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A new species of *Loimia* (Annelida, Terebellidae) from Papua New Guinea, with comments on other species recorded in the region

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Abstract

We describe a new species of *Loimia*, from shallow waters off Northern Papua New Guinea and compare morphologically to other species recorded from the region and a key is provided. We provide a Maximum likelihood tree for species of *Loimia* for which we have data and it forms a distinct clade from other species. Finally, we discuss characters that we consider as useful specific characters in this large genus, which includes many poorly described species.

Keywords: 16S, COI, New species, *Loimia*, Molecular, Morphology, Taxonomy

INTRODUCTION

The genus *Loimia* Malmgren, 1866 belongs to the Terebellidae, a very diverse family of tubiculous polychaetes characterized by the presence of numerous grooved buccal tentacles used for selective deposit feeding. Terebellids are found in all marine environments, from the intertidal to the abyss, and are common worldwide, distributed from polar to tropical regions (Hutchings et al., 2021). The genus *Loimia* comprises 31 valid species (Read and Fauchald, 2023), mostly distributed in the southern hemisphere, and living in coastal and intertidal habitats (Hutchings et al., 2021). Although new species have recently been described from Australia (Nogueira et al., 2015), Brazil (Carrerette and Nogueira, 2015), China (Wang et al., 2020), and Europe (Lavesque et al., 2017; Martin et al., 2022), the systematics of this genus is still quite challenging. Indeed, many species have been described a long time ago with original descriptions being inadequate and type specimens often lost or not designated (Lavesque et al., 2021). Moreover, for decades, the type species of the genus, *Loimia medusa* (Savigny, 1818), described from the Red Sea, was considered a cosmopolitan species and then reported all over the world (Hutchings and Glasby, 1988; Hutchings and Kupriyanova, 2018; Hutchings et al., 2021).

Despite being considered as a marine biodiversity hotspot, the polychaete fauna of

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Papua New Guinea (PNG) is poorly known (Lavesque et al., 2022). A few years ago, we had the opportunity to collect a species of *Loimia* from intertidal area in far eastern and northern PNG, which, in this study, we describe as a new species, *L. lanai* n. sp., using both morphology and molecular data. We also provide, for the first time, 16S sequences of species described from the Great Barrier Reef: *Loimia juani* Nogueira, Hutchings & Carrerette, 2015 and *Loimia tuberculata* Nogueira, Hutchings & Carrerette, 2015. We also compare *L. lanai* n. sp. with nine other species that occur in nearby Australian and Indonesian waters and provide a key to these species. We also discuss the important morphological characters for distinguishing species in this genus and highlight that many of them are poorly known and only a few have associated molecular data.

METHODS

Sampling and morphologal analysis

The specimens of this new species were collected under rocks in shallow subtidal areas of northeastern Papua New Guinea, at Lissenung Island in 2015 and 2016 and from Kranket Island, near Madang in 2012.

All material was fixed in 96% ethanol. A few parapodia were removed from several specimens for both molecular and scanning electron microscope analysis. Specimens were examined under an Olympus S7X7 stereomicroscope and BX53 microscope, being photographed with an Olympus DP74 camera. Methyl green, which can be washed out, was used to reveal the abundant glandular areas and to highlight the ornamentation of these areas that are difficult to observe otherwise.

Some parapodia along the body were removed from the type material, dehydrated in ethanol, critical point dried, covered with 20 nm of gold, examined under the scanning electron microscope (JEOL JSM 6480LA), and imaged with a secondary detector at Macquarie University, Sydney, Australia.

The photo of the live animal was taken with a Canon Rebel T3i with a 100 mm macro lens.

The material is deposited at the Australian Museum, Sydney (AM) and Scripps Institution of Oceanography, San Diego (SIO).

MOLECULAR DATA AND ANALYSES

Extraction of DNA for most specimens was performed with ISOLATE II Genomic DNA kit (BIOLINE), following manufacturers' instructions. Approximately 450 bp of 16S RDNA gene was amplified, using primers 16Sannf (GCGGTATCCTGACCGTRCWAAGGTA) (Sjölin et al., 2005) and 16SbrH (CCGGTCTGAACTCAGATCACGT) (Palumbi, 1996). Approximately 600 bp of COI gene was amplified using primers polyLCO (GAYTATWTTCAACAAATCATAAAGATATTGG) and polyHCO (TAMACTTCWGGGTGACCAAARAATCA) (Carr et al., 2011). For one specimen (AM W.54020), these primers were unsuccessful, so alternative primers were used: LCOI (GGTCAACAAATCATAAAGATATTGG) and HCOI (TAAACTTCAGGGTGACCAAAAAATCA), which are modified from Folmer et al., 1994. Polymerase Chain Reaction (PCR) was performed with Taq DNA Polymerase QIAGEN Kit in 20 µL mixtures containing: 2 µL of 10X CoralLoad PCR Buffer (final concentration of 1X), 1.5 µL of MgCl2 (25 mM) solution, 1.5 µL of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 0.4 µl of each primer (final concentration of 0.2 µM), 0.1 µl of Taq DNA Polymerase (5U/µl), 1 µl template DNA, and 13.1 µL of nuclease free water. The temperature profiles were as follows 94 °C / 10 min – (94 °C / 60 s–59 °C / 30 s–72 °C / 90 s)*40 cycles– 72 °C / 10 min – 4 °C (16S); 94 °C / 1 min – (94 °C / 40 s–45 °C / 40 s–72 °C / 60 s)*5 cycles – (94 °C / 40 s–51 °C / 40 s–72 °C / 60 s)*35 cycles– 72 °C / 5 min (polyLCO/PolyHCO); and 94 °C / 2 min – (94 °C / 30 s–50 °C / 45 s–72 °C / 60 s)*35 cycles– 72 °C / 3 min (LCOI/HCOI). PCR success was verified by electrophoresis using 1% p/v agarose gel stained with Gelred. Amplified products were sent to Macrogen Company to obtain sequences, using same set of primers as used for PCR. The paratype SIO-BIC A15677 COI was sequenced using methods in Stiller et al. (2020).

A total of 14 16S sequences were downloaded from GenBank or obtained during this study,

with 13 sequences belonging to *Loimia* species, whereas the remaining one was of the closely related genus (*Lanice*), used as outgroups (Table 1). All 16S sequences were aligned in Geneious Prime 2019.0.4 using the MAAFT plugin and default settings. The maximum likelihood analysis was performed in IQ-TREE 2.2.0 (Trifinopoulos et al. 2016) with the best fitting evolutionary model TIM2+F+G4 selected. Bootstrap support was estimated using an ultrafast bootstrap algorithm (UFBoot) (Minh et al. 2013) for 1000 replicates. Pair-wise Kimura 2-parameter (K2P) genetic distance was performed using MEGA version 7.0.26.

Table 1. Terminal taxa used in the molecular section of the study (16S and COI genes), with voucher specimens, collection and type localities, GenBank accession numbers, and references.

Species	Voucher specimen	Type locality	Collection locality	16S	COI	References
Lanice conchilega	SMA-New-Lan-02	Netherlands, North Sea	Belgium, North Sea	MZ648390	MZ622192	Lavesque et al., 2020
Loimia bermudensis	SIO-BIC A9451	Bermuda	Belize	MT166815	MT167000	Stiller et al., 2020
Loimia lanai n. sp.	AM W.51456	Lissenung, PNG	Lissenung, PNG	OR353515	OR345063	This study
Loimia lanai n. sp.	AM W.54019	Lissenung, PNG	Lissenung, PNG	OR353514	OR345062	This study
Loimia lanai n. sp.	SIO-BIC A15677	Lissenung, PNG	Madang, PNG		OR345064	This study
Loimia tuberculata	MNHN--IA-2017-3587	Lizard Island, Australia	Madang, PNG	OR353518		This study
Loimia tuberculata	AM W.54517	Lizard Island, Australia	Heron Island, Australia	OR353520		This study
Loimia tuberculata	AM W.54516	Lizard Island, Australia	Heron Island, Australia	OR353519		This study
Loimia tuberculata	AM W.52886	Lizard Island, Australia	Lizard Island, Australia	OR353521		This study
Loimia ramzega	MNHN-IA-TYPE 1788	Brittany, France	Brittany, France	KY555058	KY555061	Lavesque et al., 2017
Loimia ramzega	MNHN-IA-TYPE 1790	Brittany, France	Brittany, France	KY555060	KY555063	Lavesque et al., 2017
Loimia ramzega	MNHN-IA-TYPE 1789	Brittany, France	Brittany, France	KY555059	KY555062	Lavesque et al., 2017
Loimia juani	AM W.52882	Lizard Island, Australia	Lizard Island, Australia	OR353516		This study
Loimia juani	AM W.52883	Lizard Island, Australia	Lizard Island, Australia	OR353517		This study
Loimia minuta	SIO:BIC:A9452	South Florida, USA	Belize: Carrie Bow Cay	MT166816	MT167001	Stiller et al., 2020

Most of the *Loimia* COI sequences stored in GenBank belong to questionable specimens, often sampled far away from type locality. For this reason, a COI molecular tree was not justified.

RESULTS

Taxonomic account

Family Terebellidae Johnston, 1846
***Loimia* Malmgren, 1866**
Type species: *Loimia medusa* Savigny 1818, by monotypy.
Diagnosis (after Hutchings et al., 2021).

Transverse prostomium attached to dorsal surface of upper lip, thick crested basal part, eye spots present or absent. Peristomium restricted to lips, short button–like upper lip, and a midventral lower lip. Well-developed lateral lobes on segments 1, 3, and sometimes 4. A total of three pairs of arborescent branchiae on segments 2–4. Notopodia from segment 4 to segment 20. Neuropodia from segment 5 to pygidium. Neurochaetae as short-handled uncini throughout, initially arranged in single rows, then in double rows in back-to-back arrangement from segment 11 to 20, partially intercalated to separate double rows, then in single rows. Uncini with several lateral teeth and high pectinate crest. Nephridial papillae on segment 3, genital papillae on segments 6–8, inserted posterior to notopodia. Pygidium smooth to papillate.

Loimia lanai n. sp.

MATERIAL EXAMINED. Holotype. AM W.54019, coll. 1st April 2016, Hutchings, Papua New Guinea, Lissenung Island, Home Reef (2°39' S, 150° 44' E), 2 m depth, hand collected by Hutchings, underneath boulder on reef flat; fixed in 95% alcohol, tissue sample taken for DNA, and chaetigers 16, 27, and far posterior abdominal neuropodia removed for SEM. **Paratypes** AM W.51456, same location as holotype, in two pieces, broke as being removed from tube, coll. 4th July 2015; SIO-BIC A15677, coll. 5th December 2012, Rouse, Summers & Anker Papua New Guinea, Madang Lagoon, North Kranket Island (5.189°S,145.8242°E), 1–20 m depth, hand collected, fixed in formalin, subsample in 95% ethanol, tissue sample taken for DNA.

DESCRIPTION. Holotype is divided in two pieces; the anterior part 14 cm of length and 1 cm of max width, along with 17 pairs of notopodia and 31 abdominal segments. The posterior part 21 cm of length and 10 mm of max width tapering towards pygidium with 125 neuropodia (broke in two as being extracted from tube). The paratype anterior part is 11 cm in length without tentacles, with 8 mm max width and a posterior part of approximately 20 cm. Alcohol preserved material white. The live paratype SIO-BIC A15677 had bright red branchiae, thoracic region pale purple dorsally, deep red ventrally, tan colored abdominal region, with cream colored buccal tentacles presenting faint brown markings (Figure 1).

Numerous buccal tentacles of two lengths; short ones with faded transverse stripes, long ones mainly colorless, except for few with basal stripes, with conspicuous groove (Figure 1).

Three pairs of highly branched branchiae on segments 2–4, which are very short and strongly contracted (Figures 1, 2A–C). The first pair is larger than the second and third. Moreover, the second pair is inserted more laterally than the others. Short thick stem that branches almost immediately into two dichotomous branches and then repeatedly branches.

Transverse prostomium attached to dorsal surface of upper lip and basal part without eyespots. Peristomium with upper lip recurved and glandular, with numerous vertical shallow grooves; lower lip small and rectangular.

Segment 1 reduced dorsally, laterally forming a rectangular lateral lobe, with thinner and curled over margins that form a thin connecting strip across ventrum with thinner crenulated margins. Segment 2 reduced ventrally and laterally, dorsally not visible since it is covered by strongly contracted branchiae (Figure 2A-C). Segment 3 with large dorsally inserted lateral lobe with conspicuous flag-like dorsal extension (Figure 2A), not vertically aligned with those on segment 1. Segment 4 without lateral lobes.

Nephridial papillae present on segment 3, at base of branchiae and dorsal margins of lateral lobe. Genital papillae visible on segments 6 and 7, being small and inserted posteriorly to base of notopodia, none of the examined material was gravid.

Ventral pads present from segment 2 to 13, becoming increasingly wider and then decreasing in size; smooth rectangular pads can be found in the most anterior pads, whereas numerous transverse ridges are present on the posterior pads, followed by a mid-ventral groove that narrows towards posterior abdominal segments and then completely disappears as body tapers to the pygidium (Figure 1). Dorsum and ventrum are smooth (Figure 1).

Notopodia from segment 4, with 17 pairs, are all vertically aligned, although the first notopodium is slightly smaller than subsequent ones. Notopodia present swollen bases that are basally attached to neuropodial tori. Furthermore, they have capillary chaetae, smooth with narrow wings tapering to fine tips arranged in two tiers, with each tier graded in length (Figure 3A, B).

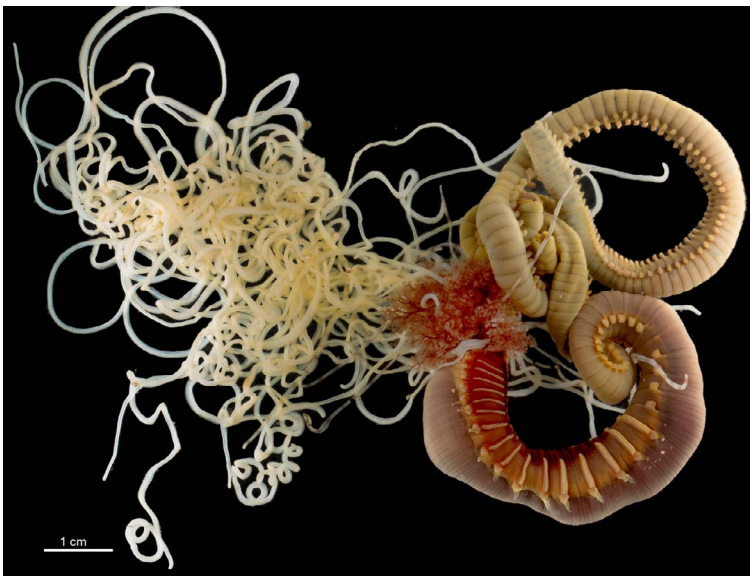

Figure 1. *Loimia lanai* **n. sp.** paratype SIO-BIC A15677. Live specimen.

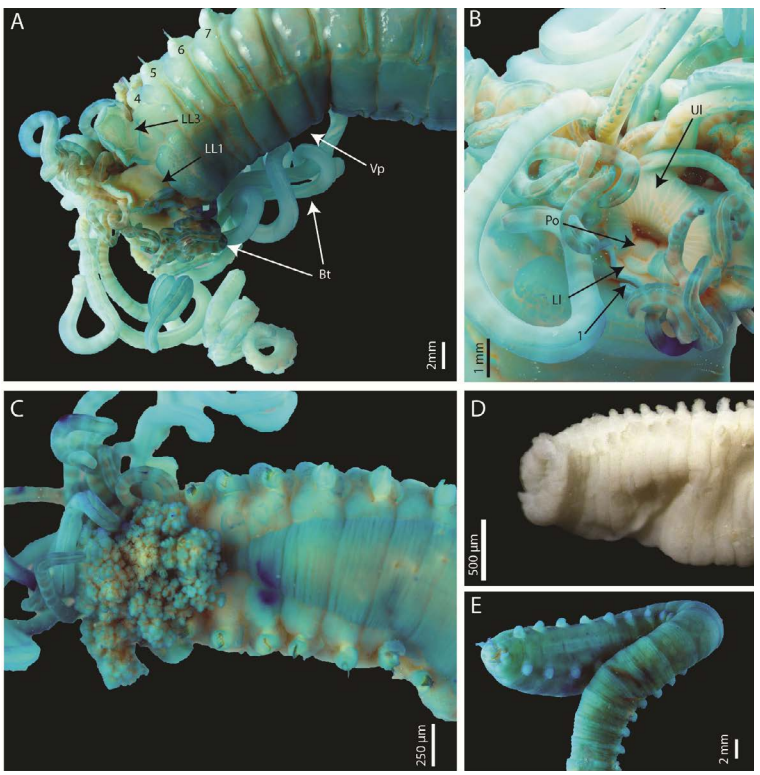

Figure 2. *Loimia lanai* **n. sp.** holotype AM W.54019. A. Anterior end, lateral view. B. Anterior end, ventral view. C. Anterior end, dorsal view. D. Pygidium, lateral view. E. Posterior end, lateral view. Abbreviations: Bt, buccal tentacles; LI, lower lip; LL1, lateral lobe SG1; LL3, lateral lobe SG3; Po, pharyngeal organ; UI, upper lip; Vp, ventral pad. Numbers referring to segments.

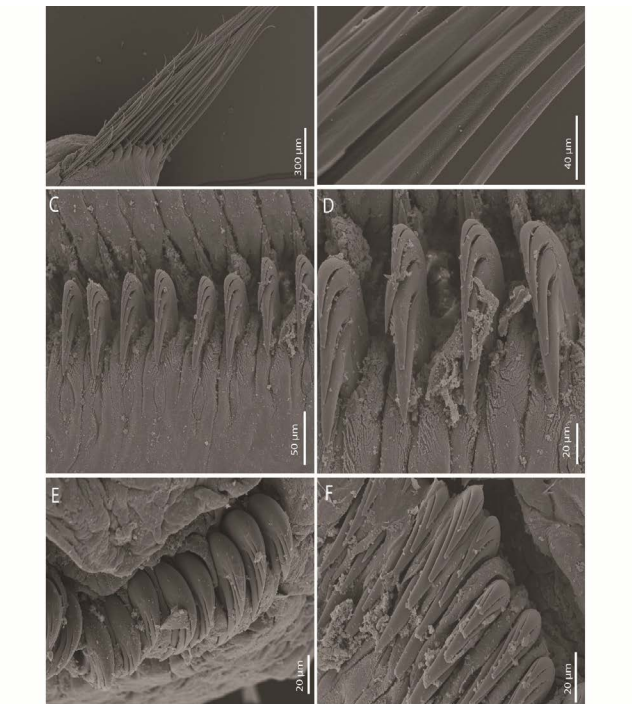

Figure 3. SEM images of *Loimia lanai* **n. sp.** holotype AM W.54019. A–B. Notochaetae, thoracic segments; C–D. Uncini, thoracic segments; E–F. Uncini, abdominal segment.

Neuropodia from segment 5 continuing to pygidium, and thoracic neuropodia with elongated rows of uncini on slightly glandular raised tori. The abdominal neuropodia becomes progressively smaller and erect (Figures 1, 2D, E).

Thoracic neuropodia with short-handled avicular pectinate uncini, with 3 to 4 major teeth above main fang and 1 or 2 smaller apical teeth (Figure 3C, D), initially arranged in single rows, from segment 11 until termination of notopodia arranged in partially intercalated to completely separated double rows, in back-to-back arrangement. Abdominal neuropodia with similar pectinate uncini but are arranged in single rows throughout, with 3 to 4 major teeth above the main fang and at least 1 or 2 smaller apical teeth (Figure 3E, F).

Margins of pygidium with 20 uniform sized small lobes (Figures 1, 2D).

Methyl green staining highlights the ventral pads (Figure 2A–C) and the glandular nature of the parapodia both noto- and neuropodia also shown in Figure 1 of live specimen.

HABITAT

Under rocks in shallow subtidal areas.

DISTRIBUTION

Known only from the type locality, Lissenung, Kavieng, New Ireland, North Kranket Island, Madang Lagoon, Papua New Guinea.

ETYMOLOGY

The species is named after Paulo Lana, a distinguished Brazilian polychaete worker who mentored many students and ran the 6th International Polychaete Conference held in Curitiba, Brazil in August 1998.

REMARKS

Loimia lanai n. sp. is characterized by segment 1 with a rectangular lobe that continues ventrally as a thin connecting strip; segment 3 with large laterally inserted lobe with a dorsally flag-like extension; and segment 4 with no lateral lobe. No eye spots, ventral pads from segment 2 to 13, ventrum and dorsum smooth, and pygidium with about 20 uniformly small papillae. The genus currently consists of 31 accepted species according to the World Register of Marine Species (WoRMS), with ten species recorded from Australia and Indonesia and

no species previously reported from Papua New Guinea. The absence of lateral lobes on segment 4 on *L. lanai* n.sp., clearly distinguishes it from *L. triloba*, *L. juani* (Nogueira et al., 2015), and *L. pseudotriloba* (Noguiera et al., 2015), which all have lateral lobes on segments 1, 3, and 4. *Loimia lanai* n.sp. can also be distinguished from *L. keablei* (Nogueira et al., 2015), *L. tuberculata* (Nogueira et al., 2015), and *L. verrucosa* (Caullery, 1944) by having a smooth dorsum, whereas these three species all have well developed rows of rounded tubercles. *Loimia lanai* n. sp. can be distinguished from *L. batilla* (Hutchings and Glasby, 1988) by the lateral lobe of segment 1 not continuing as a shallow ridge across the dorsum. It can also be distinguished from *L. ingens* (Grube, 1878), in which the second lateral lobe arises from the junction of segment 2/3, and from *L. nigrifilis* (Caullery, 1944) by the shape of the lateral lobe on segment 3 that is ear-shaped, contrasting with those on the new species that has a distinctive flag shaped extension to the lobe. Finally, *L. lanai* n. sp. can also be distinguished from *L. ochracea* (Grube, 1878), which has semi-circular lateral lobes on segment 3 with no flag shaped extension and ventral pads forming a U-shaped ventral structure to chaetiger 16, whereas, in the new species, ventral pads only occur on chaetiger 10 and then rapidly contracting to forming a ventral groove.

However, it should be noted that some of the above species are poorly known, such as *L. ingens*, which was originally described from the Philippines and has been widely reported from Australian water. Hutchings and Glasby (1988) state that it certainly represents a species complex and, since no type material is known, fresh material must be collected and a neotype designated. Similarly, the two species described by Caullery from Indonesian waters need to be re-examined and fully described. In fact, the entire genus needs a revision.

Based on 16S, *L. lanai* n. sp. clearly differs from species with available sequences, especially species from the same region (Figure 4). The Pair-wise Kimura 2-parameter (K2P) between *L. lanai* n. sp. and other species varies between 14 and 20%, which confirms it represents a new species. Based on COI, *L. lanai* n. sp. matches with a specimen sampled from a reef in Madang, Papua New Guinea (Plaisance et al., 2021).

We suggest that useful characters to separate these species include good descriptions of the lateral lobes on segments 1–4, not only their shape but their points of insertion on the body. Other useful characters are the ornamentation of the pygidium, dorsum, and ventrum, as well as molecular data from the type locality.

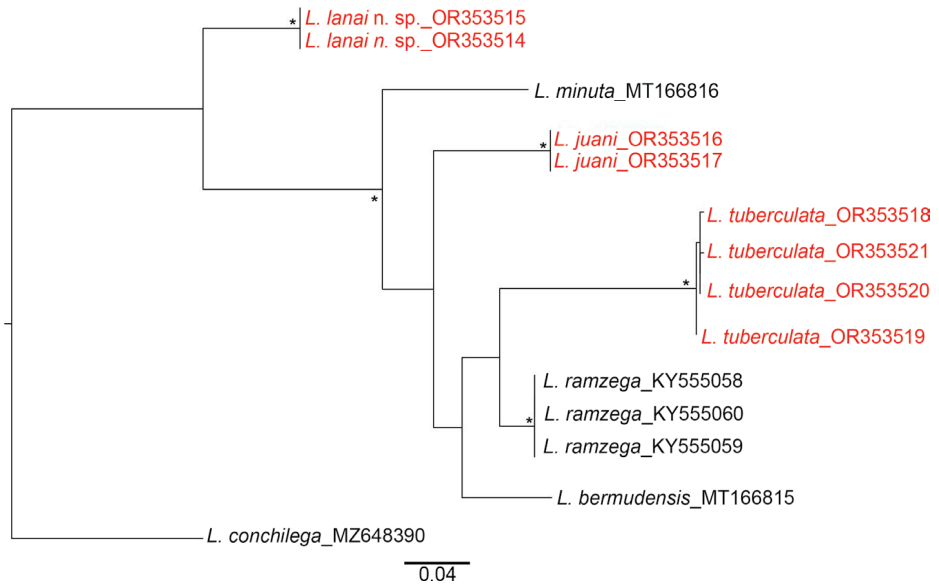

Figure 4. Maximum likelihood tree of *Loimia* species, based on 16S gene sequences. Asteriks correspond to the bootstrap support values of the ML analysis >80%. Text in red indicates specimens used in this study.

Key to the species of *Loimia* from Australia and South East Asia

1A. Dorsum with rounded tubercles… 2
1B. Dorsum smooth… 4
2A. Dorsum with tubercles present from segment 5 (after the last branchiae)… *L. tuberculata* Nogueira et al., 2015
2B. Dorsum with tubercles present from after the last pair of notopodia… 3
3A. Eyespots present; segment 1 with large anteriorly directed lobes, segment 3 with short almost square lobes with rounded corners… *L. keablei* (Nogueira et al., 2015)
3B. Eyespots absent; lateral lobes on segment 1 poorly developed, compared to those on segment 3… *L. verrucosa* (Caullery, 1944).
4A. Segment 4 with lateral lobes… 5
4B. Segment 4 without lateral lobes…7
5A. Eyespots present in 2 rows; segment 3 with nearly circular lateral lobes; pygidium with 15 elongate papillae…*L. triloba* (Hutchings and Glasby, 1988)
5B. Eyespots absent; lateral lobes otherwise… 6
6A. Segment 3 with almost circular lobes, dorsal margins of lobes of segment 4 reaching level of notopodia; margins of pygidium crenulate to slightly papillate… *L. juani* (Nogueira et al., 2015)
6B. Segment 3 with elongate and distally rounded lobes; dorsal margins of lobes of segment 4 at level of mid-length of neuropodia; pygidium with digitiform papillae… *L. pseudotriloba* (Nogueira et al., 2015)
7A. Lateral lobe of segment 3 forming dorsal flag like extension ….. *L. lanai* n. sp.
7B. Lateral lobe of segment 3 without dorsal flag like extension … 8
8A. Lateral lobes present on segment 1 and 3…. 9
8B. Lateral lobes present on segment 1, 2 and 3… 10
9A. Segment 1 with semi-circular lateral lobes connecting mid ventrally by U shaped glandular ridge, segment 3 with semi-circular lobes directed dorso-laterally with fine convoluted margins connected across ventrum by connected ridge; uncini with 5 teeth; ventral pads discrete forming U-shaped ventral structure to segment 20… *L. ochracea* (Grube, 1878)
9B. Segment 1 with lobes well separated ventrally by deep medio ventral notch, segment 3 with ear shaped lateral lobes; uncini with 3–4 teeth; pygidium with 12 short papillae… *L. nigrifilis* (Caullery, 1944)
10A. Segment 1 with large lateral lobes united ventrally, forming anteriorly directed scoop; lateral lobes on segment 2/3 extending ventrally forming glandular scoop, 1st pair of branchiae 2x size of 2nd & 3rd pair…*L. batilla* (Hutchings & Glasby, 1988)
10B. Segment 1 with lateral lobes which continue across dorsum as a small collar; lobes well developed on segments 2/3, all branchiae similar in size…*L. ingens* (Grube, 1878) ***

***Hutchings & Glasby (1988) comment that *L. ingens* is a species complex, and material from the type locality Philippines needs to be redescribed and a neotype erected as Grube's description is minimal.

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PH first met Paulo at the 2nd International Polychaete Conference held in Copenhagen, Denmark in 1986 and at all subsequent meetings, including the one held in Sydney in 2013. Paulo was always willing to review papers and provide advice on taxonomic and ecological matters, and engaged with students, as seen during the mid-week excursion during the Curitiba meeting. I certainly missed his friendly face and conversations at this year's Polychaete conference in South Africa. We have certainly lost a major polychaete worker, and far too early.

NL met Paulo during Cardiff and Los Angeles IPC. NL will always remember enthusiastic stories about polychaetes (in French) over a beer. To him, Paulo was a great specialist of polychaetes, both in taxonomy and ecology, and it is a great loss to our community.

GWR knew Paulo since 1998, when they worked together on a polychaetes workshop in Curitiba. To him, Paulo was very kind, tolerant, and polite and mentored a generation of students, leaving us far too early.

AUTHOR CONTRIBUTIONS

P.H.: Conceptualization; Investigation; Writing – Original Draft; Writing – Review & Editing.
B.F.; G. D.: Formal Analysis.
G. W. R.: Data Curation; Methodology; Writing – Review & Editing.
N.L.: Methodology; Writing – Original Draft.

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