

Alentiana palinpada, a new commensal polynoid species from a seamount in the Northwest Pacific Ocean

Yueyun Wang^{1, 2†}, Hong Cheng^{1, 2†}, Chunsheng Wang^{1, 2, 3, 4*}

¹Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China

²Key Laboratory of Marine Ecosystem Dynamics, Ministry of Natural Resources, Hangzhou 310012, China

³School of Oceanography, Shanghai Jiao Tong University, Shanghai 200030, China

⁴Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519080, China

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Abstract

Alentiana has only one member, *A. aurantiaca* (Verrill, 1885), commensal with sea anemone (*Bolocera tuediae*). Here, *Alentiana palinpada* sp. nov., a second member in the genus *Alentiana* (subfamily Lepidastheniinae), a new commensal polynoid is described, based on a specimen collected from a deep-water seamount in the Northwest Pacific Ocean. The new species is characterized by large elytra that completely cover the dorsum and are present in every third segment in the posterior regions. Neuropodia are truncated, with two types of neurochaetae; supraacicular chaetae toothed on one side with a slightly inflated end, and subacicular chaetae that are heavy and smooth; the prechaetal lobe is larger than the postchaetal lobe, with the ventral part rolling upward and backward.

Key words: Polynoidae, new species, systematics, taxonomy, commensalism

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1 Introduction

Among polychaetes, polynoids have the highest number of symbiotic species found living with a wide variety of marine invertebrates, including other polychaetes (Salazar-Vallejo et al., 2015). *Alentiana aurantiaca*, the first and only member in this genus, living among the tentacles of the sea anemone *Bolocera tuediae*, was first described by Verrill (1885) as a member of *Polynoe*. After reviewing the type specimens, Hartman (1942) erected the genus *Alentiana* and recombined this species. *Alentiana* differs remarkably from the other genera of the family in having reduced notopodia without chaetae, and elytra present in every third segment in medial and posterior regions, neuropodial chaetae of two kinds: fine supraacicular chaetae with few rows of spines and single or few stouter, spine-like subacicular ones. *Alentiana aurantiaca* is described from northeastern coast of America (Verrill, 1885; Pettibone, 1963), and reported in European waters (Bellan, 2001).

Norlinder et al. (2012) analyzed the phylogenetic relationship of Polynoidae and other Aphroditiformia scale worms via the morphology and molecular sequences of 18S rDNA, 28S rDNA, 16S rDNA and the cytochrome c oxidase subunit I (COI) gene. There were 30 species of the Polynoidae family used in their study, but only two species, *Hyperhalosydna striata* and *Lepidasthenia elegans* belonging to the subfamily Lepidastheniinae, had reported as having those four marker genes. This study is the first report of molecular marker genes: two nuclear genes (18S rDNA and 28S rDNA) and two mitochondrial genes (16S rDNA and the COI gene) in the genus *Alentiana* (Lepidastheniinae). The marker-gene data

set was retrieved from next-generation sequencing (NGS) and whole genome survey sequences (GSS) and provided a method to obtain the gene information from the NGS and GSS data.

In this contribution, *Alentiana palinpada* sp. nov., the second member of this genus living as a commensal among the tentacles of the large sea anemone (Actiniidae), is described based on specimens collected from a seamount in the Northwest Pacific Ocean. This is the first report on the four molecular marker genes in the *Alentiana* species which can supplement the gene information of family Polynoidae in the public gene database.

2 Materials and methods

The specimen was collected by *Hailong III* ROV (remotely-operated vehicle) DY56 Cruise (the 56th China Ocean Scientific Research) in September 2019. When photographs were taken of the sea anemone sample, this living scale-worm suddenly slipped out from the socket among the tentacles of Actiniidae. The iridescence of its elytra surfaces was remarkable and impressive.

The specimen was preserved in a 95% ethanol solution and deposited in the Sample Repository of Second Institute of Oceanography (RSIO), Ministry of Natural Resources, Hangzhou, China. The characteristics of the whole body were observed with appendages dissected under a stereomicroscope (Zeiss Stemi 305). Parapodium and neurochaetae were dissected, and mounted onto temporary slides using glycerol or permanent slides using Canada Balsam. Methyl green was used for staining in order to distinguish papillae on the Pharynx. Photographs of the chaetae were captured using light microscopy (Zeiss Axio Imager A2).

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*Corresponding author, E-mail: wangsio@sio.org.cn

†These authors contributed equally to this work.

The total genomic DNA of this specimen was extracted from its muscle appendage using QIAGEN DNeasy® Blood and Tissue Kits (Qiagen, CA, USA), according to the manufacturer's protocol. Attempts to extract DNA from the elytra were unsuccessful, with little or nothing when we detected in the DNA concentration using a NanoDrop 2000 Spectrophotometer and a Qubit fluorometer. The qualified DNA was stored in sterilized Milli-Q® water at -80°C before sequencing. The entire genome was obtained by Illumina sequencing. One pair-end (PE150) library was constructed with insert size of approximately 350 bp, and sequenced on HiSeq™ X-Ten platform (Illumina, CA, USA) at Zhejiang Tianke High Technology Development Co., Ltd. A total of 61 640 260 sequence clean reads and 8.86 Gbp clean bases

(Q20 ≥ 98.96%) were obtained after quality trimming, and then de novo assembled by the SPAdes genome assembler (Bankevich et al., 2012). In order to pick out the nuclear 18S rDNA, 28S rDNA and mitochondrial 16S rDNA, and COI genes, the assembled contigs were aligned against the downloaded reference sequences of those four genes in the GenBank database via the NCBI local *blastn* program (Cock et al., 2015). The complete length of the COI gene sequence was checked, based on the result of invertebrate mitochondrial genome prediction by the MITOS server online (Bernt et al., 2013). The start and end of target sequences were checked by the reported universal primer pairs (Norlinder et al., 2012). The accession number of sequences used in phylogenetic analysis is listed in Table 1.

Table 1. List of information of 76 species and GenBank accession numbers of marker genes

Family	Species name	Origin	Voucher	Accession number				Reference
				18S rDNA	28S rDNA	16S rDNA	cox1 gene	
Polynoidae	<i>Abyssarya acus</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1811	MH233231	-	MH233179	MH233277	Bonifácio and Menot (2019)
	<i>Acholoe squamosa</i>	France	SMNH118959	AY839567	JN852850	JN852888	AY839576	Norlinder et al. (2012)
	<i>Alentia gelatinosa</i>	Trondheimsfjord, Norway	-	AY839566	-	-	AY839577	Gonzalez et al. (2018)
	<i>Alentiana palinopoda</i>	Pacific Ocean	B6317500003	MW397195	MW405100	MW397208	MW374288	this study
	<i>Antarctinoe ferox</i>	Ross Sea	-	KF713423	-	KF713463	KF713373	Gonzalez et al. (2018)
	<i>Bathyedithia retierei</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1814	MH233215	-	MH233157	-	Bonifácio and Menot (2019)
	<i>Bathyeliasona mariaae</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1815	MH233204	-	MH233149	MH233249	Bonifácio and Menot (2019)
	<i>Bathyfauwelia glacigena</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1817	MH233218	-	MH233160	MH233274	Bonifácio and Menot (2019)
	<i>Bathyfauwelia ignigena</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1819	MH233246	-	MH233200	MH233262	Bonifácio and Menot (2019)
	<i>Bathymoorea lucasi</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1822	MH233223	-	MH233165	MH233266	Bonifácio and Menot (2019)
	<i>Bathypolaria</i> sp. 173	Clarion-Clipperton Fracture Zone	MNHN-IA-PNT 63	MH233206	-	MH233151	MH233281	Bonifácio and Menot (2019)
	<i>Branchinotogluma bipapillata</i>	Southwest Indian Ridge	RSIO3530	MH717079	-	MH717078	MH202755	Zhou et al. (2018)
	<i>Branchinotogluma elytrapapillata</i>	Okinawa Trough	NSMT-Pol P-668	MG799378	MG799380	MG799377	MG799387	Zhang et al. (2018a)
	<i>Branchinotogluma ovata</i>	Manus Back-Arc Basin	MBM286062-sps2	MK211411	MK211413	MK211416	MK357896	Wu et al. (2019)
	<i>Branchinotogluma sandersi</i>	Juan de Fuca Ridge	SMNH118960	JN852821	JN852851	JN852889	JN852923	Norlinder et al. (2012)
	<i>Branchinotogluma segonzaci</i>	Manus Back-Arc Basin	MBM286042	MK211412	-	MK211418	MK357906	Wu et al. (2019)
	<i>Branchinotogluma trifurcus</i>	Manus Back-Arc Basin	MBM286046	MK211410	-	MK211415	MK357905	Wu et al. (2019)
	<i>Branchipolynoe longqiensis</i>	Longqi field, Southwest Indian Ocean Ridge	-	KY753847	KY753847	KY753826	KY753826	Zhang et al. (2018b)
	<i>Branchipolynoe pettiboneae</i>	Seep, South China Sea	-	KY753840	KY753840	KY753825	KY753825	Zhang et al. (2018b)
	<i>Bruunilla nealae</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1824	MH233216	-	MH233158	-	Bonifácio and Menot (2019)
	<i>Bylgides elegans</i>	Sweden	SMNH118962	JN852822	JN852852	JN852890	JN852924	Norlinder et al. (2012)
	<i>Bylgides sarsi</i>	Sweden	SMNH118961	JN852823	JN852853	JN852891	JN852925	Norlinder et al. (2012)
	<i>Capitulatinoe</i> cf. <i>cupisetis</i>	Clarion-Clipperton Fracture Zone	-	KF919301	-	KF919303	-	Bonifácio and Menot (2019)
	<i>Eunoe nodosa</i>	Norway	SMNH118963	JN852824	JN852854	JN852892	JN852926	Norlinder et al. (2012)
	<i>Gastrolepidia clavigera</i>	Papua New Guinea	SMNH118964	JN852825	JN852855	JN852893	JN852927	Norlinder et al. (2012)
	<i>Gattyana ciliata</i>	San Juan Channel, WA	USNM1077218	AY894297	DQ790035	-	AY894312	Gonzalez et al. (2018)
	<i>Gattyana cirrhosa</i>	Sweden	SMNH118965	JN852826	JN852856	JN852894	JN852928	Norlinder et al. (2012)
<i>Gesiella jameensis</i>	Lanzarote, Canary Islands	-	KY454403	KY823476	KY454412	KY454429	Gonzalez et al. (2018)	
<i>Halosydna brevisetosae</i>	California	SMNH118966	JN852827	JN852857	JN852895	AY894313	Norlinder et al. (2012)	

to be continued

Continued from Table 1

Family	Species name	Origin	Voucher	Accession number				Reference
				18S rDNA	28S rDNA	16S rDNA	<i>coxI</i> gene	
	<i>Halosydnella australis</i>	Baia de Paranagua, Pontal do Sul, Brazil	-	KY823449	KY823463	KY823480	KY823495	Gonzalez et al. (2018)
	<i>Harmothoe glabra</i>	England	SMNH118967	JN852828	JN852858	JN852896	JN852929	Norlinder et al. (2012)
	<i>Harmothoe imbricata</i>	Bohuslan, Sweden	-	AY340434	AY340400	AY340463	AY839580	Gonzalez et al. (2018)
	<i>Harmothoe impar</i>	Sweden	SMNH118968	JN852829	JN852859	JN852897	JN852930	Norlinder et al. (2012)
	<i>Harmothoe oculinarum</i>	Norway	SMNH118969	AY894299	JN852860	JN852898	AY894314	Norlinder et al. (2012)
	<i>Harmothoe rarispina</i>	Disko Island, Greenland	-	KY657611	KY657624	KY657641	KY657659	Gonzalez et al. (2018)
	<i>Hermenia verruculosa</i>	Belize	SMNH118970	JN852830	JN852861	JN852899	JN852931	Norlinder et al. (2012)
	<i>Hodor anduril</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1826	MH233240	-	MH233191	MH233288	Bonifácio and Menot (2019)
	<i>Hodor hodor</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1825	MH233238	-	MH233189	MH233257	Bonifácio and Menot (2019)
	<i>Hyperhalosydna striata</i>	Japan	SMNH118971	JN852831	JN852862	JN852900	JN852932	Norlinder et al. (2012)
	<i>Intoshella dictyaulus</i>	near Mariana Trench	MBM 304 568	MG519807	-	-	MG519808	Sui et al. (2019)
	<i>Lepidasthenia elegans</i>	France	SMNH118973	JN852832	JN852863	JN852901	JN852933	Norlinder et al. (2012)
	<i>Lepidonotus clava</i>	England	SMNH118974	JN852833	JN852864	JN852902	JN852934	Norlinder et al. (2012)
	<i>Lepidonotus squamatus</i>	Sweden	SMNH118975	AY176290	JN852865	JN852903	AY894316	Norlinder et al. (2012)
	<i>Lepidonotus sublevis</i>	Mumlo Cove, Griffin Bay, WA	USNM107222	AY894301	DQ790039	-	AY894317	Gonzalez et al. (2018)
	<i>Levensteiniella undomarginata</i>	Okinawa Trough	CBM-ZW 1118	MG799379	MG799381	MG799376	MG799385	Zhang et al. (2018a)
	<i>Macellicephala clarionensis</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1828	MH233233	-	MH233183	MH233269	Bonifácio and Menot (2019)
	<i>Macellicephala parvafauces</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1831	MH233210	-	MH233153	MH233282	Bonifácio and Menot (2019)
	<i>Macellicephalooides moustachu</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1832	MH233212	-	-	-	Bonifácio and Menot (2019)
	<i>Malmgreniella mcintoshii</i>	Sweden	SMNH118976	JN852834	JN852866	JN852904	JN852935	Norlinder et al. (2012)
	<i>Melaenis loveni</i>	Svalbard	SMNH118977	JN852835	JN852867	JN852905	JN852936	Norlinder et al. (2012)
	<i>Neopolynoe paradoxa</i>	Norway	SMNH118978	JN852836	JN852868	JN852906	JN852937	Norlinder et al. (2012)
	<i>Nu aakhu</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1836	MH233209	-	-	-	Bonifácio and Menot (2019)
	<i>Paradyte crinoidicola</i>	Papua New Guinea	SMNH118979	JN852837	JN852869	JN852907	JN852938	Gonzalez et al. (2018)
	<i>Paralepidonotus ampulliferus</i>	Papua New Guinea	SMNH118980	JN852838	AF185164	JN852908	JN852939	Gonzalez et al. (2018)
	<i>Pelagomacellicephala cf. iliffii</i>	Eleuthera, Bahamas	ZMUC-POL-2394	KY454408	KY823474	KY454420	KY454435	Gonzalez et al. (2018)
	<i>Polaruschakov lamellae</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1837	MH233205	-	MH233150	MH233250	Bonifácio and Menot (2019)
	<i>Polynoe scolopendrina</i>	England	SMNH118981	JN852839	JN852870	JN852909	JN852940	Norlinder et al. (2012)
	<i>Thormora jukesii</i>	Japan	SMNH118983	JN852840	JN852871	JN852910	JN852941	Norlinder et al. (2012)
	<i>Yodanoe desbruyeresi</i>	Clarion-Clipperton Fracture Zone	MNHN-IA-TYPE 1843	-	-	MH233156	MH233251	Bonifácio and Menot (2019)
Outgroup								
Sigalionidae	<i>Neoleanira tetragona</i>	Sweden	SMNH118984	AY839570	JN852872	JN852911	AY839582	Norlinder et al. (2012)
	<i>Psammolyce</i> sp. EN 2012	Belize	SMNH118989	JN852843	JN852877	JN852916	-	Norlinder et al. (2012)
	<i>Sigalion spinosus</i>	-	-	AY894304	DQ790062	-	AY894319	Norlinder et al. (2012)
Iphionidae	<i>Iphione</i> sp.	Papua New Guinea	SMNH118972	JN852819	-	JN852886	JN852921	Norlinder et al. (2012)
	<i>Thermiphione</i> sp. EN 2012	Fiji, Lau Basin	SMNH118982	JN852820	JN852849	JN852887	JN852922	Norlinder et al. (2012)
Chrysopetalidae	<i>Chloeia flava</i>	Japan	SMNH95025	EF076780	EF076781	JN852917	JN852944	Norlinder et al. (2012)
Euphrosinidae	<i>Euphrosine foliosa</i>	Banyuls,	SMNH95028	EF076784	EF076785	JN852918	JN852945	Norlinder et al. (2012)
	<i>Mexieulepis weberi</i>	Belize	SMNH118958	JN852818	-	JN852885	JN852920	Norlinder et al. (2012)

to be continued

Continued from Table 1

Family	Species name	Origin	Voucher	Accession number				Reference
				18S rDNA	28S rDNA	16S rDNA	cox1 gene	
Nereididae	<i>Nereis pelagica</i>	Sweden, paragenophore	SMNH118992	AF474279	AY612636	AY340470	JN852947	Norlinder et al. (2012)
Syllidae	<i>Eusyllis blomstrandii</i>	Sweden, paragenophore	SMNH118994	EF123887	JN852880	EF123788	EF123749	Norlinder et al. (2012)
Glyceridae	<i>Glycera alba</i>	Sweden, paragenophore	SMNH118991	AY176287	DQ779689	DQ779615	JN852946	Norlinder et al. (2012)
Aphroditidae	<i>Aphrodita aculeata</i>	Sweden	SMNH118956	AY176281	JN852846	JN852882	AY839578	Norlinder et al. (2012)
	<i>Laetmonice filicornis</i>	Sweden	SMNH118955	JN852816	JN852847	JN852883	JN852919	Norlinder et al. (2012)
Pholoidae	<i>Pholoe baltica</i>	Sweden	SMNH118985	AY839573	JN852873	JN852912	AY839585	Norlinder et al. (2012)
	<i>Pholoe pallida</i>	Sweden	SMNH118986	AY894302	JN852874	JN852913	AY894318	Norlinder et al. (2012)
	<i>Pisione remota</i>	-	-	AY525628	-	-	AF221575	Norlinder et al. (2012)
	<i>Pisionidens</i> sp.	Mexico	SMNH118988	JN852842	JN852876	JN852915	JN852943	Norlinder et al. (2012)

Note: - means the data had not reported in the database.

Nuclear and mitochondrial sequences were aligned using the ClustalW algorithm with default settings (15/6.66 as gap/gap length penalties) in Geneious Prime software (Biomatters Ltd., New Zealand). The alignments of nuclear 18S rDNA, 28S rDNA and mitochondrial 16S rDNA and COI genes were performed separately, and then concatenated into one file after manually trimming unalignable regions on both flanks. The phylogenetic tree was performed with Maximum Likelihood analysis (ML), using the IQ-TREE Web Server (Trifinopoulos et al., 2016) with auto-selected substitution model, default parameters and 1 000 bootstrap replicates (command line: path_to_iqtree -s concatenated_alignments.phy -spp concatenated_alignments.partition.txt -pre concatenated_alignments.phy -m TEST -bb 1000 -alrt 1000). The SH-aLRT test (Guindon et al., 2010) was performed with the manual's recommendation of adding "-alrt 1000" into the IQ-TREE command line.

3 Systematics

Family Polynoidae Kinberg, 1856

Subfamily Lepidastheniinae Pettibone, 1989

Genus *Alentiana* Hartman, 1942

Alentiana palinpada sp. nov. (Fig. 1)

Material examined. Holotype, catalog number B6317500003, unnamed seamount in the Northwest Pacific Ocean, 15°31'N, 161°48'E, 1 156 m depth, commensally with a sea anemone (Actiniidae), September 20, 2019.

Diagnosis. Body short with 37 segments. Elytra large, completely covering dorsal surface, present in every third segment in posterior regions. Notopodia reduced, without notochaetae. Neuropodia truncated, with two types of neurochaetae; prechaetal lobe larger than postchaetal lobe, with ventral part rolling upward and backward.

Description. Holotype complete, ca. 32 mm long, 13 mm wide, including parapodia, with 37 segments (including tentacular segment), last two small. Body dorsoventrally flattened, not tapering posteriorly, with very long parapodia.

Prostomium bilobed, wider than long, without cephalic peaks. Two pairs of conspicuous dark eyes, anterior pair largest, situated laterally on widest part of prostomium, posterior pair dorsally, near hind margin of prostomium. Median and lateral antennae present; median antenna with bulbous ceratophore in anterior notch of prostomium and a long style about two times the length of lateral ones; lateral antennae inserted terminally on prostomium, with ceratophores formed by anterior prolongations of prostomial lobes. Styles smooth, tapering. Palps stout, 1.5 times thicker than antennae, tapered, smooth, and slightly shorter than median antenna. Facial tubercle absent. Pharynx with two pairs of jaws without any teeth (Figs 1h and i) and encircled distally by nine pairs of bulbous papillae (Figs 1b, c, and g). Two pairs of lateral papillae on subterminal pharynx (Figs 1b, c, and

g). Tentacular segment (Segment 1) with two pair tentaculophores directed anteriorly, lateral to prostomium, without chaetae (Fig. 1j); tentacular cirri longer than median antenna. Second segment with first pair of elytra, sub-biramous parapodia, and ventral cirri obviously longer than the following ones. Parapodia same from second segment to the end of the body.

Sixteen pairs of large, bulbous elytraphores present on Segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, 32, and 35. Elytra large, completely covering the dorsum, oval to subreniform in shape, without papillae or microtubercles on margin and surface (Figs 1p and q). Dorsal tubercles on cirriferous segments, indistinct (Fig. 1k). Parapodia sub-biramous (Figs 1m and n) throughout body, with embedded noto- and neuroacacula (Fig. 1n). Dorsal cirri on cirriferous segments elongate, about six to seven times longer than ventral cirri. Notopodia reduced to a small digitate lobe, without chaetae (Figs 1k and m). Neuropodia distally truncate, elongate, notched dorsally and ventrally (Fig. 1o), prechaetal lobe larger than postchaetal lobe, forming upward flanged structure in ventral side, and enveloping ventral side of postchaetal lobe (Figs 1l and o). Neurochaetae of two kinds, including six to ten supraacicular chaetae toothed on one side (Fig. 1s) with a slightly inflated end and a heavy, smooth, subacicular chaeta (rarely two in a parapodium), with falcate tip and smooth sides (Fig. 1r). All chaetae and acicula yellow. The ventral nephridial papillae small, beginning on Segment 4.

Color: body and pharynx red, elytra dark brown in live specimen; body pale yellow with dark pigments (Figs 1a–d), elytra white in ethanol-preserved specimen (Figs 1e–m).

Etymology. The specific name *palinpada* is a combination of the Greek *palin-* (backward) and *poda* (foot), referring to the ventral side of neuropodia of the new species rolling upward and backward.

Distribution. Only known from the type locality.

Remarks. *Alentiana aurantiaca* (Verrill, 1885) was the only known member of the genus *Alentiana* prior to the report of this new species. The major characteristics of *Alentiana palinpada* sp. nov. are mostly similar to those of *A. aurantiaca*, originally described from northeastern coast of America (Verrill, 1885; Hartman, 1942). Both species are commensal among the tentacles of Actiniidae (sea anemone). However, *Alentiana palinpada* sp. nov. clearly differs from *A. aurantiaca* in the number of chaetigers, the arrangement of elytra, neuropodium, and subacicular chaeta as follows. (1) About 39 chaetigers are present in *A. aurantiaca*, whilst there are 37 in *A. palinpada* sp. nov. (2) Large elytra completely cover the dorsum in *A. palinpada* sp. nov., whereas those of *A. aurantiaca* leave the median dorsum broadly exposed. (3) The neuropodium of *A. aurantiaca* has triangular chaetal lobes, and the prechaetal lobe slightly exceeds the postchaetal lobe in length. However, that of *A. palinpada* sp. nov. is truncated with pre- and postchaetal lobes equal; the prechaetal lobe of *A. palinpada* sp. nov. is larger than the post-

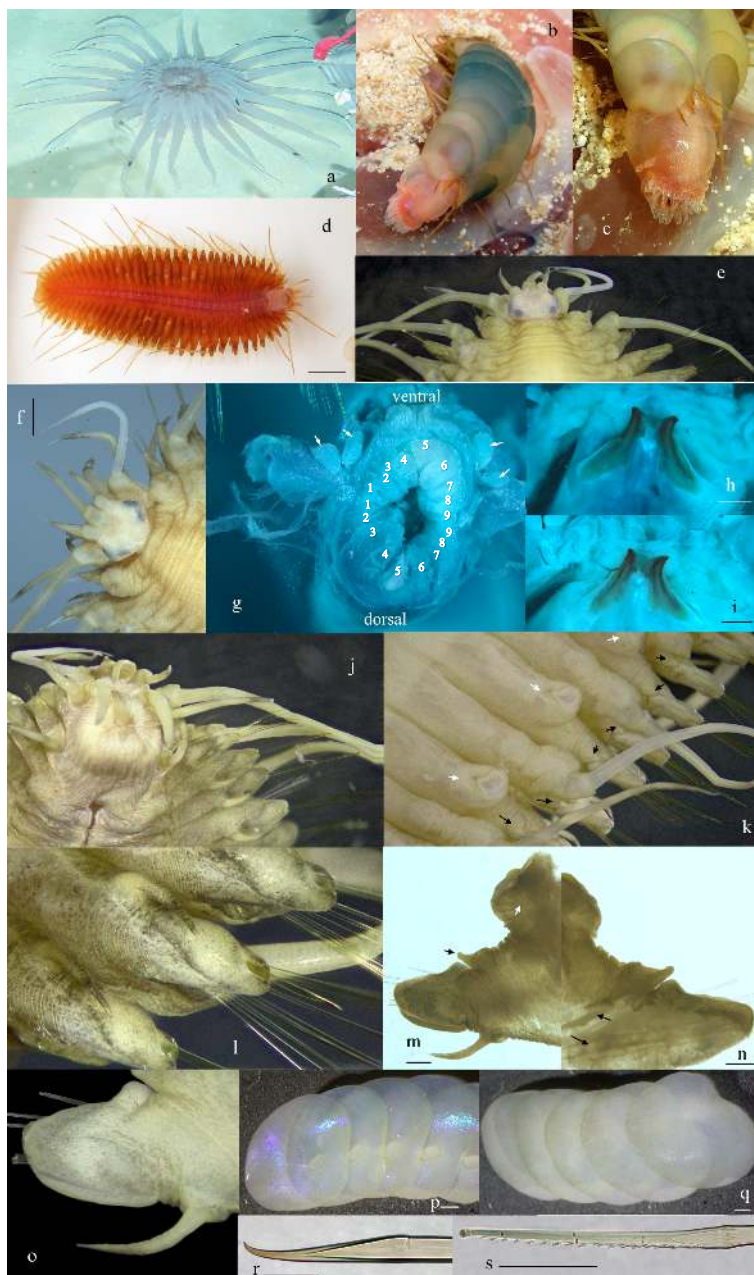


Fig. 1. Holotype of *Alentiana palinpoda* sp. nov. a. Living *Alentiana* and sea anemone *in situ*; b. dorsal view of living specimen (pharynx introverted); c. pharynx; d. ventral view of specimen; e and f. dorsal view of head (elytra removed); g. opening of the pharynx distally encircled by nine pairs of bulbous papillae, white arrows show the subterminal lateral papillae; h and i. two pairs of dorsal and ventral jaws respectively; j. ventral view of head; k. dorsal view of segments in mid-body (black arrows show notopodia, white arrows show elyptrophores); l. ventral view of the 6th parapodium; m. posterior view of the 15th parapodium (black arrows show notopodium, white arrows show elyptrophore); n. anterior view of the 15th parapodium (black arrows show noto- and neuroacacula); o. posterior view of the 15th neuropodium; p and q. ventral and dorsal view of elytra respectively; r. subacicular chaeta; s. supraacicular chaeta. g-i were stained with methyl green. Scale bars: 0.5 cm (d), 1.0 mm (f), 0.5 mm (h-i), 100 μ m (m-n and p-s).

chaetal lobe in width, and rolls upward and backward on the ventral side. And (4) the distal end of the subacicular chaeta is more curved in *A. palinpoda* sp. nov.

4 Results and discussion

The information of selected taxa for phylogenetic analysis is presented in Table 1. The length of 18S rDNA, 28S rDNA and 16S rDNA was confirmed by the respective sequences of PCR primers (Norlinder et al., 2012). The primer-sequence alignments of PCR

primers located in the position of related genes are shown in the schematic map (Fig. A1 in Appendix). This study can also provides base information to design or edit primers when we need to amplify the gene fragments of relevant species. The data set of 18S rDNA, 28S rDNA, 16S rDNA partial sequence and COI complete sequence of *Alentiana palinpoda* sp. nov. (voucher B6317500003) contained 1 013 bp (1 013 bp used in phylogenetic analysis, total length 1 481 bp), 1 108 bp, 527 bp and 1 524 bp, respectively.

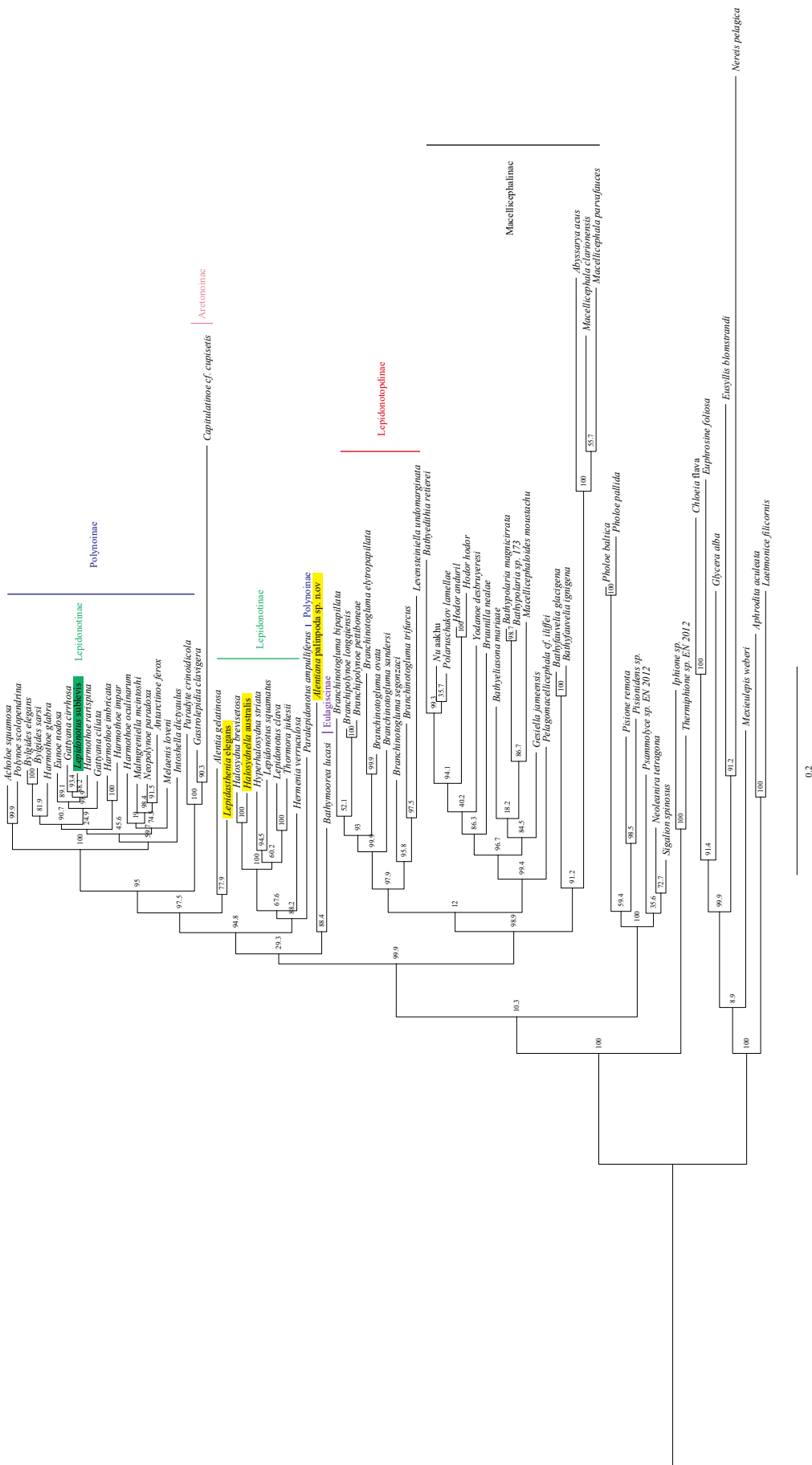


Fig. 2. Maximum-likelihood phylogenetic tree of 59 polynoid species from six subfamilies of Polynoidae, showing the relationships of the novel species *Alentiana palipoda* and related taxa. The tree is assessed from the concatenated alignments of 18S rDNA, 28S rDNA, 16S rDNA and COI gene. Each branch was assigned SH-aLRT supports. Numbers near the branch nodes refer to SH-aLRT support values (all based on 1 000 replicates). Lepidosthinae genera are highlighted with yellow color. The clade appeared to be more reliably supported when its SH-aLRT value is equal or greater than 80%.

The ML tree was constructed based on the concatenated alignment file of *Alentiana palinpoda* sp. nov. and 75 reference species (Fig. 2). Molecular phylogeny revealed the subfamily Lepidonotinae Willey, 1902 is a polyphyletic group. *Lepidasthenia elegans* and *Hyperhalosydna striata* are clustered with members of Lepidonotinae. Both of the two genera belong to the subfamily Lepidastheniinae (Pettibone, 1989). This topology suggests a closed relationship between Lepidonotinae and Lepidastheniinae and the monophyly of Lepidonotinae is doubtful. Lepidonotinae differs from Lepidastheniinae in the form of neuropodia and elytra (Wehe, 2006). Neuropodia are deeply incised dorsally and ventrally in Lepidastheniinae, whilst not deeply incised in Lepidonotinae. Elytra are usually small, not covering the mid-dorsum and smooth in Lepidastheniinae while they are usually large, covering the mid-dorsum and ornamented in Lepidonotinae. Elytra of *Alentiana palinpoda* sp. nov. are large, covering the mid-dorsum. This characteristic makes it easily distinguishable from other members of Lepidastheniinae. *Alentiana palinpoda* sp. nov. and *Bathymoorea lucasi* (subfamily Eulagiscinae) form a well supported clade. *Bathymoorea* has one pair of large eyes, elongate acicular lobe and numerous notochaetae. Bonifácio and Menot (2019) synonymize all these subfamilies lacking lateral antennae (Bathyedithinae, Bathymacellinae, Branchinotogluminae, Branchiplicatinae, Branchipolynoinae, Lepidonotopodinae, Macellicephalinae, Macellicephaloidinae, Macelloidinae, Polaruschakovinae and Vampiropolynoinae) with *Macellicephalinae sensu* Hartmann-Schröder, 1971. Hatch et al. (2020) reinstated Lepidonotopodinae comprised of *Branchipolynoe*, *Branchinotogluma*, *Bathykurila*, *Branchiplicatus*, *Lepidonotopodium*, *Levensteiniella*, *Thermopolynoe*, and *Peinaleopolynoe*. In our tree, Lepidonotopodinae clustered with partial members of Macellicephalinae with low supported value. Our results support the phylogenetic proposal of Bonifácio and Menot (2019). The phylogenetic relationship between Lepidastheniinae and other polynoid subfamilies has not been fully investigated yet. Lepidastheniinae comprised of 10 genera (*Alentiana*, *Benhamipolynoe*, *Hyperhalosydna*, *Lepidasthenia*, *Lepidastheniella*, *Parahalosydna*, *Perolepis*, *Pseudopolynoe*, *Showapolynoe*, *Telolepidasthenia*), while only three of them included in our analysis. Further analyses would be required to assess the taxonomic relationship between Lepidastheniinae and Lepidonotinae.

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Appendix:

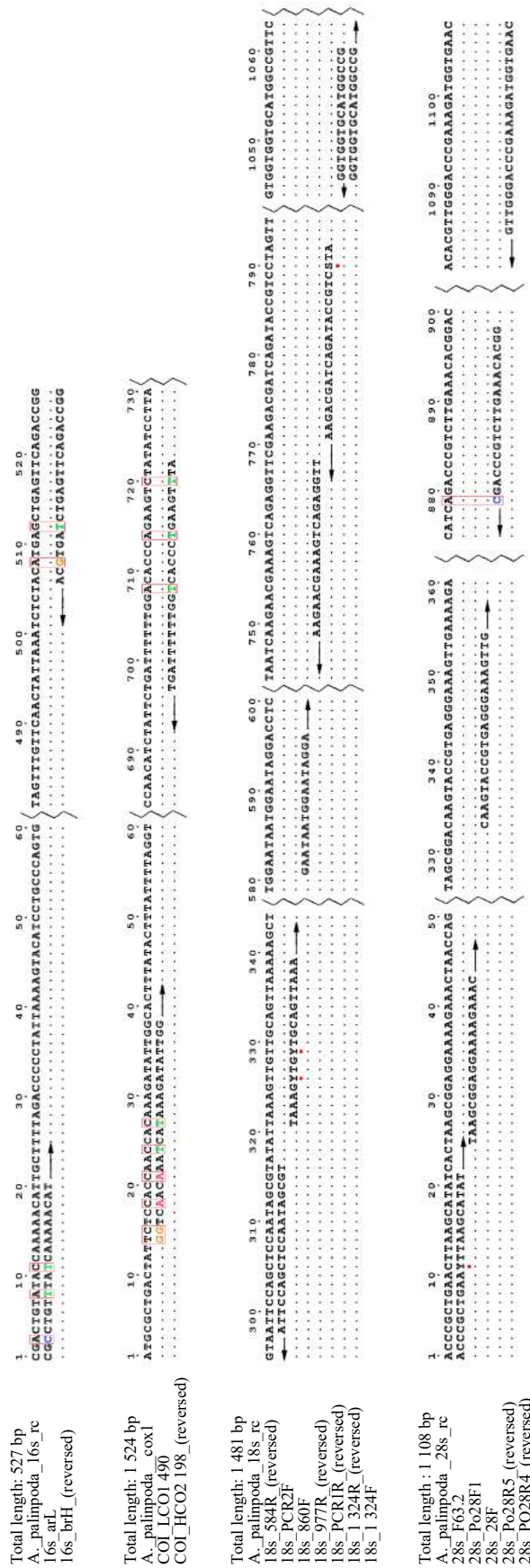


Fig. A1. The schematic map of PCR primers locate position in the target marker genes. The arrows the amplified orientation in the sequence. The vertical wavy lines present the abridged nucleotide base. The colorful base and red pane show the discrepancy between gene and primer sequence. Red-filled circles show the wobble bases in the primers. The universal name of primers is listed in the left edge. This alignment information can provide the evidence to design or edit the primers when amplifying the gene fragments of relevant species.