921.5.1.1613–1614, 1 specimen; 1885.12.1.177, 2 specimens) (see Materials Examined).

Polychaetes were also newly collected from the Knysna Estuary, Western Cape Province, South Africa, in austral summer (10–17 January 2021), autumn (2–16 May 2021) and winter (23–30 July 2021), at 10 intertidal sites along the estuary, from the mouth to the river (Figure 2; Table 2). Three sediment samples were collected from each site at low tide, using a circular PVC core of 0.007 m^2 and 10-cm diameter, to a depth of 10 cm. The sediment samples were sifted on-site (0.5-mm mesh sieve), stored in estuary water, and later sorted. The freshly collected polychaetes were kept in estuary water or in 7% magnesium chloride solution, and identified to genus level according to Day (1967) under a stereoscopic microscope; the specimens were then fixed individually in 96% ethanol for morphological examination and molecular processing. The samples were collected under permits issued to CAS (RES2021/04: Department of Forestry, Fisheries and the Environment; SIMO-CR/2019-006: South African National Parks).

Morphological examination

Using the identification keys in Day (1967) and Carrera-Parra (2006a), the characters examined were: lengths of the pre- and post-chaetal lobes; prostomium shape; presence of simple and/or composite multidentate hooded hooks (SMHH and CMHH, respectively) and limbate chaetae along the body; aciculae colour; and number and colour of teeth on all maxillae (i.e. MI–MV, indicating the position of different maxillary parts from posterior to anterior). Additional characters examined following Carrera-Parra (2006a) were: total length of complete specimens (TL); length from the prostomium to the 10th chaetiger (L10); width of the 10th chaetiger (W10), excluding parapodia; length of the peristomium compared with the prostomium; ventral buccal lips (sometimes called “bosses”, as in McIntosh [1885]); presence of dorsal cirri and notoaciculae; numbers of teeth on SMHH and CMHH; size of the maxillary carriers; and size of the MII connecting plates. All measurements were taken by means of a graduated eyepiece on the stereomicroscope.

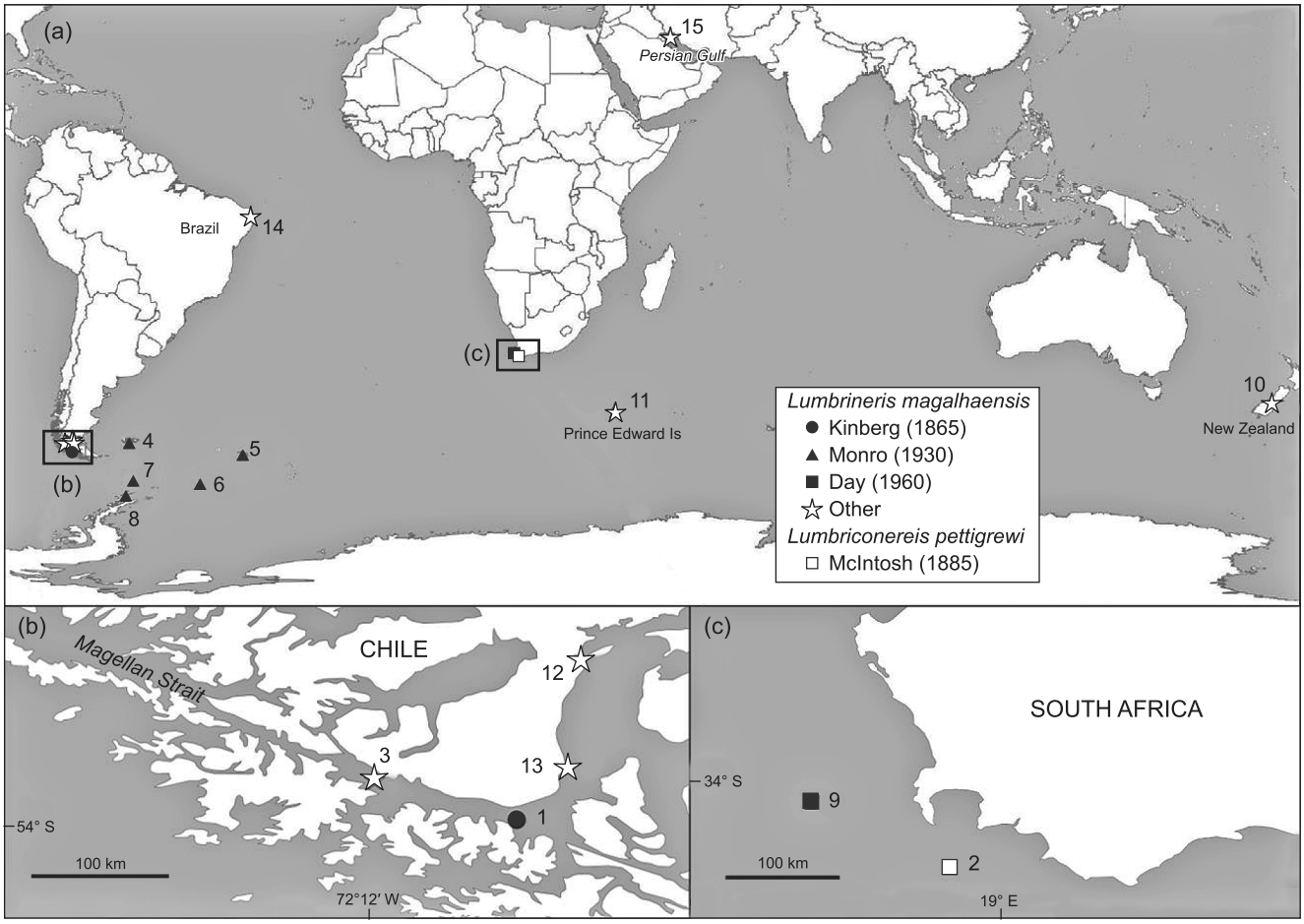

Figure 1: (a) Apparent distribution of *Lumbrineris magalhaensis*: black circle = holotype, Kinberg (1865); black triangles = specimens of Monro (1930); black square = specimen of Day (1960); white stars = *L. magalhaensis* specimens identified using the key in Day (1967); white square = *Lumbriconereis pettigrewi* McIntosh (1885) specimens. Close up of reported localities in (b) Magellan Strait, Chile, and (c) South Africa. Note: Ehlers (1897) did not provide geographic coordinates, thus we chose random coordinates within the Magellan Strait to best fit the map. Map created using the QGIS geographic information system (http://qgis.org)

Table 1: List of historic collection sites for *Lumbrineris magalhaensis* and *Lumbrineris pettigrewi* comb. nov. reinst., as referred to in Figure 1, organised by year of publication

Label on map	Collector/Author	Publication date	Collection locality	Coordinates
1	Kinberg [type locality]	1865	Strait of Magellan, Chile	53˚58′ S, 70˚75′ W
2	McIntosh [type locality]	1885	South Africa	34˚41′ S, 18˚36′ E
3	Ehlers	1897	Strait of Magellan, Chile	–
4	Monro	1930	Falkland Islands	51˚79′ S, 59˚52′ W
5	Monro	1930	South Georgia and South Sandwich Islands	54˚41′ S, 36˚58′ W
6	Monro	1930	South Orkney Islands	60˚35′ S, 45˚30′ W
7	Monro	1930	South Shetland Islands	62˚59′ S, 59˚91′ W
8	Monro	1930	South Shetland Islands	62˚59′ S, 59˚91′ W
9	Day	1960	South Africa	34˚17′ S, 17˚53′ E
10	Probert and Wilson	1984	South Island of New Zealand	45˚53′ S, 170˚48′ E
11	Branch	1994	Marion Island	46˚90′ S, 37˚73′ E
12	Ríos et al.	2007	Strait of Magellan, Chile	52˚56′ S, 70˚50′ W
13	Ríos et al.	2007	Strait of Magellan, Chile	53˚38′ S, 70˚55′ W
14	Corrêa et al.	2014	Brazil	08˚30′ S, 35˚00′ W
15	Lozano-Cortes et al.	2019	Persian Gulf	27˚42′ N, 48˚93′ E

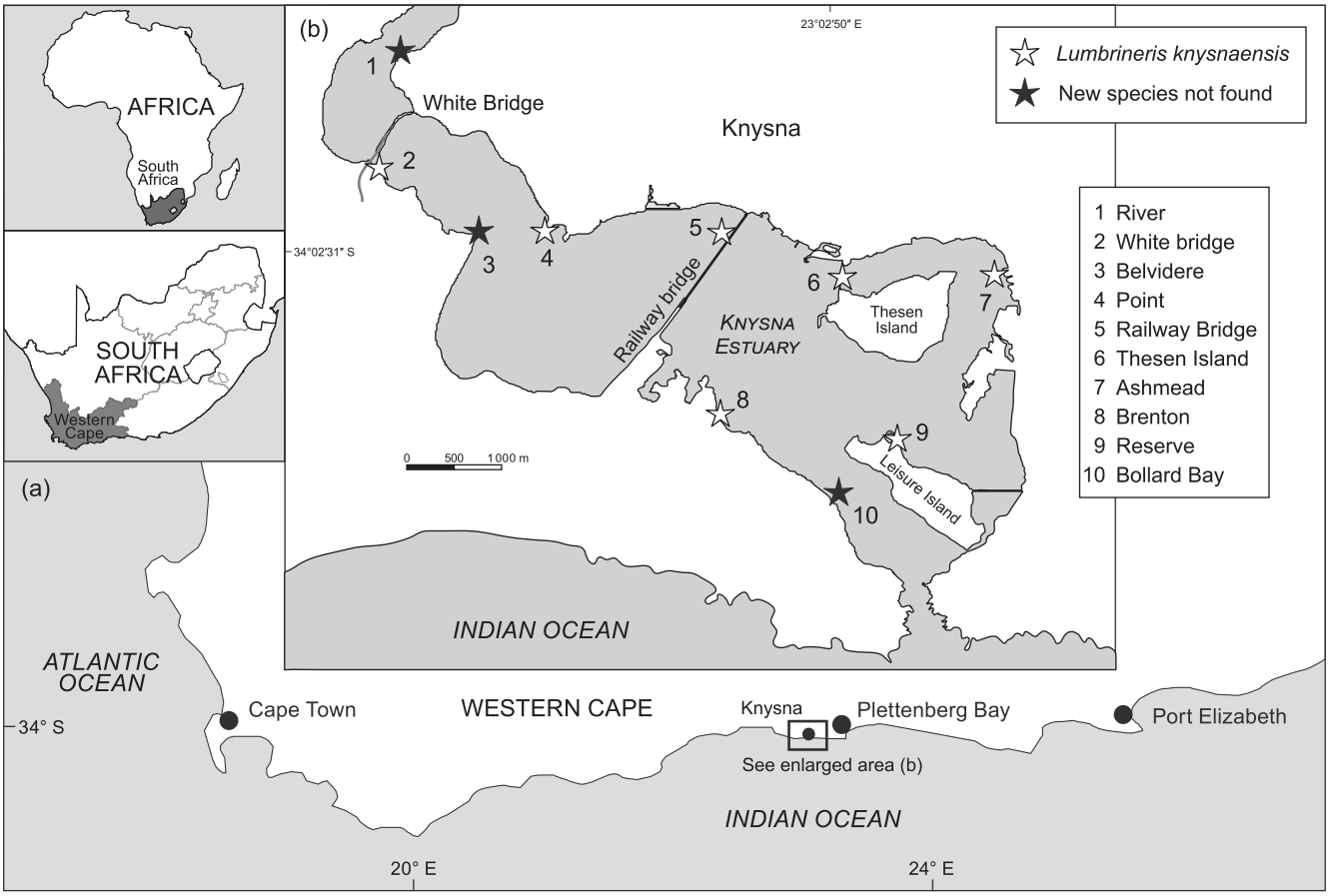

Figure 2: Locations of the 10 intertidal sites in the Knysna Estuary, South Africa, where sampling for polychaetes was carried out (see Table 2 for coordinates). Map created using the QGIS geographic information system (http://qgis.org)

Table 2: Locations and geographic coordinates of the collection sites at the Knysna Estuary, Western Cape Province, South Africa, as labelled in Figure 2, listed from the upper estuary to the mouth

Site no.	Site name	Coordinates
1	River	34°01′22.1″ S, 22°59′33.5″ E
2	White Bridge	34°01′56.6″ S, 22°59′30.2″ E
3	Belvidere	34°02′22.4″ S, 23°00′15.6″ E
4	Point	34°02′21.8″ S, 23°00′44.7″ E
5	Railway Bridge	34°02′22.7″ S, 23°02′04.3″ E
6	Thesen Island	34°02′32.5″ S, 23°02′56.1″ E
7	Ashmead	34°02′38.6″ S, 23°03′58.5″ E
8	Brenton	34°03′31.6″ S, 23°02′08.8″ E
9	Reserve	34°02′38.6″ S, 23°03′58.5″ E
10	Bollard Bay	34°04′08.8″ S, 23°03′01.3″ E

The fresh specimens collected in this study were examined under a stereo microscope (Leica MZ75) and compound microscope (Leica DM1000). Photographs were taken with a microscope camera (Leica EC3) attached to each microscope, using the Leica LAZ EZ imaging software and the focus stacking software Helicon Focus 7, and plates were prepared in Photoshop 23. Three parapodia were removed from each specimen: one each from the anterior (the first 10 chaetigers), middle (~20th–40th chaetigers) and posterior (40th chaetiger onwards), depending on where the chaetal types changed. These regions were defined as ‘anterior’ based on limbate chaetae and CMHH present; ‘middle’ based on limbate chaetae and SMHH present; and ‘posterior’ based on only SMHH present (Carrera-Parra 2006a). The parapodia were stained with methyl green and then set in semi-permanent slides. After examining the anterior of the specimen, an anterodorsal dissection was made to view the maxillae without removing them.

Molecular analysis

DNA was extracted from ~25 mg of tissue per sample following the instructions of the ZR Genomic DNA–Tissue MiniPrep Kit. Universal mitochondrial primers (LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′; HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) were used to amplify a fragment of the mitochondrial gene. The mixture used to perform polymerase chain reactions (PCR) was composed of 12.5 µl of OneTaq Quick-Load Master Mix (New England Biolabs), 9.5 µl of molecular biology grade water, 0.5 µl of each the forward and reverse primers (10-µM concentration), 1 µl of 1% bovine serum albumin, and 1 µl

of template DNA. The thermal conditions for all samples were as follows: 95 °C for 2 min, 35 cycles at 94 °C for 20 s, 47 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR amplicons were sequenced using the forward and reverse primers at the Central Analytical Facility at Stellenbosch University, South Africa. All newly generated sequences were submitted to GenBank, with accession numbers OR666956–OR666976.

Phylogenetic analysis

Raw sequences were edited using BioEdit (Hall 1999) and aligned with the ClustalW multiple alignment method, and then blasted with the BLAST algorithm in GenBank. A DNA sequence translation was executed through EMBOSS Transeq using the invertebrate mitochondrial codon table (Madeira et al. 2022). The UniProt Knowledgebase (UniProtKB) (The UniProt Consortium 2023) was used to find a COI gene sequence to compare with the newly designated *L. knysnaensis* sp. nov., and hence to verify that no pseudo genes had been sequenced. *Mesochaetopterus xerecus* Petersen & Fanta, 1969 of the family Chaetopteridae was selected as the closest polychaete species with an available COI gene sequence that had been verified to transcript level (the second-strongest level of evidence for the existence of a protein on UniProtKB, since the first level was not available for the COI gene in Annelida) (The UniProt Consortium 2023).

The pairwise sequence alignment tool on EMBOSS Needle was used to compare the COI gene sequences of *M. xerecus* and *L. knysnaensis* sp. nov. The level of intraspecific and interspecific sequence divergence was calculated using MEGA11 version 11.0.13 (Tamura et al. 2011). Several species belonging to *Lumbrineris* were used as ingroup taxa (Table 3). *Arabella semimaculata* (Moore, 1911) of family Oenonidae was used as the outgroup to root the phylogeny (Zhou et al. 2010).

MrModeltest 2.4 (Nylander 2008) was used to find the best-fit model of evolution; using the Akaike information criterion method, the GTR+G model was selected. Using the selected best-fit model of evolution, a phylogenetic tree was reconstructed in MrBayes 3.2.7 (Ronquist et al. 2012) following the Bayesian inference (BI) algorithm. The BI tree was obtained using four Markov chains of 700 000 generations sampled simultaneously, with every 1 000th tree sampled. The first 25% of trees were discarded as burn-ins, and the remaining trees were used to create a 50% majority-rule consensus tree. Tracer 1.7.2 (Rambaut et al. 2013) was used to examine and investigate convergence between runs. Effective sample size (ESS) values of >200 indicated good mixing quality of the parameters, which was satisfied, in addition to the average standard deviation of split frequencies which was <0.01. Figtree 1.4.4 (Rambaut 2018) was used to analyse and edit the tree. MEGA11 11.0.13 was used to find the best DNA/protein model for a maximum-likelihood (ML) analysis (all parameters left standard). To confirm the best model, the COI gene was partitioned into the three codon positions, and optimal models for each codon were determined using PartitionFinder (Lanfear et al. 2012, 2016). Partitioned COI data analyses were performed in RAxML and MrBayes (Guindon et al. 2010). The AIC criterion was used to select

Table 3: GenBank accession numbers, collection locations, and reference data of cytochrome *c* oxidase subunit 1 (COI) sequences of the *Lumbrineris* ingroup taxa, and *Arabella semimaculata* as the outgroup

Species	GenBank accession no.	Collection locality	Reference
Lumbrineris knysnaensis sp. nov.	OR666956–OR666976	Knysna Estuary, South Africa: 34°02′ S, 23°03′ E	This study
Lumbrineris aberrans	KJ466054	India	Sivaraj et al. (2014)
Lumbrineris erecta	HM473450	Bamfield, north of Sandford Islands, Canada: 48°53′24″ N, 125°10′48″ W	Carr et al. (2011)
Lumbrineris japonica	HQ932605; HQ932665	Canada: 54°1′22.8″ N, 132°5′56.4″ W; 54°1′58.8″ N, 132°3′10.8″ W	International Barcode of Life (2018)
Lumbrineris latreilli	KR916859	Estuário do Sado, Alentejo Litoral, Portugal: 38°29′3.2″ N, 8°53′6″ W	Lobo et al. (2015)
Lumbrineris mixochaeta	OM237807.1; OM237808.1	Norway: 69°22′51.6″ N, 19°2′38.4″ E	Borisova and Budaeva (2022)
Lumbrineris perkinsi	KP254185	Indian River Lagoon, Florida, United States: 27°29′16.8″ N, 80°18′50.4″ W	Larey and Knowlton (2015)
Arabella semimaculata	MK550647; MK550651	White Point, San Pedro, California, United States: 33°42′50.4″ N, 118°19′1.2″ W	Wetzer et al. (2020)

the most-likely model of sequence evolution for each codon. The ML analysis was run in MEGA11, with 1 000 bootstrap replicates.

Results

A total of 177 specimens of the genus *Lumbrineris* de Blainville, 1828, and tentatively identified as representing one species, were collected from the Knysna Estuary, Western Cape Province, South Africa. Molecular analyses (described below) were conducted for species delimitation. A new species is described based on 25 specimens from Knysna, inclusive of the holotype, paratypes and additional material. *Lumbrineris knysnaensis* sp. nov. most closely resembles *L. magalhaensis* Kinberg, 1865 (see Appendix Table 1). Finally, we present evidence to support *Lumbrineris pettigrewi* comb. nov. reinst. as a valid species.

Molecular characterisation, sequence analysis and taxonomic description

Mitochondrial DNA sequences were generated for 23 of the specimens collected from the Knysna Estuary: 21 sequences were 637–639 bp long, 1 sequence was 621 bp, and 1 sequence was 601 bp. Sequences were translated and, according to where sequences had been cut off while cleaning, 11 sequences were shown to be frame 1, 7 sequences were frame 2, and 3 sequences were frame 3. No stop codons interrupted the gene (Madeira et al. 2022). GenBank sequence OR666956 (reading frame 1), with an open reading frame (ORF) start of ATC and an end ORF of ACC, was used for further analysis. EMBOSS Needle pairwise sequence alignment between *M. xerecus* and *L. knysnaensis* sp. nov. indicated 81.1% similarity (which would likely be higher if our sequences had been longer or of equal lengths for comparison), with no disagreements.

The best model for the ML analysis according to MEGA11 was indicated to be HKY+G+I; the optimal models for the first and second codon positions were determined to be GTR+G; and the third codon position was characterised by SYM+G. Thus, GTR+G was selected initially, but the tree did not in any way agree with that of the BI tree, with the outgroup taxon (*Arabella semimaculata*) being inside of the ingroup, and low posterior probability support at all sites. Therefore, the GTR+G+I model was run next to include unchanging sites, as this was the third-best model as indicated by the AIC score. The partitioned dataset using the ML and BI analyses revealed similar results, with the exception of *Lumbrineris latreilli* Audouin & Milne Edwards, 1833 moving from a sister species of *L. knysnaensis* sp. nov. in the BI tree to sister of all other *Lumbrineris* species in the ML tree. The latter node had low bootstrap and posterior probability support ($p < 0.45$, <60%).

Phylogenetic tree reconstruction revealed 100% support for a genus *Lumbrineris*, with *A. semimaculata* used to root the tree. Both the BI tree (Figure 3) and ML tree demonstrated that *L. knysnaensis* sp. nov. forms an independent species clade with strong Bayesian posterior probability (1.0) and strong likelihood (99%) support.

Intraspecific sequence divergence in *L. knysnaensis* sp. nov. was extremely low — that is, five orders of magnitude lower than interspecific distances with the other lumbrinerid species included in this study (Table 4). Other species for which more than one specimen per species was included in the phylogenetic tree also had low intraspecific sequence divergence. All interspecific sequence variations between *L. knysnaensis* sp. nov. and each of the other species used in the phylogenetic tree had similarly low levels of variation ($p = 0.241–0.292$) that did not stand out among the level of variation between the other *Lumbrineris* species sequences from GenBank ($p = 0.159–0.293$).

Systematics

***Lumbrineris knysnaensis* van Niekerk, Kara, Alvarez-Aguilar & Simon sp. nov.**

(Figure 4a–i)

Non *Lumbrineris magalhaensis* Day (1960, 1967); Carrera-Parra (2006a).

Holotype: Brenton, Knysna Estuary, Western Cape Province, South Africa, 27 July 2021 (SAMC-A089237); coll. AM van Niekerk, D Beeslaar and A Alvarez-Aguilar.

Paratypes: South Africa, Western Cape, Knysna Estuary – Railway Bridge, group 1 (2 specimens: SAMC-A089238, SAMC-A089239), 34°02′22.7″ S, 23°02′04.3″ E, 11 January 2021; White Bridge, group 2 (2 specimens: SAMC-A089240, SAMC-A089241), 34°01′56.6″ S, 22°59′30.2″ E, 16 January 2021; Brenton, group 3 (3 specimens: SAMC-A089242, SAMC-A089243, SAMC-A089244), 34°03′31.6″ S, 23°02′08.8″ E, 17 January 2021; Brenton, group 4 (6 specimens: SAMC-A089245, SAMC-A089246, SAMC-A089247, SAMC-A089248, SAMC-A089249, SAMC-A089250), 34°03′31.6″ S, 23°02′08.8″ E, 9 May 2021; Railway Bridge, group 5 (1 specimen: SAMC-A089251), 34°02′22.7″ S, 23°02′04.3 ″E, 10 May 2021; Ashmead Channel, group 6 (1 specimen: SAMC-A089252), 34°02′38.6″ S, 23°03′58.5″ E, 11 May 2021; Railway Bridge, group 7 (2 specimens: SAMC-A089253, SAMC-A089254), 34°02′22.7″ S, 23°02′04.3″ E, 11 May 2021; Reserve, group 8 (3 specimens: SAMC-A089255, SAMC-A089256, SAMC-A089257), 34°02′38.6″ S, 23°03′58.5″ E, 24 July 2021; Thesen Island, group 9 (2 specimens: SAMC-A089258, SAMC-A089259), 34°02′32.5″ S, 23°02′56.1″ E, 26 July 2021; Brenton, group 10 (1 specimen: SAMC-A089260), 34°03′31.6″ S, 23°02′08.8″ E, 27 July 2021; Point, group 11 (1 specimen: SAMC A089261), 34°02′21.8” S, 23°00′44.7” E, 28 July 2021; coll. AM van Niekerk, D Beeslaar and A Alvarez-Aguilar.

Additional material examined

South Africa, Western Cape, Knysna Estuary – September 2020 collection (22 specimens): Brenton, Thesen Island and Railway Bridge; January 2021 collection (33 specimens): Reserve, Brenton, Thesen Island, Railway Bridge and Point; May 2021 collection (53 specimens): Brenton and Thesen Island; July 2021 collection (41 specimens): Reserve, Brenton, Thesen Island and Railway Bridge; coll. A Alvarez-Aguilar and E Castillo-Olguin.

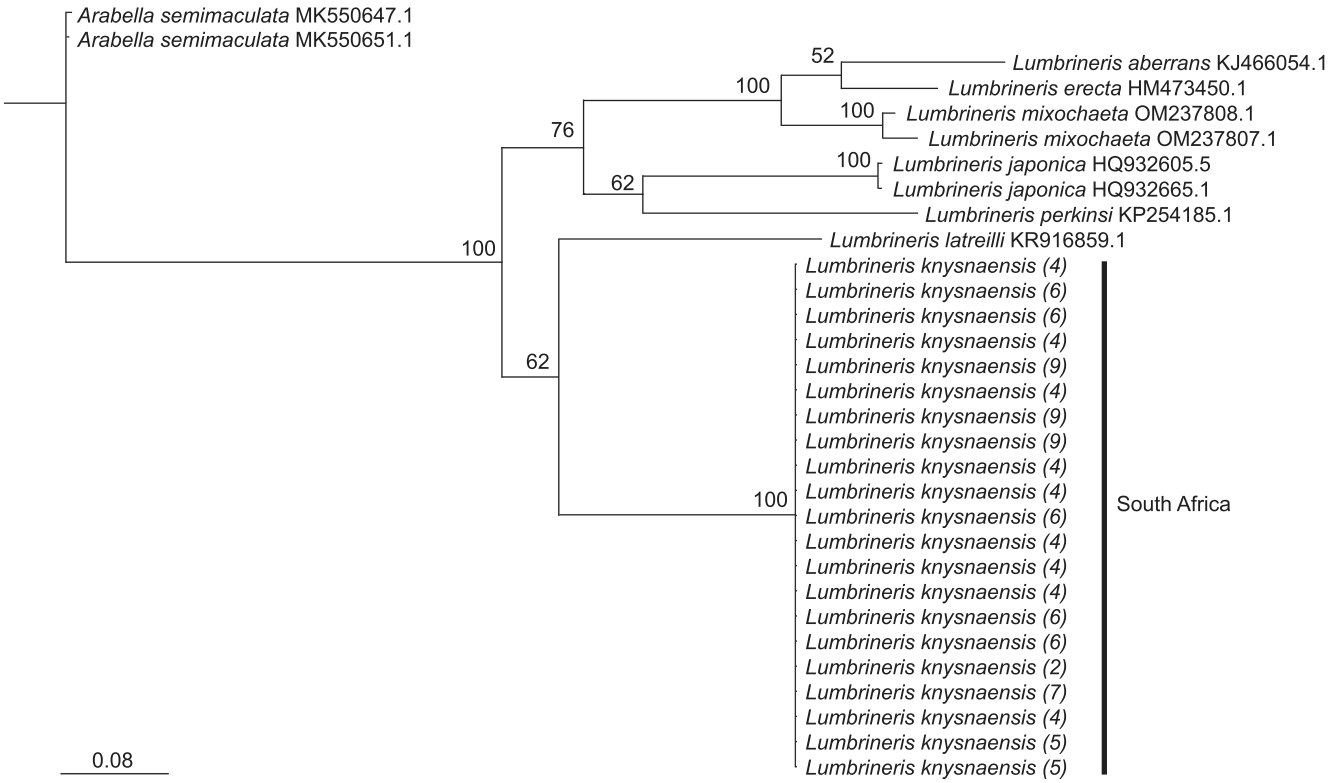

Figure 3: Bayesian tree of COI sequences generated for *Lumbrineris knysnaensis* sp. nov. collected from the Knysna Estuary, South Africa, followed by the collection locality (refer to the number labels in Figure 2), together with other *Lumbrineris* sequences obtained from GenBank. *Arabella semimaculata* was used as the outgroup. Values at nodes represent Bayesian probability support followed by maximum-likelihood bootstrap support, in percentages. Scale bar represents the number of nucleotide substitutions per site (see Table 4)

Iziko South African Museum collection
Lumbrineris magalhaensis: A20561 (1 specimen), Atlantic Ocean, South Africa, 34°17' S/17°53' E, depth 320 m, August 1960, coll. J Day; A21131 (10 specimens), southern Indian Ocean, Marion Island, Bullard Bay, stations BB10.3 + BB11.1, 46°90′ S, 37°73′ E, depth 15 m, 1994; coll. GM Branch.

Description: Data for the holotype are followed by data for the paratypes and additional material in parentheses where characters deviate from the holotype. Holotype complete with 140 chaetigers, TL = 59.81 mm, L10 = 4.25 mm, W10 = 1.56 mm (paratypes: with 77–200 chaetigers, TL = 10.1–86.8 mm, L10 = 2.03–5 mm, W10 = 0.6–2.32 mm; additional material: TL = 16.5–121.6 mm, L10 = 3–10.8 mm, W10 = 0.63–4.63 mm). Colour iridescent pink to beige, retained after preservation (Figure 4a–c). Prostomium conical and short, as long as or slightly longer than wide (Figure 4c); pair of ventral pads and defined buccal lips (Figure 4b). Peristomium shorter than prostomium, two segments, first segment twice as long as second (Figure 4c). Maxillary apparatus black (Figure 4d); 5 pairs of maxillae (here MI–MV); short maxillary carriers narrowing to point posteriorly, half length of MI (paratypes: half to equal length of MI). MI forceps-like (single tooth), attachment lamella present from middle to anterior of MI. MII three-fourths length of MI, with 4 or 5 teeth; silver-white connecting plates, one-quarter length of maxillary carriers. MIII bidentate; MIV unidentate; MV kite-shaped with rounded points, free, lateral to MIII and MIV (Figure 4d).

Pre-chaetal lobe small, round, shorter than post-chaetal lobe in all parapodia (Figure 4c,e,f,g). Post-chaetal lobe digitiform with tapering end, shortens from mid-body to end (Figure 4e,f,h). Tiny dorsal cirri with 3 or 4 notoaciculae (Figure 4h). Cmhh (Figure 4e,h) on chaetigers 1–22 (paratypes: on chaetigers 1 to 6–35, depending on body length [Figure 5a], do not always end on left and right parapodia of same chaetiger). Cmhh replaced by SMHH (Figure 4e–g) at chaetiger 23 (paratypes: at chaetigers 7–36), sometimes the transition parapodium has CMHH and SMHH that continue to last chaetiger. Limbate chaetae from chaetigers 1–33 (paratypes: from chaetigers 1 to 9–40 [Figure 4e,f]). Anterior parapodia with 4 CMHH and 5 limbate chaetae (paratypes: with 2–5 CMHH and 2–6 limbate chaetae), both reducing in number along body (Figure 4e,f). Where limbate chaetae are present, 2 SMHH and 2 limbate chaetae (paratypes: with 1–4 SMHH per parapodium, and 1–3 limbate chaetae) (Figure 4f). Posterior parapodia with 3 SMHH (paratypes: with 3 or 4 SMHH) (Figure 4g). Smhh with 1 large crenulated tooth and 7–12 progressively smaller teeth (Figure 4i); CMHH with 7–9 equally sized teeth (Figure 4h). Aciculae yellow, 2 per parapodium (paratypes: 2 or 3 aciculae in posterior parapodia, and one specimen with 4 aciculae in anterior parapodia) (Figure 4e,g). Pygidium with two pairs of similarly sized anal cirri.

Table 4: Interspecific and intraspecific (bold font) sequence divergence *p*-values for species of *Lumbrineris*, with *Arabella semimaculata* as the outgroup. n/c = not calculated

	L. knysnaensis sp. nov.	*L. erecta*	*L. japonica*	*L. aberrans*	*L. perkinsi*	*L. latreilli*	*L. mixochaeta*	*A. semimaculata*
L. knysnaensis sp. nov.	$7e^{-6}$							
L. erecta	0.261	**n/c**						
L. japonica	0.272	0.235	**0.003**					
L. aberrans	0.292	0.159	0.293	**n/c**				
L. perkinsi	0.252	0.250	0.239	0.287	**n/c**			
L. latreilli	0.241	0.275	0.280	0.287	0.274	**n/c**		
L. mixochaeta	0.249	0.162	0.273	0.167	0.267	0.269	**0.0345**	
A. semimaculata	0.281	0.313	0.280	0.353	0.329	0.298	0.351	**0.0044**

Etymology: Named for the Knysna Estuary where the specimens were collected.

DNA barcode: Type locality: Knysna Estuary, Western Cape, South Africa (SAMC-A089237). GenBank accession no. OR666974: a 638-bp fragment isolated using the universal mitochondrial cytochrome *c* oxidase subunit 1 gene, with primer pair LCO1490 and HCO2198 (Folmer et al. 1994).

Distribution and ecology: *Lumbrineris knysnaensis* sp. nov. is so far known only from the Knysna Estuary, Western Cape Province, South Africa. Specimens were collected from the intertidal, in sandy or muddy flats, at 8 of 10 sites sampled (not at Bollard Bay and Belvidere: see Figure 2). At sites where collected, the surficial water temperature was 10.4–23.8 °C (mean 17.71 °C), salinity 28–38 g l^{-1} (mean 32.2 g l^{-1}), dissolved oxygen 4.57–9.27 mg g^{-1} (mean 7.31 mg g^{-1}), and organic matter in the sediment 0.76–2.17% (mean 1.45%) (A Alvarez-Aguilar, pers. obs.). Sediment grain size at the sampling sites was ~0.125–0.25 mm, denoting fine to medium sand (Wentworth 1922).

Remarks: *Lumbrineris knysnaensis* sp. nov. most closely resembles *L. magalhaensis* as it matches the redescription of the type material (2 specimens) of *L. magalhaensis* (Carrera-Parra 2006a) as follows: MIII bidentate, MIV unidentate, well-developed buccal lips, and tiny dorsal cirri with 3 to 4 notoaciculae (Appendix Table 1). Differences include a prostomium that is rounded in *L. magalhaensis* but conical in *L. knysnaensis* sp. nov.; MII with 4 teeth in *L. magalhaensis*, but 5 teeth in *L. knysnaensis* sp. nov.*;* MII connecting plates are half the length of the maxillary carriers in *L. magalhaensis* (Carrera-Parra 2006a, p 39), but one-fourth the length in *L. knysnaensis* sp. nov. (Figure 4d). In the type specimens of *L. magalhaensis*, CMHH are present only from the 1st to 11th chaetigers, whereas in *L. knysnaensis* sp. nov. the CMHH are present from the 1st to the 6–35th chaetigers (Figure 5a). Limbate chaetae occur from the 1st to 75th chaetigers in *L. magalhaensis*, but only from the 1st to between the 9th and 40th chaetigers in *L. knysnaensis* sp. nov. (Figure 5b). The distributions of the multidentate hooded hooks and limbate chaetae depend on body size; thus, for specimens of similar body width in the two species, the distribution is similar for the CMHH, except that *L. magalhaensis* has nearly twice as many limbate chaetae (Figure 5).

Lumbrineris knysnaensis sp. nov. and specimens identified by Day (1960) as *L. magalhaensis* (but here recognised as representing the reinstated *L. pettigrewi*) are both described as having a short and conical prostomium. One specimen collected by Day (1960) (A20561, from depth of 320 m off the Cape of Good Hope, South Africa) was re-examined in this study, but the chaetae were too damaged to confirm the distribution of the CMHH. A single limbate chaeta was found on the 40th parapodium, but this is not enough to compare the character to the range in *L. knysnaensis* sp. nov. The main differences are as follows: in *L. knysnaensis* sp. nov. the MII connecting plates are ~25% the length of the maxillary

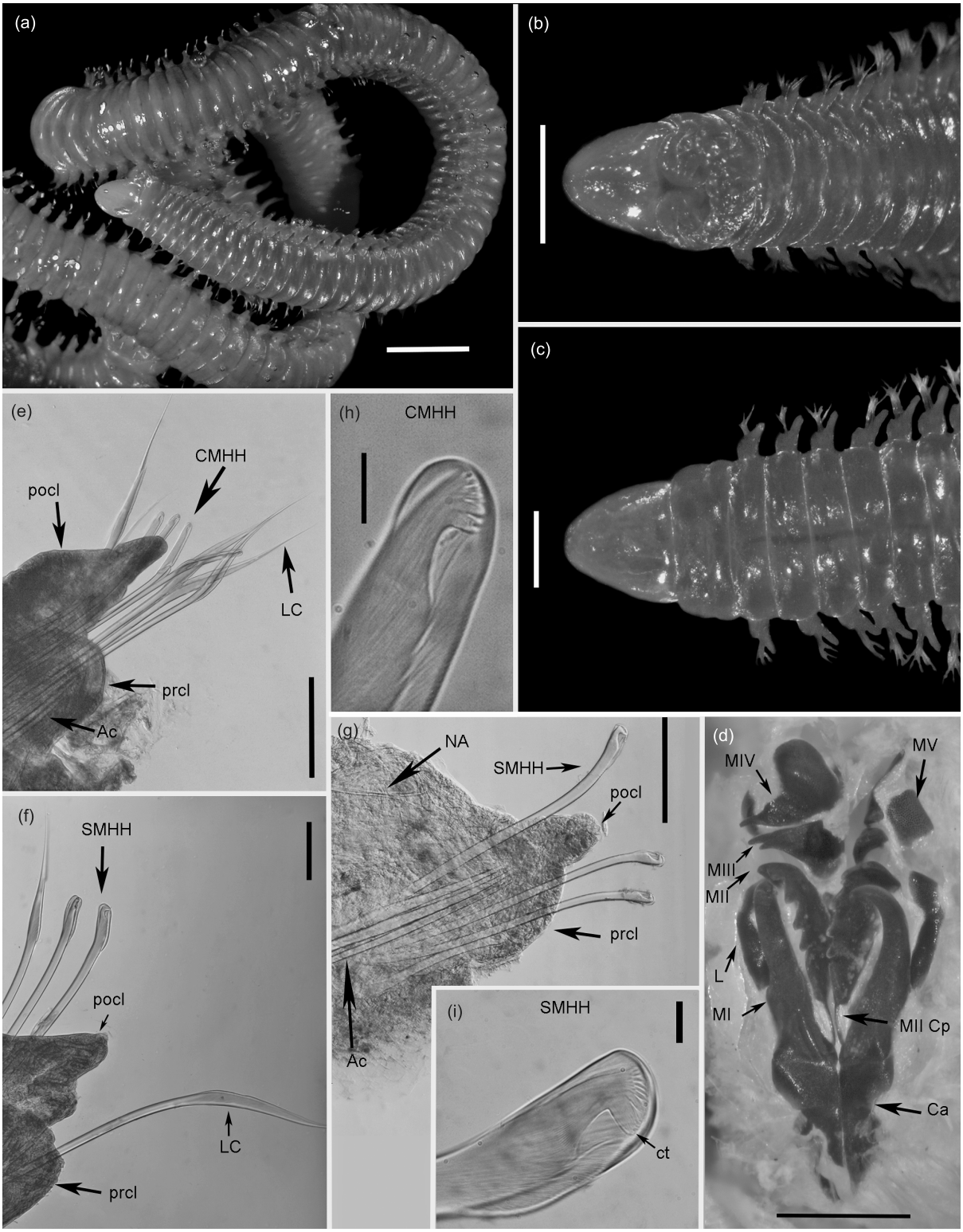

Figure 4: Images of *Lumbrineris knysnaensis* sp. nov.: (a) whole body (SAMC-A089245); (b) anterior end, ventral view, showing pair of ventral pads (SAMC-A089237); (c) anterior end, dorsal view (SAMC-A089237); (d) maxillary apparatus (SAMC-A089247); (e) parapodium 8 (right) (SAMC-A089257), with composite multidentate hooded hooks (CMHH) and limbate chaetae (LC); (f) parapodium 25 (left) (SAMC-A089261), with simple multidentate hooded hooks (SMHH) and limbate chaetae (LC); (g) parapodium 48 (right), showing dorsal cirri with notoaciculae (SAMC-A089255); (h) teeth of CMHH chaeta on parapodium 8 (right) (SAMC-A089257); (i) teeth of SMHH chaeta parapodium 26 (right) (SAMC-A089259). Abbreviations: Ac = acicula; Ca = carriers; ct = crenulated hook; L = lamella; MI–MV = maxillae 1–5; MII Cp = maxilla 2 connecting plates; NA = notoaciculae; pocl = post-chaetal lobe; prcl = pre-chaetal lobe. Scale bars: (a) = 2 mm; (b,c) = 1 mm; (d,h,j) = 0.2 mm; (e,g) = 0.005 mm; (f) = 0.02 mm; (i) = 0.5 mm

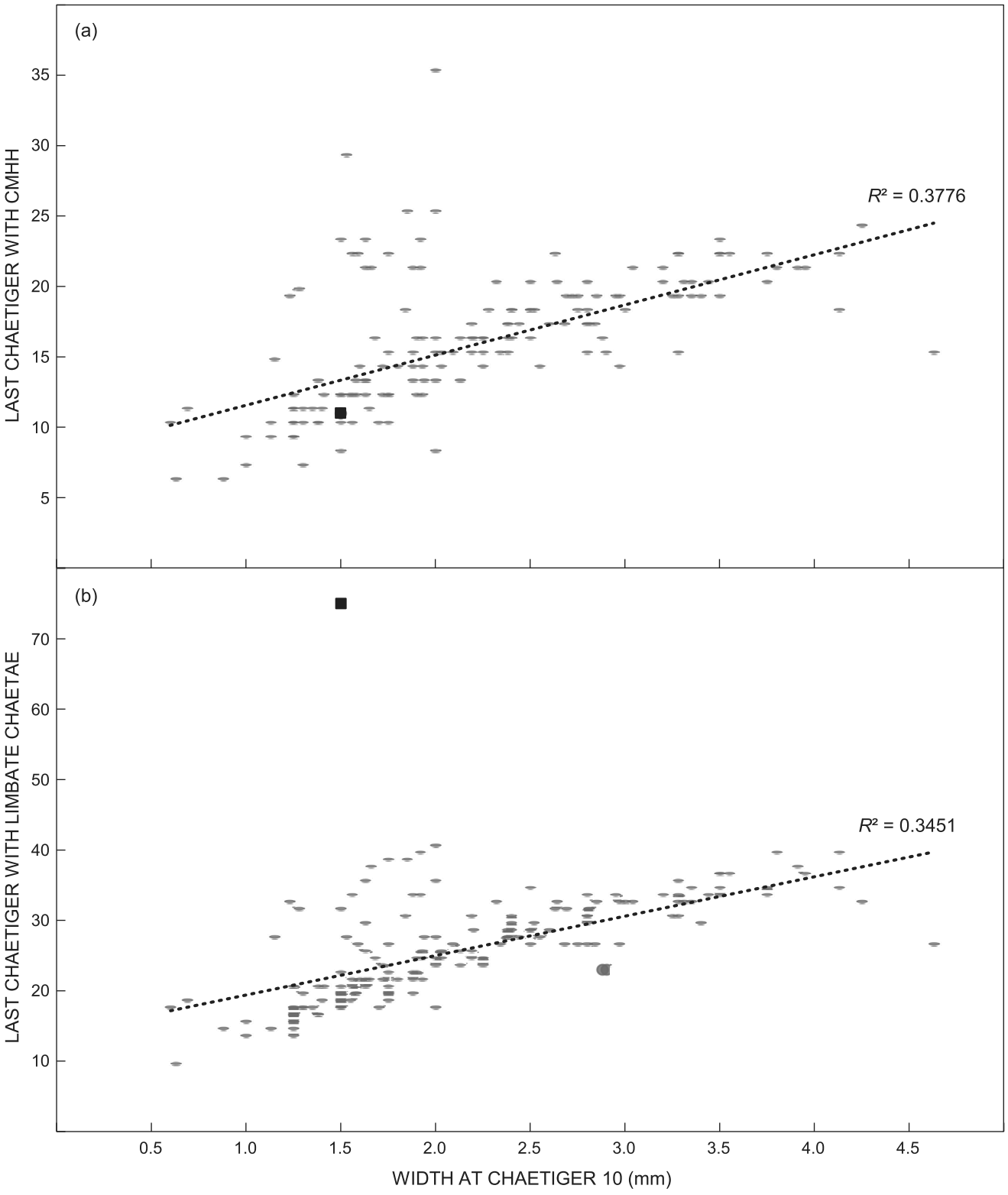

Figure 5: Relationship between the size of the specimen (represented by width of the 10th chaetiger) and the chaetiger where the (a) composite multidentate hooded hooks (CMHH), and (b) limbate chaetae, end. Grey circles represent specimens of *Lumbrineris knysnaensis* sp. nov. collected at the Knysna Estuary, South Africa; black squares represent type specimens of *Lumbrineris magalhaensis* re-examined by Carrera-Parra (2006b)

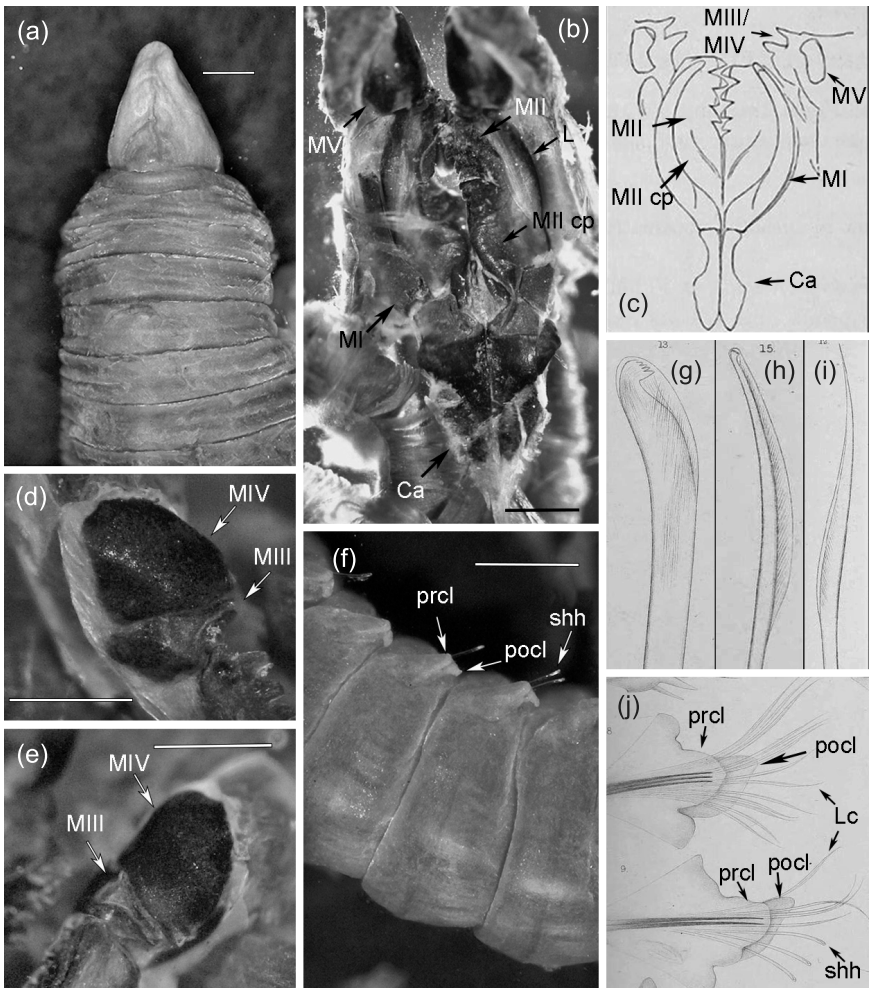

Figure 6: Photographs of the type specimen of *Lumbrineris pettigrewi* McIntosh, 1885 (museum number: 1921.5.1.1613-1614) and original illustrations from McIntosh (1885: pl. XXXVI, and pl. XVII: C, G–J]). (a) Anterior end, dorsal view; (b) maxillary apparatus; (c) maxillary apparatus, original illustration (had been misnamed as "*Lumbriconereis capensis*, Grube" as stated in McIntosh [1885, p. vii]); (d) MIII and MIV on left side (viewed from above); (e) MIII and MIV on right side (viewed from above); (f) section of body showing parapodia and last few remaining simple multidentate hooded hooks (SMHH) remaining on the type specimen; (g) simple hooded hook from a posterior parapodium; (h) "winged hook with elongated tip" from the 10th parapodium; (i) limbate chaeta from the 10th parapodium; (j) 10th parapodium (top) and 30th parapodium (bottom). Abbreviations: Ca = carriers; Lc = limbate chaetae; MI–MV = maxillae 1–5; pocl = post-chaetal lobe; prcl = pre-chaetal lobe; shh = simple [multidentate] hooded hooks. Other abbreviations used in McIntosh (1885): G = n.13; H = n.15; I = n.12; J = n.8 & n.9

carriers (Figure 4d), there are 5 teeth on the MII, and the MIV are unidentate, whereas in the specimens collected by Day in 1960, the MII connecting plates are 75% the length of the maxillary carriers, the MII have 4 teeth, and the MIV are bidentate.

Lumbrineris knysnaensis sp. nov. further differs from *L. magalhaensis* as described by Ehlers (1897), Monro (1930) and Branch (1994) — descriptions that also do not agree with the most-recent redescription of the type material of *L. magalhaensis* by Carrera-Parra (2006b). *Lumbrineris knysnaensis* sp. nov. is unlike *L. magalhaensis* as described by Ehlers (1897) with regard to the following characters: in Ehlers (1897), the SMHH are described as from the 1st to the 47th–59th parapodia, instead of starting at or before the 36th parapodia and continuing to the end of the body, whereas the limbate chaetae continue to the end of the body instead of ending at around the 40th parapodia; furthermore, *L. knysnaensis* sp. nov. has bidentate MIII and unidentate MIV, whereas Ehlers (1897) describes unidentate MIII and bidentate MIV.

The description of *L. magalhaensis* by Monro (1930) also differs from *L. knysnaensis* sp. nov. with respect to the MIII and MIV in the former being described as "undulating cutting plates" (though likely caused by weathering). Other characters described by Monro (1930) are not detailed enough for further comparison.

Specimens identified as *L. magalhaensis* by Branch (1994) collected at Marion Island in the sub-Antarctic southern Indian Ocean were also re-examined in this study. These specimens differ from *L. knysnaensis* sp. nov. in having unidentate MIII (instead of bidentate), a rounded prostomium (instead of conical), and the MII connecting plates almost equal in size to the MII (versus the MII plates one-fourth the size of the MII in *L. knysnaensis* sp. nov.).

The two species also differ with respect to habitat type and geographic locations. *Lumbrineris knysnaensis* sp. nov. was collected in fine to medium sandy sediment (up to only 10 cm deep) in the intertidal of a temperate estuary (Figures 1, 2), whereas one of the specimens identified as *L. magalhaensis* by Day (1960, 1967) (but see below) was collected in the Atlantic Ocean off the coast of South Africa at a depth of 320 m. Recorded locations of both species differ from the type locality of *L. magalhaensis*, which was sampled from floating algae in the sub-Antarctic Magellan Strait, Chile (Kinberg 1865; Ehlers 1897), ~7 000 km away. Similarly, the collection done by Branch (1994) at sub-Antarctic Marion Island was made a vast distance from the type locality of *L. magalhaensis*, thus those specimens should be referred to as *L.* cf. *magalhaensis*.

***Lumbrineris pettigrewi* (McIntosh, 1885) comb. nov. reinst.**

(Figure 6a–i)
Lumbriconereis pettigrewi McIntosh 1885: pp. 239–241, Pl. XXXVI (Figs. 7–9), Pl. XVII (Figs. 11–15).
Lumbrineris magalhaensis Day 1960: pp. 359 (Fig. 12h–j), 362–363; Day 1967: pp. 432–433, Figs. 15a–g, 17.

Material examined: *Lumbriconereis pettigrewi*, London Natural History Museum collection: 1921.5.1.1613–1614 and 1885.12.1.177 (3 syntypes), 34°41′ S, 18°35′ E, off the Cape of Good Hope (station 141), South Africa, 17 December 1873, at 180 m; coll. William C McIntosh.

Comparative material examined: *Lumbrineris magalhaensis*, Iziko South African Museum collection: A20561 (1 specimen), 34°17′ S, 17°53′ E, 8 October 1960, at 320 m; coll. John Day.

Description: Based on the three syntypes of *L. pettigrewi* (McIntosh 1885) – Incomplete, with 20, 129 and 300 chaetigers, respectively; TL = 10.04 mm, 58.08 mm, 83.94 mm; L10 = 4.91 mm, 5.66 mm, 5.15 mm; W10 = 2.21 mm, 2.43 mm, 1.91 mm. Prostomium conical and small (Figure 6a); peristomium with two rings, dorsally 2nd ring half the length of 1st, laterally equal in length, and merges ventrally to form buccal lips equal in length to 1st peristomial ring. Prostomium twice the length of peristomium.

Maxillary apparatus dark brown (Figure 6b); 5 pairs of maxillae (i.e. MI–MV); carriers triangular, cinched near anterior end, short and wide, width equal to two-thirds the length at cinched part. MI forceps-like (single tooth), attachment lamella present from base of MI to anterior of MII. MII half the length of MI, blunted straight anterior end, with 5 teeth. MII connecting plates almost equal in size to MII, opaque white outside edge to pale red-brown middle with inner white edge. MIII bidentate; MIV large, circular, bidentate, teeth to MIII side; MV circular, with indent to articulate with MIV, free, lateral to MIII and MIV (Figure 6b).

Pre-chaetal lobe small, rounded, always shorter than post-chaetal lobe (Figure 6f,j). Post-chaetal lobe ovate, lengthens slightly to midbody, shortening again to be barely longer than pre-chaetal lobes in posterior. Specimens with CMHH and limbate chaetae damaged, and hence their distributions could not be confirmed; in the longest specimen, SMHH present on chaetigers 147–149 (left) and on chaetigers 186 and 187 (right) (Figure 6f).

Remarks: Day (1960) re-examined the type material of *L. pettigrewi* and synonymised the species with *L. magalhaensis* based on similarities in: prostomium shape; numbers of teeth on the MII, MIII and MIV; and the presence of limbate chaetae and CMHH anteriorly. Our re-examination of the syntypes of *L. pettigrewi* designated by McIntosh (1885) and specimens collected and identified as *L. magalhaensis* by Day (1960) indicated high similarity, supporting the idea that these specimens are the same species — but not *L. magalhaensis* sensu stricto. Important similarities include the size and colour of the MII connecting plates (opaque white outside edge to red-brown middle with inner white edge).

Carrera-Parra (2006a) described *L. magalhaensis* as having a rounded prostomium, MII connecting plates half the length of MII, and carriers ~1.5-times as long as wide; in contrast, McIntosh (1885) described *L. pettigrewi* as having an extremely conical prostomium (though this feature has likely shrunk over time because of age and preservation; see Figure 6a), MII connecting plates and MII equal in length, but carriers slightly longer than wide. The specimens from South Africa and from the Magellan Strait, Chile, differ with respect to habitat: the type material of *L. magalhaensis* was collected from floating algae in the sub-Antarctic, whereas the South African specimens were collected in a temperate region, at depths of 180 m and 320 m off the Cape of Good Hope (McIntosh 1885; Day 1960, specimen A20561) and at 88 m in False Bay (Day 1960). *Lumbriconereis pettigrewi* should therefore be removed from synonymy with *L. magalhaensis* and re-instated as *L. pettigrewi* (not as "*Lumbriconereis*" which was considered an "improved" spelling of *Lumbrineris* created by Grube [1840] and not as a different genus, according to Read and Fauchald [2022]).

Similarities between *L. knysnaensis* sp. nov. and *L. pettigrewi* comb. nov. reinst. include the presence of CMHH up to at least the 12th chaetiger, and SMHH as early as on the 30th chaetiger and up to at least the 187th chaetiger. In *L. pettigrewi*, limbate chaetae occur on the 10th and 30th parapodia (Figure 6j), but decrease in number along the length of the body; in this study, SMHH were found on the 147th–149th and 186th–187th chaetigers, thus it is assumed that SMHH are present from at least the 147th–187th chaetae. Here, *L. pettigrewi* is further described as having: a conical prostomium (Figure 6a); MII connecting plates almost equal to MII in size, and with opaque-white outer edge, red-brown middle and white inner edge (Figure 6i); MIV bidentate; maxillary carriers almost as wide as long (Figure 6i); and a winged hook with an elongated tip described for the 10th chaetiger (Figure 6d). In contrast, *L. knysnaensis* sp. nov. has a less conical prostomium; silver-white MII connecting plates, one-quarter the size of the MII (Figure 4d); MIV unidentate (Figure 4d); maxillary carriers ~1.5–2-times as long as wide (Figure 4d), and no winged hook.

Unfortunately, some chaetae and hooded hooks of the specimens collected by McIntosh (1885) were either missing or damaged (Day 1960; this study), thus their shape and exact distribution could not be determined. This prevented a thorough comparison between the specimens collected by John Day and specimens designated *L. pettigrewi.* However, our comparison of the characters that could be examined suggested that specimen A20561 is not *L. magalhaensis* but probably *L. pettigrewi*, and that both species differ from *L. knysnaensis* sp. nov. More specimens are required from the localities where Day (1960) and McIntosh (1885) sampled to address this uncertainty.

Discussion

We confirm that the specimens collected in the Knysna Estuary are not *L. magalhaensis* if following the descriptions of Kinberg (1865), Ehlers (1897) or Monro (1930) or as redescribed by Carrera-Parra (2006a), nor do they match the original description of *L. pettigrewi* by McIntosh (1885). Specimens collected off the coast of South Africa and identified as *L. magalhaensis* by Day (1960) also differ from the specimens collected at Knysna. Therefore, the specimens from the Knysna Estuary are here described as a separate species, *L. knysnaensis* sp. nov.

The specimens collected in this study display many similarities to descriptions of *L. magalhaensis* (i.e. Kinberg 1865; Monro 1930; Hartman 1948; Day 1967; Carrera-Parra 2006a) and *L. pettigrewi* (McIntosh 1885; Day 1960). However, our Knysna specimens do not fully match the original or updated descriptions of either of those nominal species, nor the specimens collected and described by Day (1960) or Branch (1994) that were re-examined here. *Lumbrineris magalhaensis* and *L. knysnaensis* sp. nov. differ in prostomium shape, the size and colour of MII connecting plates, the arrangement of limbate chaetae, and the dentition on MIII and MIV. Furthermore, sufficient differences (i.e. in prostomium shape, dentition on MIV, size and colour of MII connecting plates, and size of the maxillary carriers) were detected between specimens previously identified as *L. pettigrewi* or *L. magalhaensis* to support the reversal of the synonymy of those species, and thus support *Lumbrineris pettigrewi* comb. nov. reinst. as a valid species.

In this study, the arrangement of chaetae relative to the width of the specimen (here represented by width of the 10th chaetiger) is the most important character distinguishing between our fresh specimens of *L. knysnaensis* sp. nov. and the *L. magalhaensis* syntypes described by Carrera-Parra (2006a), confirming that these are separate species. Although preserved specimens are liable to shrink (Thibault-Botha and Bowen 2004; Lucas 2009), shrinkage is unlikely to have influenced the conclusions drawn here. In fact, even if the old syntype material had shrunk to as little as 20–25% of its original size (when the animal was alive or freshly fixed), as found in those studies, the differences between the species would be even more obvious, as there would be no overlap with *L. knysnaensis* sp. nov. with respect to the distributions of the chaetigers with the CMHH and limbate chaetae.

Separation of these taxa based on the morphological differences detected here is supported by differences in the geographic locations and environmental conditions at the habitats they occupy. In this study, we scrutinised the descriptions and/or examined specimens of *L. magalhaensis* from the Atlantic Ocean (inclusive of the type locality: Kinberg 1865; Ehlers 1897) and from the Indian Ocean (McIntosh 1885; Day 1960, 1967; Branch 1994). Not only are these locations up to 7 000 km apart, but the various specimens were collected from different latitudes, depths and habitats. Therefore, these taxa can be divided into four geographical groups (described below) and, accordingly, at least four species (see Appendix Table 1).

Group 1 includes specimens of *L. magalhaensis* from the sub-Antarctic/Antarctic Atlantic Ocean (Kinberg 1865; Ehlers 1897; Monro 1930). Though previous descriptions lack information regarding many of the characters now used to distinguish between species of *Lumbrineris*, comparisons of the descriptions of *L. magalhaensis* by Ehlers (1897) and Monro (1930) with that of the type material re-examined by Carrera-Parra (2006a) also suggest that these specimens could represent more than one species. Carrera-Parra (2006a) describes the type specimens as having a rounded prostomium, MII connecting plates half the length of MII, bidentate MIII, unidentate MIV, and limbate chaetae up to the 75th parapodium; however, in the older descriptions (Ehlers 1897; Monro 1930) the specimens are described as having a conical prostomium, “small connecting plates” (Ehlers 1897), unidentate MIII (Ehlers 1897), bidentate MIV (Ehlers 1897), and limbate chaetae up to the end of the body (Ehlers 1897). Furthermore, the specimens described by Monro (1930) may represent several species; not only were they collected 780–2 200 km from the type locality and intertidally (versus sub-tidally to a depth of 270 m), they were collected in sub-Antarctic and Antarctic regions of the southern Atlantic Ocean that straddle the Polar Front (i.e. at the Falkland and South Georgia islands to the north; at the South Shetland and South Orkney islands to the south), which is known to break connectivity between benthic polychaete communities in the region (Montiel et al. 2016).

Group 2 is represented by *L.* cf. *magalhaensis* collected at a depth range of 150–300 m at Marion Island in the sub-Antarctic southern Indian Ocean by Branch (1994), and it differs from *L. magalhaensis* with respect to the prostomium shape and unidentate MIII.

Groups 3 and 4 are represented by two species from the temperate south coast of South Africa, but from different depths and habitats. The temperate subtidal *L. pettigrewi* (McIntosh 1885; Day 1960; this study, Group 3) was collected at depths of 88–320 m — where water temperatures are about 7 °C lower, and salinity is on average 3 g l^{-1} higher than in the Knysna Estuary, where the temperate intertidal *L. knysnaensis* sp. nov. (this study, Group 4) was collected (McIntosh 1885; Knauth 2011; A Alvarez-Aguilar, pers. obs.). Such a scenario was previously demonstrated for *Petta assimilis* McIntosh, 1885 of family Pectinariidae, which had been reported from various depths and locations (i.e. at 2 926 m,

between Prince Edward Island and Kerguelen Islands; at 1 800–2 000 m, off Cape Horn, Chile; and at 360–376 m, off the south coast of South Africa), but the specimens were eventually shown to be different species, each with narrower depth ranges (Zhang and Hutchings 2021).

Additional material of *L. pettigrewi* from the type locality (Cape of Good Hope, South Africa) is needed to prepare a more comprehensive description of this species, while an inclusion of genetic data will further elucidate the distinction between *L. pettigrewi* and *L. knysnaensis* sp. nov. and relationships among species in the *L. magalhaensis* species complex.

There is some disagreement between the ML and BI phylogenetic trees depicting the placement of one species, namely *L. latreilli*, but all else is similar, including *L. knysnaensis* sp. nov. samples being grouped as one species, with strong support. This inconsistency may be cleared up with the use of more *Lumbrineris* DNA sequences, especially *L. latreilli*. Intraspecific and interspecific variation further supports *L. knysnaensis* sp. nov. as a single species, with very low intraspecific sequence divergence observed across our samples from the Knysna Estuary, and normal interspecific sequence divergence when compared with other *Lumbrineris* species. The COI sequences generated in this study were determined to not be pseudo-genes, as there were no stop codons within the gene, and had a high similarity to another polychaete species (*M. xerecus*) with a high protein-existence score (evidence at transcript level).

This study did not examine putative specimens of *L. magalhaensis* with localities outside of South Africa; however, it is probable that those collected far from the type locality (Magellan Strait, Chile), such as from the sub-Antarctic Pacific Ocean (New Zealand: Probert and Wilson 1984) and from the tropical Persian Gulf (Lozano-Cortés et al. 2019), also represent different species. However, the possibility that a species may disperse passively across long distances should not be discounted; notably, *L. magalhaensis* has been found on floating algae and on brown alga holdfasts in southern oceans (Kinberg 1865; Pabis and Sicinski 2010). Future genetic studies of specimens of *L. magalhaensis* collected from different locations in the Southern and Northern Hemispheres would greatly help to clarify its taxonomic position and to understand its distribution range.

Acknowledgements — The authors would like to thank the National Research Foundation for funding (grant no. 118560, issued to CAS), and the Knysna Basin Project and South African National Parks (Garden Route Park) for logistical support. Danné Beeslaar, Hendré van Rensburg, Stephanie Schoeman, Evangelina Castillo-Olguin and Iker Alvarez-Castillo are thanked for providing field assistance. We also thank two anonymous reviewers and Conrad Matthee whose comments greatly improved the manuscript.

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Manuscript received: 20 October 2023, revised: 16 June 2024, accepted: 23 November 2024
Associate Editor: A Davinack

Appendix Table 1: Comparison of features of *Lumbrineris magalhaensis* and *Lumbriconereis pettigrewi* in earlier descriptions of specimens from various localities, and *Lumbrineris knysnaensis* sp. nov. collected in this study

	Kinberg (1865)	Hartman (1948) [based on type material]	Carrera-Parra (2006a) [based on type material]	Ehlers (1897)	Monro (1930)	Branch (1994)	McIntosh (1885) [based on syntypes: 1921.5.1.1613–1614 and 1885.12.1.177]	Day (1960)	Current study: specimen A20561 of Day (1960)	Current study: freshly collected specimens
Species name in paper	*Lumbriconereis magalhaensis*	*Lumbrineris magalhaensis*	*Lumbrineris magalhaensis*	*Lumbriconereis magalhaensis*	*Lumbrineris magalhaensis*	*Lumbrineris magalhaensis*	*Lumbriconereis pettigrewi*	*Lumbrineris magalhaensis*	*Lumbrineris magalhaensis*	*Lumbrineris knysnaensis* sp. nov.
Locality	Magellan Strait, Chile	Magellan Strait, Chile	Magellan Strait, Chile	Magellan Strait, Chile	South Georgia, Falkland, South Shetland, and South Orkney islands	Marion Island, sub-Antarctic Indian Ocean: 46°90' S, 37°73' E	Off Cape of Good Hope, South Africa: 34°41' S, 18°36' E	Off Cape of Good Hope, South Africa	Off Cape of Good Hope, South Africa: 34°17' S, 17°53' E	Knysna Estuary, south coast of South Africa
Depth	On floating algae	On floating algae	On floating algae	Not recorded	0–270 m	150–300 m	180 m (10 °C)	88 m	320 m	Intertidal, in sediment
Prostomium shape	Rounded	Conical	Rounded	Conical, but with short and long variations, as well as some rounded	Conical	Rounded to conical	Long and conical	Short and conical	Short and conical	Short and conical
MII connecting plates	Not recorded	Not recorded	Half-length of MII	Small connecting plates	Not recorded	Not recorded	Almost as long as MII	Half-length of MII	Almost as long as MII	Quarter length of MII
MIII	Unidentate	Uni- and bidentate	Bidentate	Unidentate	Undulating cutting plate	Uni- and bidentate	Bidentate	Curved cutting plate	Unidentate	Bidentate
MIV	Not recorded	Not recorded	Up to 2 teeth	Bidentate	Undulating cutting plate	Unidentate	Bidentate	Unidentate	Unidentate	Unidentate
Maxillary carriers	Long and narrow	Twice as long as wide	Shorter than MI; length ~1.5-times width	Long and thin	Length ~1.5-times width	Twice as long as wide	Length and width nearly equal	Length <1.5-times width	Length ~1.5-times width	Length ~1.5–2-times width
Distribution of composite multidentate hooded hooks (CMHH)	Present	Present anteriorly	1st–11th chaetigers	Anterior to 17th chaetiger	1st–19th chaetigers	First 5 to ~15th chaetiger	147–149th and 186th or 187th chaetigers [observed in this study]	First 4 and 12–15th chaetigers	Broken	1st to 10–25th chaetigers
Distribution of limbate chaetae	Present	Present anteriorly	1–75th chaetigers	Present anteriorly; extend beyond simple multidentate hooded hooks (SMHH)	Not recorded	First 5 to ~15th chaetiger	Present anteriorly	Present anteriorly	Specimen broken (1 on 40th chaetiger)	1st to 17–40th chaetigers