

Article

First Molecular Phylogeny of Lumbrineridae (Annelida)

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Abstract: Lumbrineridae is a family of marine annelids with simple external morphology but complex and diverse jaw apparatuses consisting of paired maxillae and mandibles. Here we present the first phylogeny of lumbrinerids based on combination of nuclear (18S rDNA) and mitochondrial (COI, 16S rDNA) markers utilizing Bayesian inference and Maximum Likelihood approaches. Despite limited taxon sampling, our results support monophyly of the genera *Abyssoninoe*, *Augeneria*, *Gallardoneris*, *Lumbrineriopsis*, and *Ninoe* and indicate polyphyly of the genera *Lumbrineris* (the type genus of the family) and *Scoletoma*. None of the morphological characters traditionally used in lumbrinerid systematics, such as the presence of connecting plates, four pairs of maxillae, bidentate simple hooded hooks, colorless maxillae IV, and multidentate maxillae IV were found to be exclusive homologies for a well-supported clade and have probably evolved several times independently within Lumbrineridae

Keywords: Polychaeta; maxillae; jaws; morphology

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1. Introduction

Lumbrineridae Schmarda, 1861 [1] is a family of marine bristle worms from the larger and well-defined group of jaw bearing annelids—Eunicida. The jaws are composed of dorsal maxillae and ventral mandibles located in the ventral muscular pharynx and are used for feeding. Lumbrinerids exhibit rather simple external morphology with poorly developed parapodia and without prominent prostomial and body appendages (Figure 1). Therefore, the details of maxillary apparatus have proven to be useful as diagnostic characters at genus and species level [2,3]. The maxillae of lumbrinerids are considered to be of symmetrogath type [4] with short paired maxillary carriers, forceps-like maxillae I, multidentate massive maxillae II, and two to four pairs of smaller distal maxillary plates (maxillae III–VI). Accessory plates such as attachment lamellae and connecting plates can be found in some genera but are still poorly described for most species [5,6] (Figure 2).

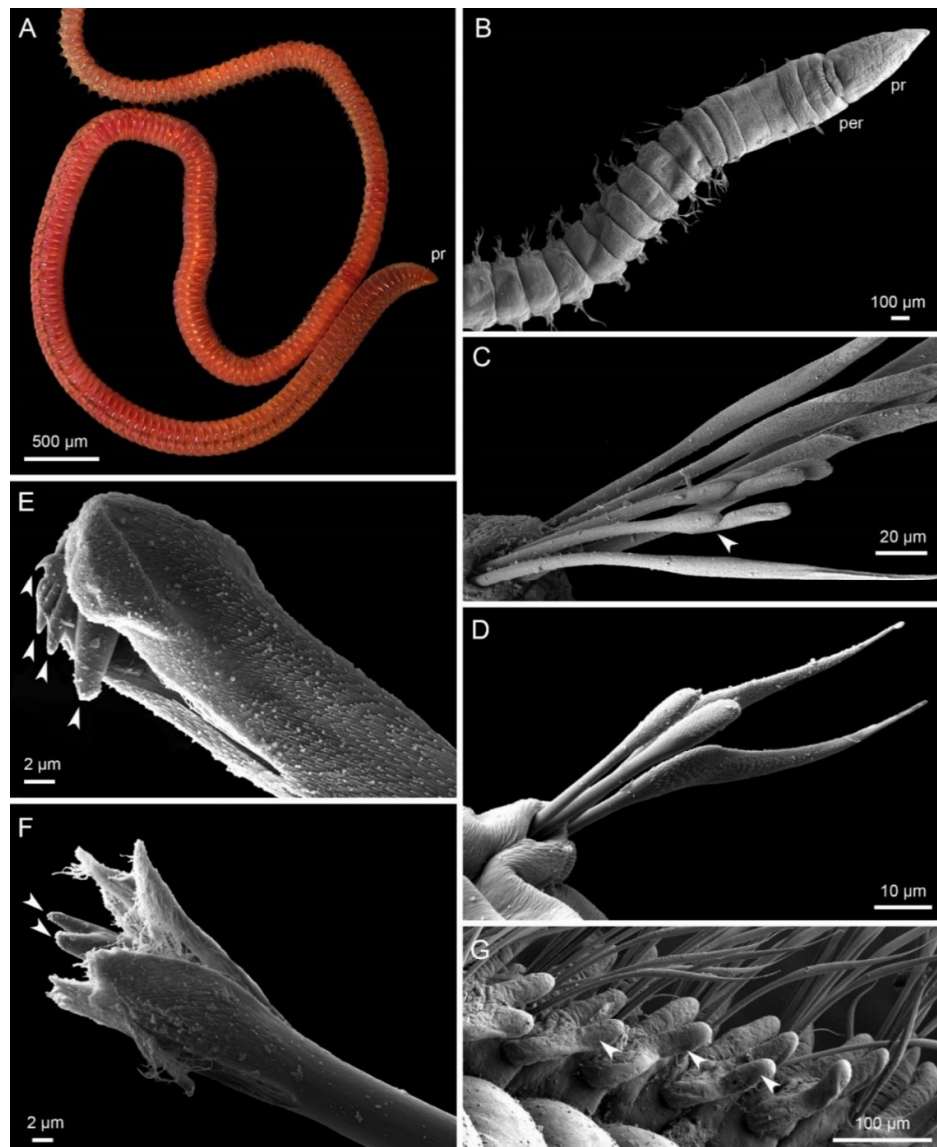


Figure 1. Morphology of Lumbrineridae, live specimen photograph (A), scanning electron microscopy micrographs (B–G). (A) *Scoletoma fragilis*, lateral view of anterior part of the body, pr—prostomium ©, Vedenin, A.; (B) *Lumbrinerides* sp., ventral view of anterior fragment, pr—prostomium, per—peristomium; (C) *Lumbrineris* sp., limbate chaetae and compound hooks from the 4th parapodium, arrowhead indicates the articulation in the compound hook; (D) *Lumbrinerides* sp., limbate chaetae and simple hooks from the 4th parapodium; (E) *Lumbrineris* sp., distal part of the multidentate hooded hook from the 30th parapodium, arrowheads indicate numerous denticles, hood is slightly open; (F) *Lumbrineriopsis* sp., distal part of the bidentate hook, arrowheads indicate two equal denticles, hood is partly removed; (G), *Ninoe armoricana*, lateral parapodia with branchiae (arrowheads) from the middle of the body.

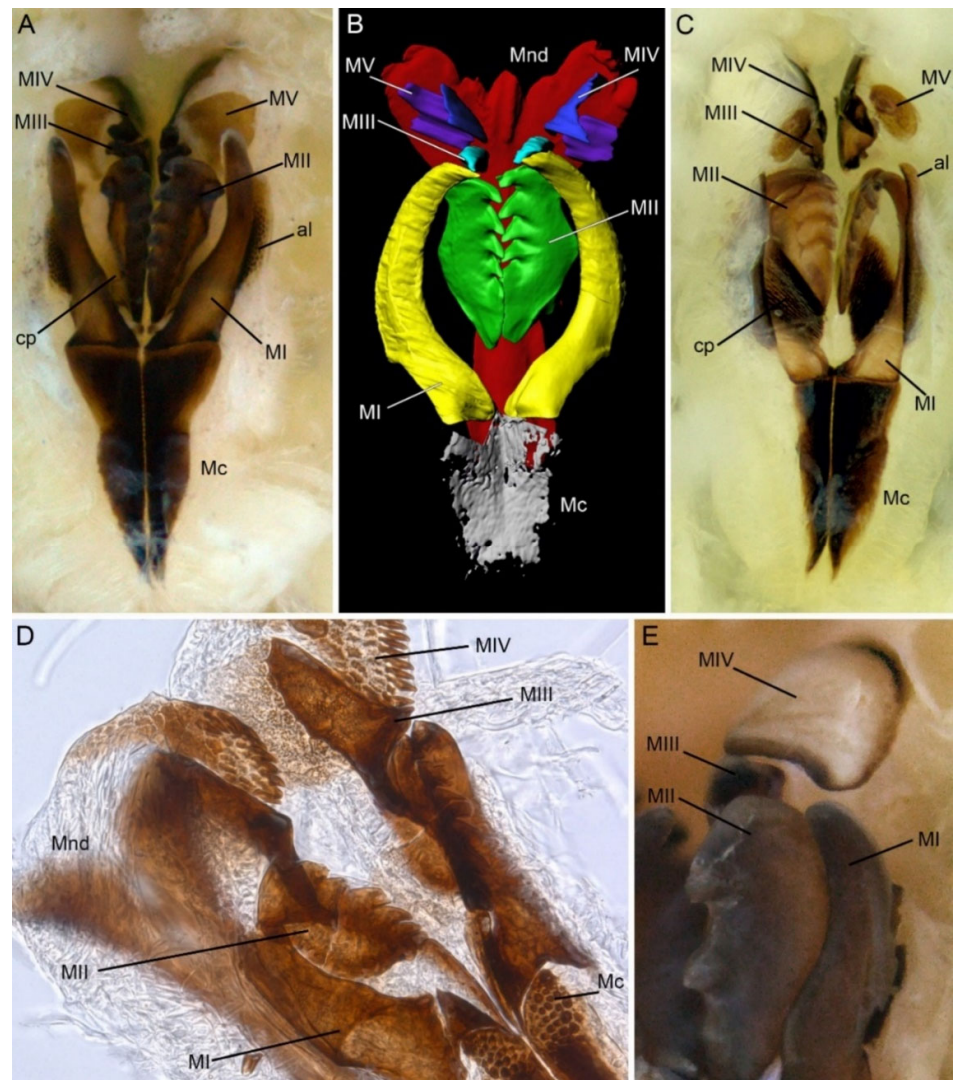


Figure 2. Morphology of lumbrinerid jaws: mandibles and maxillae, light photographs of dissected jaws (A,C–E), 3D reconstruction of jaws based on micro-CT scans. (A) *Lumbrineris* sp. showing complete set of maxillary structures; (B) *Lumbrineris* sp., maxillary carriers partly reconstructed, connecting plates and attachment lamellae omitted; (C) *Hilbigneris* sp. maxillary apparatus showing extremely sclerotized connecting plates; (D) *Lumbrineriopsis* sp. distal part of the maxillary apparatus showing multidentate maxillae IV; (E) *Augeneria albidentata*, part of the maxillary apparatus showing right massive maxilla II and maxilla IV with non-pigmented white central area. al—attachment lamella; cp—connecting plate; Mc—maxillary carriers; Mnd—mandibles; MI–MV—maxillae I–V.

The currently accepted system of Lumbrineridae was proposed by Carrera-Parra [7] who provided diagnoses for 19 genera including six new genera. Recent review of the family by Oug et al. [6] listed updated diagnoses for all known lumbrinerid genera largely based on the revision by Carrera-Parra [7] but also utilizing more recent publications and the original descriptions (Table 1). The current total species count ranges between about 200 [6] and 280 [5] species; however, even the latter number might be highly underestimated.

Table 1. Accepted genera of Lumbrineridae with the number of species in each genus and number of species used in the phylogenetic analysis in the present study. Species count for each genus follows Oug et al. [3].

Genus	Author	Type Species	Number of Species	Number of Species Used in this Study
<i>Abyssoninoe</i>	Orensanz, 1990 [2]	<i>Lumbriconereis abyssorum</i> McIntosh, 1885 [8]	8	2
<i>Arabelloneris</i>	Hartmann-Schröder, 1979 [9]	<i>Arabelloneris broomensis</i> Hartmann-Schröder 1979 [9]	2	0
<i>Augeneria</i>	Monro, 1930 [10]	<i>Augeneria tentaculata</i> Monro, 1930 [10]	9	3
<i>Cenogenus</i>	Chamberlin, 1919 [11]	<i>Cenogenus descendens</i> Chamberlin, 1919 [11]	12	0
<i>Eranno</i>	Kinberg, 1865 [12]	<i>Eranno bifrons</i> Kinberg, 1865 [12]	11	0
<i>Gallardoneris</i>	Carrera-Parra, 2006 [7]	<i>Lumbrineris shiinoi</i> Gallardo, 1968 [13]	3	4
<i>Helmutneris</i>	Carrera-Parra, 2006 [7]	<i>Lumbriconereis flabellicola</i> Fage, 1936 [14]	3	1
<i>Hilbigneris</i>	Carrera-Parra, 2006 [7]	<i>Hilbigneris pleijeli</i> Carrera-Parra, 2006 [7]	3	1
<i>Kuwaita</i>	Mohammad, 1973 [15]	<i>Kuwaita magna</i> Mohammad, 1973 [15]	5	0
<i>Loboneris</i>	Carrera-Parra, 2006 [7]	<i>Lumbrineris pterignatha</i> Gallardo, 1968 [13]	1	0
<i>Lumbricalus</i>	Frame, 1992 [16]	<i>Lumbriconereis januarii</i> Grube, 1878 [17]	9	0
<i>Lumbrinerides</i>	Orensanz, 1973 [18]	<i>Lumbrinerides gesae</i> Orensanz, 1973 [18]	16	1
<i>Lumbrineriopsis</i>	Orensanz, 1973 [18]	<i>Lumbriconereis mucronata</i> Ehlers, 1908 [19]	5	2
<i>Lumbrineris</i>	Blainville, 1828 [20]	<i>Lumbrineris latreilli</i> Audouin & Milne-Edwards, 1833 [21]	~ 50	4
<i>Lysarete</i>	Kinberg, 1865 [12]	<i>Lysarete brasiliensis</i> Kinberg, 1865 [12]	3	0
<i>Ninoe</i>	Kinberg, 1865 [12]	<i>Ninoe chilensis</i> Kinberg, 1865 [12]	33	4
<i>Scoletoma</i>	Blainville, 1828 [20]	<i>Lumbricus fragilis</i> O.F. Müller, 1776 [22]	25	3
<i>Sergioneris</i>	Carrera-Parra, 2006 [7]	<i>Lumbrineris nagae</i> Gallardo, 1968 [13]	1	0
Lumbrineridae gen. sp.			-	1
			199	26

Carrera-Parra [7] suggested a phylogenetic reconstruction of Lumbrineridae based on 38 morphological characters using genera as terminal taxa. Thus, the hypotheses of monophyly of individual genera was not tested. All genera were grouped into three sub-clades, except for *Lysarete* and *Arabelloneris* lacking bi- or multidentate hooks, a character present in all other lumbrinerids. The first sub-clade was represented by *Ninoe*, a genus with well-developed palmate parapodial branchiae. The second sub-clade combined genera with connecting plates – maxillary accessory structures placed between MI and MII (Figure 2A,C): *Scoletoma*, *Lumbrineris*, *Hilbigneris*, *Kuwaita*, *Lumbricalus*, *Sergioneris* and *Eranno*. Nevertheless, the shape, position, and degree of sclerotization of the connecting plates in all seven genera varies greatly and homology of these structures is not clearly understood. The remaining nine genera (*Abyssoninoe*, *Cenogenus*, *Lumbrinerides*, *Lumbrineriopsis*, *Augeneria*, *Loboneris*, *Gallardoneris*, *Helmutneris*, and *Gesaneris*) were grouped

into the third sub-clade based on the presence of only four pairs of maxillae, although this character state was not exclusive to the clade and was also reported in *Sergioneris*.

Lumbrineris is the type and the most species-rich genus of the family. Carrera-Parra [7] recognized 36 valid species based on 57 known names but the number may well reach 50 [6]. Originally, *Lumbrineris* included only three species [20] but due to very wide definition subsequently encompassed a very large number of species [23–25]. Reevaluation of maxillary and fine chaetal structures allowed restricting of the genus diagnosis and led to transferring of part of the species to several different genera [2,7,16]. Nevertheless, many of the early described lumbrinerids lacking detailed information on their jaw morphology are still referred to *Lumbrineris* [6] making this genus a taxon of questionable monophyly.

Very little molecular data for lumbrinerids is available in public databases (e.g., GenBank, Barcode of Life Database—BOLD) mostly for the genera *Lumbrineris*, *Scoletoma* and *Ninoe*. For nine genera no genetic information is published up to date. Several species were used in reconstructing large-scale phylogenies of Eunicida [26,27] or Annelida [28–30] based on several markers or genomic data but no molecular phylogenetic reconstruction of relationships between the genera is available. In the present study, we publish new genetic data for 18 species of lumbrinerids and propose the first molecular phylogeny of Lumbrineridae based on independent evidence such as combination of mitochondrial and nuclear markers. We also discuss the importance of selected morphological characters in systematics of Lumbrineridae.

2. Materials and Methods

2.1. Taxon Sampling

We have sampled 45 specimens from 18 species of lumbrinerids representing 10 of the 19 currently known genera. Sequences for eight more species from the sampled genera were obtained from the GenBank and BOLD (Table 2). Nine genera were not included in the analysis due to unavailability of material suitable for DNA extraction. Three species from other eunicids families and *Glycera alba* (Glyceridae) were selected as outgroup taxa. Voucher specimens are deposited in the Invertebrate Collection, University Museum of Bergen, University of Bergen, Bergen, Norway (ZMBN), Zoological Museum of Moscow State University, White Sea Biological Station, Moscow, Russia (WSBS), and in the annelid collection of the Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, Russia (IORAS). Detailed information of the museum catalogue numbers and geographical localities of each specimen can be found in the Supplementary Material (Table S1).

Table 2. List of taxa used in the phylogenetic analyses with BOLD process IDs and GenBank accession numbers.

Clade	Taxon	Author	BOLD Process ID	GenBank Accession Number			Source
				COI	16S	18S	
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998 [31]	POLNB1550-15	OM237808	OM237844	OM312047	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	POLNB1560-15	OM237807	OM237843	OM312046	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	POLNB1562-15	OM237807	OM237843	OM312045	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	EUNI007-21	OM237805	OM237841	OM312044	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH004-21	OM237804	OM237840	OM312043	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH005-21	-	OM237831	OM312034	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH006-21	OM237803	OM237839	OM312042	This study

A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH007-21	OM237802	OM237838	OM312041	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH008-21	OM237801	OM237837	OM312040	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH009-21	OM237800	OM237836	OM312039	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH010-21	OM237799	OM237835	OM312038	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH011-21	OM237798	OM237834	OM312037	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH012-21	-	OM237833	OM312036	This study
A	<i>Lumbrineris mixochaeta</i>	Oug, 1998	LUPH013-21	-	OM237832	OM312035	This study
B	<i>Scoletoma fragilis</i>	(O.F. Müller, 1776) [22]	LUPH016-21	OM237813	-	OM312052	This study
B	<i>Scoletoma fragilis</i>	(O.F. Müller, 1776)	POLNB827-14	OM237814	-	-	This study
C	Lumbrineridae gen. sp.		MIWAP303-13	OM237794	OM237827	OM312031	This study
D	<i>Gallardoneris</i> sp.		MIWAP377-13	OM237790	OM237824	-	This study
D	<i>Gallardoneris</i> sp.		MIWAP379-13	OM237788	OM237822	OM312027	This study
D	<i>Gallardoneris</i> sp.		MIWAP380-13	OM237789	OM237823	OM312028	This study
D	<i>Gallardoneris</i> sp.		MIWAP376-13	OM237789	OM237823	OM312026	
E	<i>Scoletoma tetraura</i>	(Schmarda, 1861) [1]		GU362689	GU362682	-	[32]
E	<i>Scoletoma zonata</i>	(Johnson, 1901) [33]		-	HM746713	HM746727	[34]
F	<i>Hilbigneris</i> sp.		MIWAP353-13	OM237793	OM237826	-	This study
F	<i>Hilbigneris</i> sp.		MIWAP352-13	OM237792	OM237825	OM312030	This study
F	<i>Hilbigneris</i> sp.		LUPH014-21	OM237791	-	OM312029	This study
G	<i>Abyssoninoe cf. hibernica</i>	(McIntosh, 1903) [35]	SKAG032-11	OM237776	-	-	This study
G	<i>Abyssoninoe cf. scopa</i>	(Fauchald, 1974) [36]	POLNB779-14	OM237778	-	-	This study
G	<i>Abyssoninoe cf. scopa</i>	(Fauchald, 1974)	POLNB1742-15	OM237777	OM237815	OM312019	This study
H	<i>Lumbrineris inflata</i>	Moore, 1911 [37]		BOLD Process ID HZPLY357	AY838832	AY525622	[26,38]
H	<i>Lumbrineris latreilli</i>	Audouin and Milne-Edwards, 1834 [21]		KR916859	AY838833	-	[27,29]
H	<i>Lumbrineris</i> sp.		MIWAP289-13	OM237809	OM237845	OM312048	This study
H	<i>Lumbrineris</i> sp.		MIWAP291-13	OM237811	OM237846	OM312050	This study
H	<i>Lumbrineris</i> sp.		MIWAP345-13	OM237810	-	OM312049	This study
I	<i>Helmutneris vadum</i>	Borisova & Budaeva, 2020 [39]	LUPH001-20	MT763201	MT763203	-	
I	<i>Helmutneris vadum</i>	Borisova & Budaeva, 2020	LUPH002-20	MT763200	MT763202	-	
J	<i>Ninoe armoricana</i>	Glémarec, 1968 [40]	MIWAP295-13	OM237812	OM237847	OM312051	This study
J	<i>Ninoe armoricana</i>	Glémarec, 1968		KT307669	-	-	[41]
J	<i>Ninoe chilensis</i>	Kinberg, 1865 [12]		JF731019	-	-	[42]
J	<i>Ninoe leptognatha</i>	Ehlers, 1900 [43]		JF731020	-	-	[42]
J	<i>Ninoe nigripes</i>	Verrill, 1873 [44]		AY838869	AY838837	AY838852	[26,38]
K	<i>Lumbrinerides</i> sp.		MIWAP325-13	OM237795	OM237828	OM312032	This study
L	<i>Augeneria albidentata</i>	(Ehlers, 1908) [19]	MIWAP298-13	OM237780	-	OM312020	This study
L	<i>Augeneria albidentata</i>	(Ehlers, 1908)	MIWAP334-13	OM237779	OM237816	-	This study
L	<i>Augeneria albidentata</i>	(Ehlers, 1908)	MIWAP335-13	OM237781	OM237817	OM312021	This study
L	<i>Augeneria algida</i>	(Wirén, 1901)	EUNI006-21	OM237783	OM237819	OM312023	This study
L	<i>Augeneria algida</i>	(Wirén, 1901)	LUPH003-21	OM237782	OM237818	OM312022	This study
L	<i>Augeneria cf. tentaculata</i>	Monro, 1930	POLNB591-14	OM237786	-	-	This study

L	<i>Augeneria</i> cf. <i>tentaculata</i>	Monro, 1930	POLNB932-14	OM237784	OM237820	OM312024	This study
L	<i>Augeneria</i> cf. <i>tentaculata</i>	Monro, 1930	POLNB1745-15	OM237785	-	OM312025	This study
M	<i>Lumbrineriopsis</i> sp.		EUNI012-21	OM237796	OM237829	OM312033	This study
M	<i>Lumbrineriopsis</i> sp.		LUPH015-21	-	OM237830	-	This study
M	<i>Lumbrineriopsis</i> sp.		MIWAP330-13	OM237797	-	-	This study
	<i>Glycera alba</i>	(O.F. Müller, 1776) [22]		KF369131	DQ779615	DQ779651	[45,46]
	<i>Eunice rubra</i>	Grube, 1856 [12]		KF808171	GQ478132	GQ497478	[47,48]
	<i>Nothria conchylega</i>	(Sars, 1835) [49]		HQ023895	KJ027342	KJ027382	[50,51]
	<i>Tainokia logachevae</i>	Ravara & Cunha, 2017 [52]		MF795582	MF795579	MF795580	[52]

2.2. DNA Extraction, PCR Amplification and DNA Sequencing

Genomic DNA was extracted from 96% ethanol fixed samples using glass milk methods or QuickExtract™. Fragments of mitochondrial COI and 16S genes were amplified using primer pairs: 16SarL (forward) + 16Brh (reverse) [53], polyLCO (forward) + polyHCO (reverse) [50], HCO (forward) + LCO (reverse) [54]. For the nuclear marker 18S we designed specific primers (Table S2). PCR cycling protocol are shown in the Table S2. After visualization on a 1% agarose gel, the PCR products were purified using Ethanol/EDTA/Sodium Acetate Precipitation and bidirectionally Sanger sequenced using an Applied Biosystems automated sequencer. Chromatograms of forward and reverse sequences were aligned and proofread in Geneious R6 [55].

2.3. Sequence Alignment

Alignment was conducted using default parameters in MUSCLE [56] implemented in MEGA7 [57]. Aligned COI sequences were translated into amino-acid sequences using the invertebrate mitochondrial code (NCBI translation code 5) to ensure stop codons or frameshift mutations were not present. The sequences were then back translated into nucleotides for further phylogenetic analyses. For 16S and 18S markers we used Gblocks V.0.91b [58] to eliminate poorly aligned positions in the original alignments. The following parameters were used while masking: minimum number of sequences for a conserved position–49; minimum number of sequences for a flanking position–81; maximum number of contiguous non conserved positions–8; minimum length of a block–10; allowed gap positions–with half.

2.4. Phylogenetic Analyses

2.4.1. Bayesian Inference (BI)

Best-fit partitions and models were inferred with PartitionFinder2 [59] on XSEDE v1.6.10. Models were: GTR + G + I for second codon in COI and for 16S, HKY + G for 18S, SYM + G for first codon in COI, HKY + G + I for third codon in COI. Trees were reconstructed in MrBayes v. 3.2.1 [60] implementing PartitionFinder results which assigned codon position 1, 2, and 3 to different partitions. Two independent and simultaneous runs with flat prior probabilities and four chains were run for 5,000,000 generations. Trees were sampled every 1000th generation. Stationarity of each chain was checked with TRACER v.1.7 [61] and the first 25% discarded as burn-in after visualizing a plot of likelihood score. The remaining trees were summarized into a majority rule consensus tree with posterior probabilities (PP) indicating the support value for each clade.

2.4.2. Maximum Likelihood (ML)

The same dataset was used for phylogeny inference using the maximum likelihood criterion implemented in IQ-Tree on XSEDE v1.6.10 [62]. IQ-TREE was used to select models based on the Akaike information criterion (AIC), model was GTR + F + I + G4 for both

partitions COI + 16S and 18S. IQ Tree was run through the CIPRES Science Gateway V3.3. Ultrafast bootstrap was performed in 10000 iterations.

3. Results

The combined dataset had 1722 aligned positions (659 positions of COI, 552 positions of 16S rDNA, and 511 positions of 18S rDNA). After applying Gblocks, the new 16S rDNA alignment retained 415 positions (75%), 18S rDNA alignment retained 481 positions (95%), Gblocks was not applied to the COI alignment. Both Bayesian and Maximum likelihood approaches yielded trees with very similar topologies and node support (Figures 3 and S1). The following differences between the resulting trees from the two analyses were observed: the polytomy comprising clades A, B and C in the Bayesian tree versus resolved and well supported relationships (A–C) in the ML tree; the polytomy comprising clades (A–E), (F–H), and I in the Bayesian tree versus resolved but very poorly supported relationships between these clades in the ML tree. Regardless of the method used, the following clades were obtained. Lumbrineridae is monophyletic (PP 1.00, BP 98). The following genera are monophyletic: *Gallardoneris* (clade D, PP 1.00, BP 100), *Abyssoninoe* (clade G, PP 1.00, BP 100), *Ninoe* (clade J, PP 1.00, BP 100), *Augeneria* (clade L, PP 1.00, BP 96), *Lumbrineriopsis* (clade M, PP 1.00, BP 97), *Helmutneris* (clade I), *Hilbigneris* (clade F), *Lumbrinerides* (clade K), and a single specimen that could not be attributed to any of the described genera, Lumbrineridae gen. sp. (clade C), were represented by a single species, thus their monophyly was not tested. The type genus of the family, *Lumbrineris* (clades A and H), and the genus *Scoletoma* (clades B and E) appeared polyphyletic. *Lumbrineris mixochaeta* formed a well-supported (PP 1.00, BP 100) clade with *Scoletoma fragilis* and Lumbrineridae gen. sp. while *Lumbrineris latreilli*, *L. infaltata*, and *Lumbrineris* sp. formed own clade (PP 0.89, BP 73) sister to *Abyssoninoe/Hilbigneris* clade (PP 0.85, BP 81). Nevertheless, these sister relationships were poorly supported (PP 0.60, BP 52). *Scoletoma tetraura* and *S. zonata* (clade E) were sister to the clade comprising *Gallardoneris*, *L. mixochaeta*, *S. fragilis* and Lumbrineridae gen. sp. although these relationships were also poorly supported (PP 0.81, BP 81). Three highly supported sister relationships were recovered in both analyses: *Ninoe* and *Lumbrinerides* (PP 0.99, BP 87), *Augeneria* and *Lumbrineriopsis* (PP 0.96, BP 74), and *Gallardoneris* and (*L. mixochaeta*, *S. fragilis* and Lumbrineridae gen. sp.) clade (PP 1.00, BP 98).

4. Discussion

Although our dataset was rather limited, covering only 12 out of 19 described genera and 26 species (~10% of total species count), certain clades and relationships were well supported. Most genera were found monophyletic and several of them were highly supported. *Ninoe* clade (J) comprised four species, all sharing the presence of the parapodial palmate branchiae in the anterior region of the body (Figure 1G). *Augeneria* clade (L) included three species, all sharing massive maxillae II with few rounded teeth and large maxillae IV with white central areas (Figure 2E). Specimens identified as *Augeneria* cf. *tentaculata* did not form a sub-clade within the genus due to large amount of missing sequence data (Table 2). *Gallardoneris* clade (D) included four specimens with four pairs of maxillae, maxillae IV with unpigmented central areas, and maxillae II with few rounded teeth and missing attachment lamellae. These specimens were provisionally identified as *Gallardoneris iberica* Martins, Carrera-Parra, Quintino & Rodrigues, 2012 [63] collected from the western African waters. Despite very similar morphology, all four specimens showed very divergent sequences indicating a possible case of cryptic speciation in the genus which should be investigated further based on larger sampling size. *Lumbrineriopsis* clade (M) included two species, both showing similar maxillary morphology with prolonged maxillary carriers and multidentate maxillae IV (Figure 2D). *Abyssoninoe* clade (G) comprised two species sharing the presence of anterior prolonged hooded hooks and fused maxillae IV and V—the two main diagnostic characters of the genus. Notably, a single specimen that was not identified to the genus level (*Lumbrineridae* gen. sp.) also exhibited joined maxillae IV and V and elongated anterior hooks. However, this specimen could not be assigned to *Abyssoninoe* due to the presence of very strongly sclerotized connecting plates and short maxillae II (significantly shorter than maxillae I). The latter two characters are not reported for any of *Abyssoninoe* species, including the type species, *Abyssoninoe abyssorum* (McIntosh, 1885) [8]. Furthermore, Oug et al. [6] noted that the fusion of maxillae IV and V occurs also in other genera and thus should be reconsidered as the main diagnostic character in *Abyssoninoe*. *Lumbrineridae* gen. sp. could potentially represent a new genus of lumbrinerids, however we suggest that the new genera are erected after complete revision of the existing system of the family.

Two most species-rich genera within Lumbrineridae, *Lumbrineris* and *Scoletoma*, were recovered as polyphyletic taxa forming two unrelated clades each. These two genera have very similar maxillary morphology (five pairs of maxillae, maxillae I and II almost equal in length, connecting plates present) and were split based on the presence of compound multidentate hooks (Figure 1C) in the former and only simple multidentate hooks in the later (Frame 1992). *Lumbrineris latreilli*, the type species of the genus, formed a clade with two other *Lumbrineris* species and this clade presumably can be considered the part of *Lumbrineris sensu stricto*. Nevertheless, *L. latreilli* is a species with problematic status. It has been originally described from the Atlantic Ocean off France [20] but later reported widely in the world [3]. Sequences of *L. latreilli* used in our analyses were downloaded from the GenBank and although at least one of them was obtained from a specimen collected from the South European Atlantic coast, the identification of this species requires further clarification. *Scoletoma fragilis* (O.F. Müller, 1776), the type species of the genus, formed a well-supported clade with *L. mixochaeta* and *Lumbrineridae* gen. sp., however we were not able to identify morphological characters common for these three taxa. Lack of own material and reliable published sequence data on several markers obtained from the same species prevented from including more species of *Lumbrineris* and *Scoletoma* into the analyses. Although our data indicate that both genera are polyphyletic and require revision, more complete dataset is needed for formal decision on the systematics of both genera.

Augeneria, *Gallardoneris* and *Helmutneris*, together with *Gesaneris* and *Loboneris* (both absent in the present analysis), were also seen as a group of closely related genera sharing maxillae III with unpigmented white central area [7] (Figure 2E). General architecture of maxillary apparatuses in these five genera is rather dissimilar. *Loboneris* has very thin and prolonged maxillary carriers and wing-shaped colorless maxillae IV. *Gesaneris*

demonstrates maxillae II, significantly shorter than maxillae I, which is not characteristic for other genera in this group. *Helmutneris* has maxillae I and II almost equal in length but maxillae II bear numerous (6–7) pointed teeth, while in *Augeneria* and *Gallardonneris*, the maxillae II have only three rounded and widely distributed teeth. Maxillae of the latter two genera are most similar with each other, however in *Augeneria*, the basal parts of maxillae I form a clear locking system characteristic to all lumbrinerids [4] while in *Gallardonneris*, this system appears to be absent (pers. observation). Our results do not support close relationships between *Augeneria*, *Gallardonneris* and *Helmutneris* suggesting an independent origin of colorless maxillae IV at least in these three genera.

Lumbrinerides and *Lumbrineriopsis* were traditionally seen as closely related genera [7] due to their minute size (less than 1 mm in width), elongated prostomium (Figure 1B) and bidentate simple hooded hooks (Figure 1F)—a condition reported for these two genera only. Nonetheless, these genera did not form sister relationships on the present tree suggesting their independent miniaturization and acquisition of bidentate hooks. Maxillary apparatuses of *Lumbrinerides* and *Lumbrineriopsis* are also very dissimilar with short carriers, massive maxillae I and very short maxillae II in the former and elongated carriers and multidentate maxillae III in the latter. Notably, *Lumbrineriopsis* was sister to *Augeneria* while *Lumbrinerides*—sister to *Ninoe*, both relationships were highly supported on the molecular-based tree although we could not identify morphological characters supporting these two clades.

None of the morphological characters traditionally used in lumbrinerid systematics could be assigned to a clade higher than the genus level. The morphological characters used by Carrera-Parra [7] to define large groups of genera, such as the presence of connecting plates and only four pairs of maxillae did not support any of the clades recovered in our analyses. On the contrary, the genera showing these character states were scattered across the whole tree (clades A + B + C, E, F, and H with connecting plates and clades D, G, I, J + K, and L + M with four pairs of maxillae) suggesting their homoplasy (Figure 3).

5. Conclusions

Insufficient taxon sampling and moderate molecular data allowed only partial revision of Lumbrineridae based on molecular phylogeny results. Nevertheless, our data corroborate monophyletic status of the genera *Abyssoninoe*, *Augeneria*, *Gallardonneris*, *Lumbrineriopsis*, and *Ninoe* and indicate polyphyly of the genera *Lumbrineris* and *Scoletoma*. Being the type genus of the family, *Lumbrineris* requires further study with inclusion of higher number of species and subsequent definition of a monophyletic group inholding the type species, *L. latreilli*, as the carrier of the genus name.

Strongly supported relationships between several clades suggest that the characters, such as the presence of bidentate simple hooded hooks, non-pigmented whitish central areas in maxillae IV, multidentate maxillae IV, the presence of connecting plates and four or five pairs of maxillae have evolved several times independently within Lumbrineridae and do not represent exclusive homologies defining groups of closely related genera.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/d14020083/s1, Table S1: List of specimens used in this study with GenBank Accession numbers, BOLD process ID, and data on their sampling and storage, Table S2: Primer sequences and PCR parameters used for amplification of COI, 18S rDNA and 16S rDNA, Figure S1: Consensus tree from the Maximum likelihood analysis of the combined COI, 16S rDNA and 18S rDNA dataset; numbers on nodes indicate bootstrap support; capital letters correspond with the clades (A–M) discussed in the text. Colored bars indicate polyphyletic genera. Sequences of fourteen specimens of *Lumbrineris mixochaeta* were collapsed into a single triangle for better presentation.

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