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Research Article

Reevaluation of the Systematic Status of *Branchinotogluma* (*Annelida, Polynoidae*), with the Establishment of Two New Species

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Branchinotogluma is one of the most diverse genera among the polynoids inhabiting deep-sea chemosynthetic environments. A total of 14 species have been described from the hydrothermal vents and cold seeps worldwide. Our phylogenetic analyses based on four genes (COI, 16S, 18S, and 28S rRNA) recovered the current *Branchinotogluma* as paraphyletic, with seven clades scattered in two main clades. The monophyly of the main clade composed of *Branchinotogluma* (except *Branchinotogluma segonzaci*), *Branchipolynoe,* and *Peinaleopolynoe* is supported by the presence of 20–21 segments with 9–10 pairs of elytra, ventral papillae starting from segment 12 in males, and arborescent branchiae. The monophyly of another main clade composed of *B. segonzaci*, *Bathykurila*, *Lepidonotopodium*, *Levensteiniella*, and *Thermopolynoe* is supported by the presence of usually 22–30 segments with 11 pairs of elytra and the lack of ventral papillae in females. In addition, our study recognized two new species, *Branchinotogluma nanhaiensis* sp. nov. and *B. robusta* sp. nov., based on specimens collected from the deep-sea cold seeps in the South China Sea. Both the morphology and molecular phylogenetic analyses support the validity of the two new species and the sister relationships between *B. nanhaiensis* and *B. japonicus* and between *B. robusta* and *B. pettiboneae* as well.

1. Introduction

The deep-sea hydrothermal vents and cold seeps are typical extreme environments on Earth, sharing members of specialized macrofaunas, such as siboglinid tubeworms, bathymodiolin mussels, and vesicomyid clams [1]. *Polynoidae* is among the most diverse annelid families in the deep sea [2], particularly well represented in deep-sea hydrothermal vents, cold seeps, whale carcasses, and sunken wood [3].

Among the vent polynoids, *Branchinotogluma* is the most diverse genus with a total of 14 species described worldwide (Figure 1). Except the species *B. bipapillata* [4]

described from the Indian Ocean, all other species were found from the Pacific Ocean. Ten species were described from the western Pacific, viz. *B. burkensis* [5], *B. marianus* [5], *B. nikkoensis* [6], *B. sagamiensis* [6], *B. segonzaci* [7], *B. trifurcus* [7], *B. elytropapillata* [8], *B. japonicus* [9], *B. pettiboneae* [10], and *B. ovata* [10]. Three species are distributed in the eastern Pacific, including *B. hessleri* [11], *B. sandersi* [11], and *B. tunnicliffeae* [12]. Additionally, there are seven nominal species (i.e., *B.* sp1; *B.* sp2 in [13]; *B. sandersi* in [14]; *B.* sp. nov. 1; *B.* sp. nov. 2; *B.* sp. nov. 3; *B.* sp. nov. 4 in [15]) recorded in some ecological or systematics works [13–15]. Although these species have not been
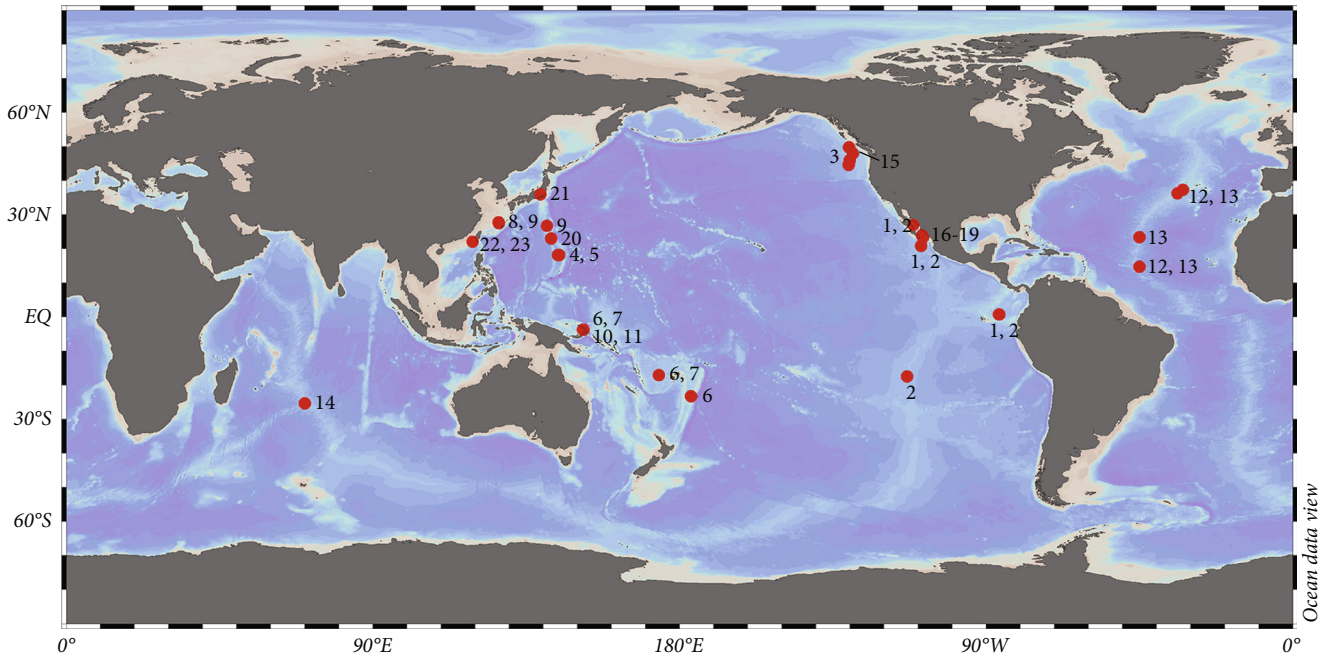
FIGURE 1: Distribution of the species of *Branchinotogluma* (1–23), based on the data of reference [6, 10] and this study. (1) *Branchinotogluma hessleri* [11]; (2) *B. sandersi* [11]; (3) *B. tunnicliffeae* [12]; (4) *B. burkensis* [5]; (5) *B. marianus* [5]; (6) *B. segonzaci* [7]; (7) *B. trifurcus* [7]; (8) *B. elytropapillata* [8, 17]; (9) *B. japonicus* [9]; (10) *B. pettiboneae* [10]; (11) *B. ovata* ([10]); (12) *B.* sp1 [13]; (13) *B.* sp2 [13]; (14) *B. bipapillata* [4]; (15) *B. sandersi* [14]; (16) *B.* sp. nov. 1 [15]; (17) *B.* sp. nov. 2 [15]; (18) *B.* sp. nov. 3 [15]; (19) *B.* sp. nov. 4 [15]; (20) *B. nikkoensis* [6]; (21) *B. sagamiensis* [6]; (22) *B. nanhaiensis* sp. nov.; (23) *B. robusta* sp. nov.

formally described, barcoding studies suggested that they likely represent different species from the 14 known species [10, 16]. Thus, the diversity of the genus *Branchinotogluma* still needs to be further investigated.

Previous studies have indicated the paraphyly of *Branchinotogluma* [8, 16, 17]. The phylogenetic relationships among the species of *Branchinotogluma*, particularly the two "weird" species, *B. segonzaci* [7] and *B. trifurcus* [7] need to be reevaluated. Both species have an indistinctly bilobed prostomium with a pair of prominently cylindrical anterior lobes. In addition, females of *B. segonzaci* have no ventral papillae, and *B. trifurcus* has reduced parapodia resembling members of *Branchipolynoe*, while most species of *Branchinotogluma* have a bilobed prostomium with triangular anterior lobes, ventral papillae in females, and a uniform pattern for parapodia. Barcoding and phylogenetic analyses have showed that both species are clearly divergent from the congeneric species [10, 16], further urging us to reevaluate the systematic status of the genus *Branchinotogluma* and related taxa.

Our examination of the specimens recently collected from the deep-sea cold seeps in the South China Sea revealed two undescribed species of *Branchinotogluma* which are herein described as *B. nanhaiensis* sp. nov. and *B. robusta* sp. nov. Thus, we reevaluated the systematic status of *Branchinotogluma* and related taxa based on four gene fragments (COI, 16S rRNA, 18S rRNA, and 28S rRNA). Moreover, we traced the evolution of the characters related to prostomium, segment, elytra, branchiae, and ventral papillae and established ancestral and derived characters of the

main clades within the polynoids from chemosynthesis-based ecosystems.

2. Materials and Methods

2.1. Specimen Collection and Morphological Examination. All specimens were obtained from five dives by the remotely operated vehicle (ROV) Fa Xian (Discovery) on board the R/V Ke Xue (Science) from a deep-sea cold seep (119°17′E, 22°074N, 1118.6–1121.8 m) in the South China Sea from July 2015 to August 2016. They were preserved in 80% (v/v) ethanol solution and later deposited in the Marine Biological Museum of the Chinese Academy of Sciences (MBMCAS), Institute of Oceanology, Chinese Academy of Sciences (IOCAS).

Morphological details of head, elytra, pygidium, ventral papillae, parapodia, branchiae, and chaetae were observed using a Zeiss Discovery V20 stereomicroscope and a Zeiss Imager Z2 compound microscope. Digital photographs were taken through an Axiocam 512 color camera mounted on the two microscopes and then stacked with images of different focal planes using Helicon Focus 7. Selected parapodia and segments were stained with methyl green in order to improve contrast. Parapodia for SEM observations were dehydrated, dried at critical point, coated with gold, and then observed and photographed using a S-3400N (Hitachi) scanning electron microscope. The morphological terminology followed Wu et al. [10] and Hatch et al. [16].

2.2. DNA Extraction, Amplification, and Sequencing. Specimens of *Branchinotogluma nanhaiensis* sp. nov., *B. robusta* sp. nov., *B. pettiboneae* [10], *Levensteiniella manusensis* [18], and *Thermopolynoe branchiata* [19] were used for gene sequencing (Table 1). Genomic DNA was extracted using the TIANamp Marine Animal DNA Kit (Tiangen Bio. Co., Beijing, China) following the instructions. Approximately, 650 bp of COI (cytochrome c oxidase subunit I), 400 bp of 16S rRNA, 1700 bp of 18S rRNA, and 1000 bp of 28S rRNA genes were amplified using the primers listed in Table S1. Polymerase chain reactions (PCR) were performed in a 25 μL reaction which contained 12 μL 2X Es Taq Master Mix (CWBIO Co., Ltd, Beijing, China), 1 μL of each primer (10 mM), 2 μL of template DNA (50 ng/μL), and 9 μL ddH$_2$O. The fragments were amplified in a T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) under the following conditions: initial denaturing at 95°C for 3 min, 40 cycles of 95°C for 30 s, 46–55°C (annealing temperatures, Table S1) for 45 s and 72°C for 40-90 s (extension time, see Table S1), and final extension step at 72°C for 7 min. The PCR products were purified and then sequenced through the ABI 3730 DNA Analyzer using the same forward and reverse primers for PCR amplification by sequencing facility of Shanghai Sangon Biological Engineering and Technical Service Company, Shanghai, China.

2.3. Species Delimitation and Phylogenetic Analyses. Three sequence datasets were created for the genetic distance and phylogenetic analyses: (1) 41 COI sequences including 21 new ones obtained from available specimens of *Branchinotogluma* for barcoding analysis, (2) 44 concatenated sequences of COI, 16S, 18S, and 28S for phylogenetic analysis, and (3) 28 concatenated sequences selected from 44 sequences for phylogenetic and character evolution analysis. *Halosydnella australis*, *Harmothoe impar*, and *Lepidonotus clava* were chosen as the outgroup (Table 1).

The newly generated sequences along with data downloaded from GenBank (Table 1) were aligned for each gene with MAFFT [25] using "—auto" strategy and normal alignment mode. Translate frame of the COI alignments was moved through discarding one base or two bases on left end and would be regarded as accurate until no stop codons are found inside the alignments. Gap sites were removed with trimAl [26] using "-automated1" command. Minimum uncorrected interspecific pairwise distances and maximum uncorrected intraspecific distances were calculated for the first dataset with MEGA X with on the Kimura 2-parameter model [27].

Aligned sequences for COI, 16S, 18S, and 28S were concatenated using PhyloSuite v1.2.2 [28]. Best partitioning models for the four predefined partitions were selected using the greedy algorithm and the corrected Akaike information criterion (AIC) in PartitionFinder2 [29]. The GTR+I+G model was selected for the COI sequences and both concatenated sequences. Maximum likelihood (ML) analysis was performed using IQ-TREE 1 [30] under Edge-unlinked partition model for 100,000 ultrafast [31] bootstraps. Bayesian inference (BI) analysis of the concatenated data were conducted using MrBayes 3.2.6 [32] under the partitioned

models (2 parallel runs, 10,000,000 generations) with sampling every 1000 generations. The initial 25% of sampled data were discarded as burn-in and the remaining trees were used to construct the 50% majority rule consensus tree and to estimate the posterior probabilities (PP). The effective sample size (ESS) values for all sampled parameters were diagnosed by Tracer v1.6 [33] to make sure convergence was reached. Finally, the phylogenetic tree was annotated with the ML bootstrap percentages and the BI posterior probabilities.

2.4. Character Evolution. Eleven morphological characters correlated with prostomium, segment, elytra, branchiae, and ventral papillae (Table 2) were summarized (Table S3) and then mapped onto a simplified BI tree. Ancestral state reconstruction (ASR) analyses were conducted using Mesquite v.3.6.1 [34] to examine the character evolutionary patterns and infer ancestral and derived characters within the studied polynoids. MK1 likelihood model was adopted because this model incorporates branch length information into the character transformation.

3. Results and Discussion

3.1. Genetic Distance and Phylogenetic Analyses. A total of 41 COI sequences including 21 new ones were obtained from 16 species of *Brachinotogluma* including two new species described. The COI sequences alignment comprises 606 nucleotide positions. Pairwise distances based on Kimura 2-parameter model were calculated within and among the 16 species of *Brachinotogluma* (Table S2). Genetic distances among the species of *Brachinotogluma* ranged from 0.084 to 0.282. Among these species, four pairs of species have relatively small interspecific distances, i.e., 0.084 between *B. elytropapillata* [8] and *B. ovata* [10], 0.084 between *B. ovata* and *B. sagamiensis* [6], 0.088 between *B. japonicus* [9] and *B. nanhaiensis* sp. nov., and 0.150 between *B. pettiboneae* [10] and *B. robusta* sp. nov. The affinities between *B. elytropapillata* and *B. ovata*, *B. ovata* and *B. sagamiensis*, *B. japonicus* and *B. nanhaiensis* sp. nov., and *B. pettiboneae* and *B. robusta* sp. nov. can also be supported by their closed resemblance in morphology ([10]; this study). In contrast, the intraspecific distances within the species of *B. japonicus*, *B. nanhaiensis* sp. nov., *B. pettiboneae*, and *B. robusta* sp. nov. were very low (0.0004–0.0038, Table S2), supporting the establishment of the two new species.

The topologies of the concatenated phylogenetic trees reconstructed from both ML and BI analyses were identical in most clades (Figures S1 and S2). The only difference is related to the polytomous branches which is resulted from the unresolved relationships between the four clades (*Branchipolynoe* and Clades1–4; Clade 5; *Peinaleopolynoe*; Clade 6). Thus, only the BI tree is presented with BI posterior probability and ML bootstrap scores indicated on each node (Figure 2). Phylogenetic trees support the establishment of the two new species and sister relationship between *B. nanhaiensis* sp. nov. and *B. japonicus* [9] and that between *B. robusta* sp. nov. and *B. pettiboneae* [10].

TABLE 1: Sampling information of the species and corresponding accession numbers used in this study. New sequences are set in boldface.

Species	Voucher	Gener	Vent and seep location	COI	16S	18S	28S	References
Bathykurila guaymasensis	—	—	Whale fall off San Diego & Santa Cruz Basin, California	DQ074766	MN428326	DQ074765	—	Glover et al. [20]; Hatch et al. [16]
Branchinotogluma bipapillata	RSIO35274	—	Southwest Indian Ocean Ridge	MH712481	MH717076	MH717080	—	Zhou et al. [4]
Branchinotogluma sandersi	SMNH118960	—	Juan de Fuca Ridge, eastern Pacific	JN852923	JN852889	JN852821	JN852851	Norlinder et al. [14]
Branchinotogluma elytropapillata	NSMT-pol P-668	Female	Sakai field, Okinawa Trough	MG799387	MG799377	MG799378	MG799380	Zhang et al. [8]
Branchinotogluma hessleri	SIO:BIC:A6316	—	Southern Gulf of California	KY684713	MH127414	MH124626	MH124616	Goffredi et al. [15]; Hatch et al. [16]
Branchinotogluma japonicus	—	—	Sakai vent field, Okinawa Trough	KY753824	KY753824	KY753841	KY753841	Zhang et al. [8]
	NSMT-Pol 113241	Male	Sakai field, Okinawa Trough, western Pacific	MG799390	—	—	—	Zhang et al. [8]
	NSMT-Pol 113242	Male	Sakai field, Okinawa Trough, western Pacific	MG799391	—	—	—	Zhang et al. [8]
	CBM-ZW 1114	Female	Aki site, Okinawa Trough, western Pacific	MG799392	—	—	—	Zhang et al. [8]
Branchinotogluma marianus	SIO:BIC:A13116	—	—	MW646931	MW654556	MW654525	—	Unpublished
Branchinotogluma nanhaiensis sp. nov.	MBM286800-sps4	—	Seep, South China Sea	**OL702996**	**OL699914**	**OL699911**	**OL699917**	This study
	MBM286796-sps3	Male	Seep, South China Sea	OL702999	—	—	—	This study
	MBM286797-sps3	Male	Seep, South China Sea	OL703002	—	—	—	This study
	MBM286797-sps4	Male	Seep, South China Sea	OL703000	—	—	—	This study
	MBM286797-sps5	Male	Seep, South China Sea	OL703001	—	—	—	This study
	MBM286800-sps1	Female	Seep, South China Sea	OL702997	—	—	—	This study
	MBM286800-sps5	Female	Seep, South China Sea	OL703003	—	—	—	This study
	MBM286800-sps6	Female	Seep, South China Sea	OL702998	—	—	—	This study
Branchinotogluma nikkoensis	—	—	Nikko seamount	ON255504	ON244617	ON244619	ON244615	Jimi et al. [6]
Branchinotogluma ovata	MBM286062-sps2	Male	Manus back-arc basin	MK357896	MK211416	MK211411	MK211413	Wu et al. [10]
	MBM286047	Female	Vent, manus back-arc basin, tropical western Pacific	**OL703004**	**OL699915**	**OL699912**	**OL699918**	This study
Branchinotogluma pettiboneae	MBM286050-sps1	Male	Vent, manus back-arc basin, tropical western Pacific	MK357901	—	—	—	Wu et al. [10]
	MBM286051-sps2	Female	Vent, manus back-arc basin, tropical western Pacific	MK357902	—	—	—	Wu et al. [10]

TABLE 1: Continued.

Species	Voucher	Gener	Vent and seep location	COI	16S	18S	28S	References
	MBM286052	Male	Vent, manus back-arc basin, tropical western Pacific	MK357903	—	—	—	Wu et al. [10]
	MBM286049-sps1	Female	Vent, manus back-arc basin, tropical western Pacific	MK357904	—	—	—	Wu et al. [10]
	MBM286805-sps2	Female	Seep, South China Sea	OL703005	OL699916	OL699913	OL699919	This study
	MBM286803-sps1	Female	Seep, South China Sea	OL703007	—	—	—	This study
	MBM286803-sps2	Female	Seep, South China Sea	OL703008	—	—	—	This study
	MBM286804-sps1	Female	Seep, South China Sea	OL703009	—	—	—	This study
	MBM286804-sps2	Female	Seep, South China Sea	OL703010	—	—	—	This study
Branchinotogluma robusta sp. nov.	MBM286805-a	Female	Seep, South China Sea	OL703012	—	—	—	This study
	MBM286805-b	Male	Seep, South China Sea	OL703013	—	—	—	This study
	MBM286805-c	Male	Seep, South China Sea	OL703014	—	—	—	This study
	MBM286805-d	Female	Seep, South China Sea	OL703015	—	—	—	This study
	MBM286805-sps3	Female	Seep, South China Sea	OL703011	—	—	—	This study
	MBM286805-sps5	Male	Seep, South China Sea	OL703006	—	—	—	This study
	MBM286806	?	Seep, South China Sea	OL703016	—	—	—	This study
Branchinotogluma sagamiensis	—	—	Off Hatsushima, Sagami Bay	ON255503	ON244616	ON244618	ON244614	Jimi et al. [6]
Branchinotogluma sandersi	SIO:BIC:A6321	—	Southern Gulf of California	KY684716	MH127416.1	MH124627.1	MH124617.1	Goffredi et al. [15]; Hatch et al. [16]
Branchinotogluma segonzaci		—	Fiji, Manus back-arc basin	MK357906	MK694858	MK211412	—	Wu et al. [10]
Branchinotogluma trifurcus	—	—	Manus back-arc basin	MK357905	MK211415	MW654529	—	Wu et al. [10]
Branchinotogluma tunnicliffeae	SIO:BIC:A7717	—	—	MW646935	MW654560	MW654530	MW654552	Unpublished
Branchiplicatus cupreus	SIO:BIC:A6160	—	Southern Gulf of California	KY684706	MH127418	MH124628	—	Goffredi et al. [15]; Hatch et al. [16]
Branchipolynoe eliseae	SIO–BIC A6548	—	Costa Rica	MH369878	MH396826	—	—	Lindgren et al. [21]
Branchipolynoe halliseyae	SIO–BIC A6532	—	Costa Rica	MH369858	MH396795	—	—	Lindgren et al. [21]
Branchipolynoe kajsae	SIO–BIC A2161	—	Costa Rica	MH369859	MH396800	—	—	Lindgren et al. [21]
Branchipolynoe longqiensis	—	—	Longqi field, Southwest Indian Ocean Ridge	KY753826	KY753826	KY753847	KY753847	Zhang et al. [17]

TABLE 1: Continued.

Species	Voucher	Gener	Vent and seep location	COI	16S	18S	28S	References
Branchipolynoe meridae	SIO–BIC A2131	—	Costa Rica	MH369884	MH396829	—	—	Lindgren et al. [21]
Branchipolynoe pettiboneae	—	—	South China Sea, western Pacific	KY753825	KY753825	KY753840	KY753840	Zhang et al. [17]
Branchipolynoe seepensis	SIO–BIC A6553	—	Gulf of Mexico, USA	MH369885	MH596848		—	Lindgren et al. [21]
Branchipolynoe symmytilida	—	—	East Pacific Rise	AY646021	AF315055			Halanych et al. [22]; Hurtado et al. [23]
Branchipolynoe tjiasmantoi	SIO–BIC A8511	—	Lau back-arc basin	MH369947	MH396830	—	—	Lindgren et al. [21]
Halosydnella australis	KW402	—	Brazil	KY823495	KY823480	KY823449	KY823463	Gonzalez et al. [24]
Harmothoe impar	SMNH118968	—	Sweden	JN852930	JN852897	JN852829	JN852859	Norlinder et al. [14]
Lepidonotopodium fimbriatum	SIO–BIC A6153	—	Gulf of California, Mexico	KY684717	MN428327	MN428333	MN428320	Goffredi et al. [15]; Hatch et al. [16]
Lepidonotopodium okinawae	—	—	Sakai vent field, Okinawa Trough	KY753828	KY753828	KY753842	KY753842	Zhang et al. [17]
Lepidonotopodium sp. n.	SIO–BIC A6317	—	Gulf of California, Mexico	KY684715	MN428328	MN428334	MN428321	Goffredi et al. [15]; Hatch et al. [16]
Lepidonotopodium williamsae	SIO–BIC A6318	—	Gulf of California, Mexico	KY684714	MN428329	MN428335	MN428322	Goffredi et al. [15]; Hatch et al. [16]
Lepidonotus clava	SMNH118974	—	England	JN852934	JN852902	JN852833	JN852864	Norlinder et al. [14]
Levensteiniella iris	—	—	East Scotia Ridge	KY753827	KY753827	KY753848	KY753848	Zhang et al. [17]
Levensteiniella manusensis	MBM286000-sps5	Juvenile	Vent, manus back-arc basin, tropical western Pacific	**OP114410**	**OP144063**	**OP144059**	**OP144061**	This study
Levensteiniella undomarginata	CBM-ZW 1118	Male	Sakai field, Okinawa Trough	MG799385	MG799376	MG799379	MG799381	Zhang et al. [8]
Peinaleopolynoe elvisi	SIO–BIC A8488	—	California, USA	MH115413	MH127422	MH124629	MH124618	Hatch et al. [16]
Peinaleopolynoe goffrediae	SIO–BIC A5485	—	California, USA	MN431782	MN428330	MN428336	MN428323	Hatch et al. [16]
Peinaleopolynoe mineoi	SIO–BIC A9919	—	Costa Rica	MN431773	MN428331	MN428337	MN428324	Hatch et al. [16]
Peinaleopolynoe orphanae	SIO–BIC A6151	—	Gulf of California, Mexico	KY684727	MH127423	MH124630	MH124619	Goffredi et al. [15]; Hatch et al. [16]

TABLE 1: Continued.

Species	Voucher	Gener	Vent and seep location	COI	16S	18S	28S	References
Peinaleopolynoe santacatalina	SIO–BIC A8487	—	California, USA	MH115411	MH127413	MH124621	MH124612	Hatch et al. [16]
Peinaleopolynoe sillardi	MNHN–IA–2010–399	—	South Atlantic Ocean	MH115414	MH127421	—	—	Hatch et al. [16]
Thermopolynoe branchiata	MBM286003	Male	Vent, Manus back-arc basin, tropical western Pacific	**OP114411**	**OP144064**	**OP144060**	**OP144062**	This study

TABLE 2: Eleven morphological characters and states used in this study.

No.	Characters	State 0	State 1	State 2	State 3
1	Shape of anterior lobes	Triangular	Prominently cylindrical	Not distinct	/
2	Shape of prostomium	Distinctly bilobed	Slightly bilobed	Not bilobed	/
3	Number of segments	20-21	22-30	>30	<20
4	Number of pairs of elytra	9-10	11	>11	<9
5	Size of elytra	Covering dorsum	Not covering dorsum	/	/
6	Presence of branchiae	Present	Absent	/	/
7	Shape of branchiae	Arborescent	Layered	Plicate	/
8	Presence of ventral papillae in males	Present	Absent	/	/
9	Start of ventral papillae in males	Segment 12	Segment 11	/	/
10	Presence of ventral lamellae in males	Present	Absent	/	/
11	Presence of ventral papillae in females	Present	Absent	/	/
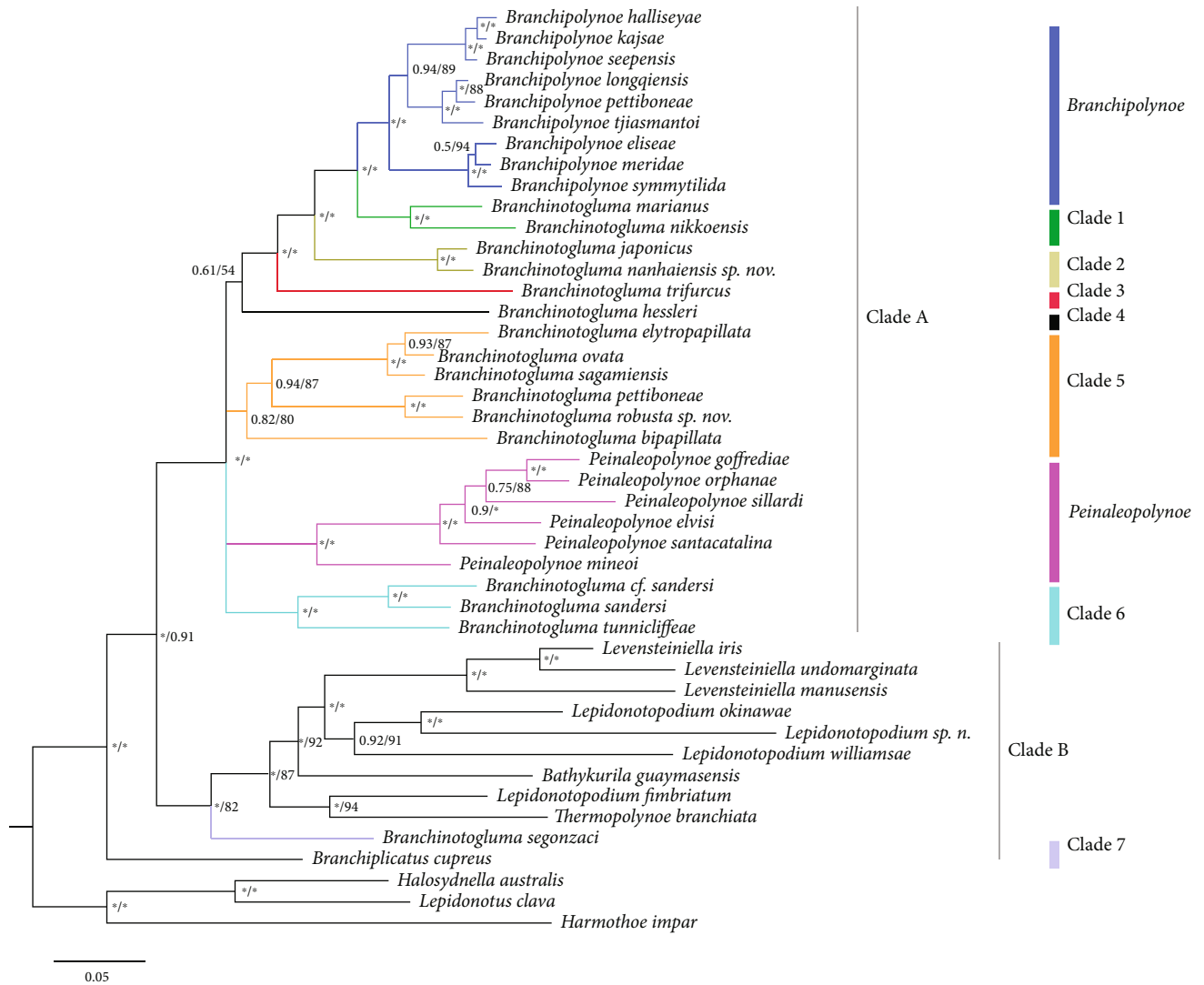
FIGURE 2: Bayesian inference (BI) tree reconstructed inferred from concatenated sequences (COI, 16S, 18S, and 28S) of *Branchinotogluma* and related genera. Numbers at the nodes represent BI posterior probability (left)/ML bootstrap scores (right), and * indicates 95% bootstrap or greater and 0.95 posterior probability or greater.

Branchinotogluma is nonmonophyletic, as indicated by both the ML and BI analyses. However, the monophyly of the two closely related genera, *Branchipolynoe* and *Peinaleopolynoe*, is well-supported, and both genera are intermingled with the clades consisting of *Branchinotogluma*. Furthermore, the phylogenetic analysis indicates that the species of *Branchinotogluma* can be divided into 7 clades.

Clade 1 (PP/BP = 1.00/100): *Branchinotogluma marianus* [5] and *B. nikkoenisis* [6] formed a well-supported clade and was recovered as the sister group to *Branchipolynoe* clade (PP/BP = 1.00/99).

Clade 2 (PP/BP = 1.00/100): *Branchinotogluma japonicus* [9] and *B. nanhaiensis* sp. nov. formed a well-supported clade and was recovered as the sister group to the clade composed of *Branchipolynoe* clade and Clade 1 (PP/BP = 1.00/99).

Clade 3: *Branchinotogluma trifurcus* [7] was recovered as a separated clade, which was the sister group to the above-mentioned clades with high supports (PP/BP = 1.00/98).

Clade 4: *Branchinotogluma hessleri* [11] formed a separated clade and was the sister group to the clade consisting of all the above-mentioned clades with low supports (PP/BP = 0.61/54).

Clade 5 (PP/BP = 0.82/80): The clade was composed of six species: *B. elytropapillata* [8], *B. ovata* [10], *B. sagamiensis* [6], *B. pettiboneae* [10], *B. robusta* sp. nov., and *B. bipapillata* [4]. The former three species formed a well-supported clade (PP/BP = 1.00/100), which was the sister group to the other well-supported clade (PP/BP = 1.00/100) composed of *B. pettiboneae* and *B. robusta* sp. nov. The species *B. bipapillata* was recovered as a basal clade.

Clade 6 (PP/BP = 1.00/100): *Branchinotogluma sandersi* sensu [14], *B. sandersi* [11], and *B. tunnicliffeae* [12] formed a clade with high-support values.

Clade 7: *Branchinotogluma segonzaci* formed a clade separated from all other species of *Branchinotogluma*, and it was the sister to the clade formed by species of *Levensteiniella, Lepidonotopodium, Bathykurila,* and *Thermopholynoe* (PP/BP = 1.00/82).

Our phylogenetic analyses also showed that the polynoids inhabiting deep-sea chemosynthetic environment can be divided into two main sister clades (Figures 2 and 3). Clade A consisted of clades 1–6, *Branchipolynoe* clade and *Peinaleopolynoe* clades. Clade B consisted of species of *Levensteiniella, Lepidonotopodium, Bathykurila, Thermopolynoe,* and *B. segonzaci.* The species *Branchiplicatus cupreus* [11] was recovered as a basal clade which is the sister to the large clade composed of Clade A and Clade B.

3.2. Character Evolution. BI molecular phylogeny was generated using the reduced concatenated dataset (Figure 3). Eleven morphological characters correlated with prostomium, segment, elytra, branchiae, and ventral papillae (Figure 4) for the 43 polynoid species were summarized (Table S3) and then mapped onto the simplified BI tree (Figures 5 and 6).

The ancestral state of shape of prostomium for the chemosynthesis-based polynoids was inferred to be distinctly bilobed prostomium with triangular anterior lobes

(Figures 4(i) and 4(l)). The slightly bilobed or nonbilobed prostomium as well as cylindrical anterior lobes (Figures 4(j) and 4(k)) are derived characters (Figures 5(a) and 5(b)).

The number of elytra and the number of segments were closely linked according to the analyses. The ancestral state for Clade A was suggested to be 20–21 segments and 9–10 pairs of elytra. In contrast, most species of Clade B have 22–30 segments with 11 pairs of elytra, which were derived from 20 to 21 segments with 9–10 elytra. However, the ancestor for the chemosynthesis-based polynoids seems to have more than 30 segments with more than 11 pairs of elytra (Figures 5(c) and 5(d)). Almost all the polynoids except *Branchipolynoe* have large elytra covering dorsum, which was considered as an ancestral state for the studied polynoids (Figure 3).

The arborescent branchiae (Figure 4(m)) was inferred to be the most likely ancestral state of the chemosynthesis-based polynoids (Figures 5(e) and 5(f)). Species of Clade B except *B. segonzaci* [7] and *Thermopolynoe branchiata* [19] lost the branchiae in the evolution. The plicate branchiae in *Branchiplicatus cupreus* [11] (Figure 4(o)) and layered branchiae in *T. branchiata* (Figure 4(n)) were likely derived from arborescent branchiae.

All the chemosynthesis-based polynoids except *Branchiplicatus cupreus* have ventral papillae in males, which was referred to be an ancestral state for the large clade composed of Clade A and Clade B (Figure 6(c)). The presence of ventral papillae in females only occurs in Clade A and was likely to be an ancestral state for Clade A (Figure 6(a)). In contrast, the absence of ventral papillae in females was inferred to be the ancestral state of Clade B (Figure 6(a)).

The ancestral state of the start of ventral papillae in males was inferred to be at segment 12 (Figures 4(a)–4(c), 4(g), and 4(h)) for both Clade A and Clade B. The start of ventral papillae from segment 11 (Figures 4(d)–4(f)) in males seems to be derived from the start from segment 12 (Figure 6(d)). The ventral lamellae (Figures 4(a), 4(b), 4(g), and 4(h)) occur in males of *Branchinotogluma* and some species of *Peinaleopolynoe*. The ancestral state was arguably inferred to be presence of ventral lamellae in males for Clade A but absence of ventral lamellae in males for Clade B (Figure 6(b)).

3.3. Systematics. Class *Polychaeta* Grube, 1850.
Order *Phyllodocida* Dales, 1962.
Family *Polynoidae* Kinberg, 1856.
Subfamily Lepidonotopodinae [36].

3.3.1. Genus Branchinotogluma [11]

(1) Type Species. *Branchinotogluma hessleri* [11], by original designation.

(2) Diagnosis. Body fusiform or ovate, 20 or 21 segments, 9 or 10 pairs of elytra. Elytra rounded to subreniform, usually smooth. Branchiae arborescent, two pairs per segment, from segment 3. Prostomium usually bilobed with anterior lobes triangular with minute or digitiform frontal filaments. Pharynx with 4–9 terminal papillae and two pairs of jaws with or without denticles. Notopodial bracts present on segment 2 or
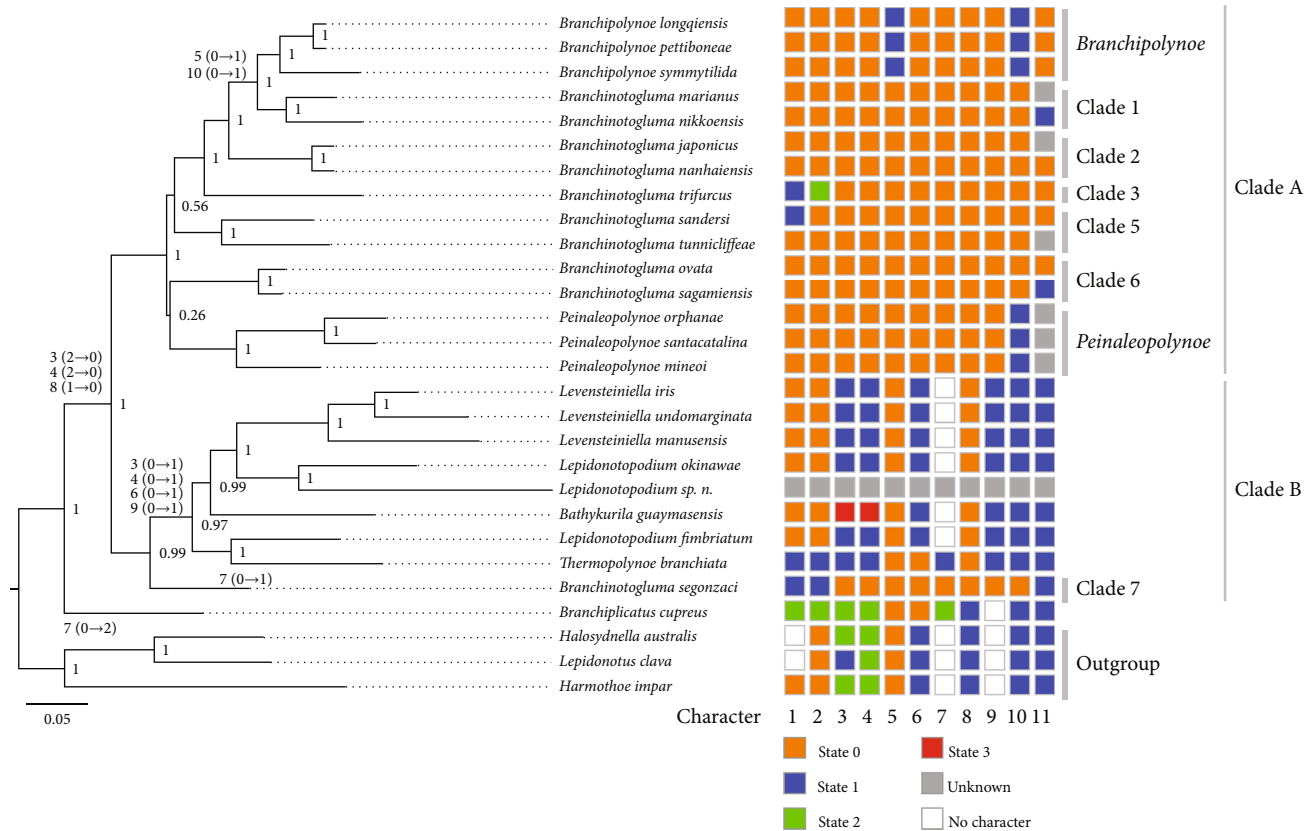
FIGURE 3: Simplified Bayesian phylogram mapped with eleven characters correlated with prostomium, segment, elytra, branchiae, and ventral papillae. Character and states follow morphological data matrix (see Table S3).

all elytrigerous segments, enclosing or fused with acicular lobes. Notochaetae usually shorter and stouter than neurochaetae, either smooth or with widely spaced spines along edge. Neurochaetae more numerous, forming a fan-shaped bundle; spinous, tips hooked or tapered. Males with paired ventral papillae from segment 12, followed by rounded lamellae. Females with 0–7 paired small, rounded ventral papillae from segment 11. Males with modified parapodia on posterior segments. Pygidium small, rectangular, usually with two anal cirri.

(3) Distribution. Deep-sea hydrothermal vents in the worldwide oceans, mainly in the Pacific Ocean. Deep-sea cold seeps in the western Pacific.

(4) Remarks. Polynoids inhabiting deep-sea chemosynthetic environments often exhibit sexual dimorphism, with distinct ventral papillae in males starting from segment 11 or 12. However, various patterns of modified posterior parapodia as well as the combination of ventral papillae and ventral lamellae (Figures 4(a), 4(b), 4(g), and 4(h)) are only found in *Branchinotogluma*. Previous authors [7, 11] did not realize the sexual dimorphism of the genus and misidentified males and females as two genera, i.e., *Branchinotogluma* and *Opisthotrochopodus*. *Opisthotrochopodus* established based on the morphology of males was synonymized with *Branchinotogluma* [37].

3.3.2. Branchinotogluma nanhaiensis sp. nov. (Figures 7–9). ZooBank LSID: http://zoobank.org.act.5B366731-583F-4522-AF62-BFCE4430138C.

(1) Material Examined. Holotype. MBM286795 (LQ16116), male, Dive 108, 119°17′08.278″E, 22°06′55.443″N, 1120.4 m, 30 August 2016.

   Paratypes. MBM286796 (LQ16116), 6 specimens (3♂3♀), same collection information as above; MBM286797 (LQ16028), 38 specimens (15♂23♀), Dive 81, 119°17′08.278″E, 22°06′55.443″N, 1120.4 m, 2 July 2016. MBM286798 (LQ16039), 6 specimens (not well-preserved), Dive 81, 119°17′08.263″E, 22°06′55.529″N, 1120.3 m, 2 July 2016. MBM286799 (LQ16069), 1 specimen, Dive 97, 119°17′08.122″E, 22°06′55.375″N, 1119.4 m, 20 July 2016. MBM286800 (L067), 8 specimens, Dive 51, 119°17′08″E, 22°06′55″N, 1118.6 m, 17 July 2015.

(2) Etymology. The specific name is derived from Nanhai, the Chinese name of the South China Sea, where the species was collected.

(3) Description. Holotype, male, well-preserved with 21 segments, length 10.8 mm, maximum width 9.1 mm (including chaetae) and 6.2 mm (excluding chaetae). Paratypes with 20
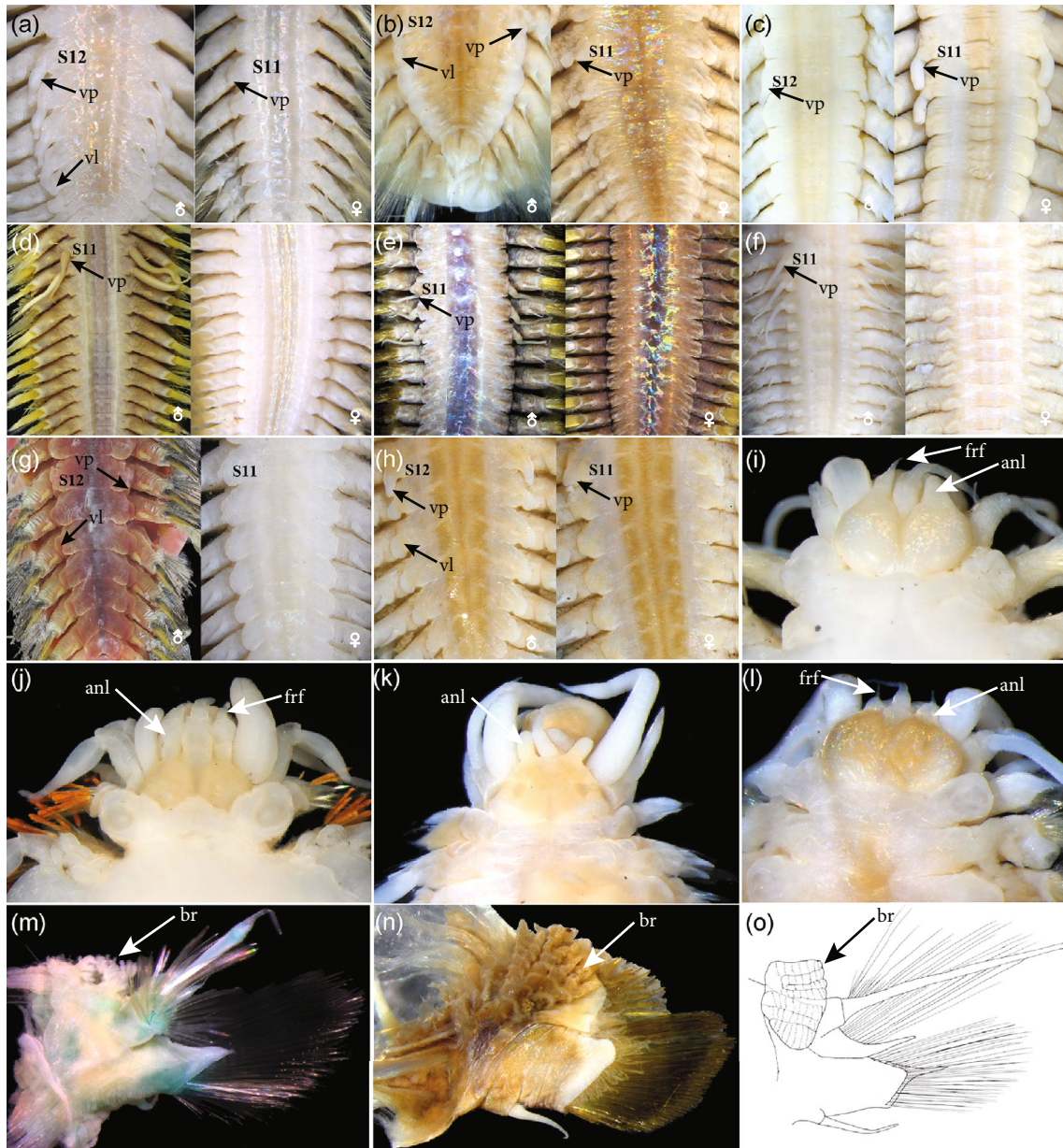
FIGURE 4: Illustrations of ventral papillae and lamellae (a–h), prostomium (i–l), and branchiae (m–o) in different genera. (a, i) *Branchinotogluma pettiboneae* [10]; (b, l, and m) *Branchinotogluma ovata* [10]; (c) *Branchipolynoe pettiboneae* [9]; (d) *Levensteiniella manusensis* [18]; (e, n) *Thermopolynoe branchiata* [19]; (f) *Lepidonotopodium okinawae* [35]; (g, j) *Branchinotogluma segonzaci* [7]; (h, k) *Branchinotogluma trifurcus* [7]; (o) *Branchiplicatus cupreus* [11]. (a, b, and g–m) modified from [10]; (o) modified from [11]. anl: anterior lobe; br: branchiae; frf: frontal filament; S11: segment 11; S12: segment 12; vl: ventral lamella; vp: ventral papilla.

or 21 segments, length 10.1–12.0 mm, width 6.8–9.2 mm (including chaetae) and 5.2–7.0 mm (excluding chaetae).

Body ovate, depressed (Figures 7(a)–7(h)). Ten pairs of elytra attached to prominent elytrophores on segments 2, 4, 5, 7, and alternate segments to 19 (Figures 7(a), 7(c), 7(e), and 7(g)), completely covering dorsum; elytra delicate, rounded to ovate, surface smooth without tubercles or papillae, with branched veins (Figures 8(h) and 8(i)). Dorsal cirri present on segments without elytra; cirrophores long and cylindrical, styles easily detached, slender with filamentous tips, extending beyond tips of chaetae (Figure 9(d)). Branchiae arborescent, small, with short fil-

aments (Figures 8(b) and 8(g)), attached to dorsal bases of notopodia; starting from segment 3 as two small groups (Figure 9(b)), slightly larger on middle segments (Figures 9(c) and 9(d)), progressively reduced in posterior end (Figure 9(e)).

Prostomium bilobed, wider than long; anterior lobes triangular with frontal filaments (Figure 8(a)). Median antenna with ceratophore inserted in anterior notch, style filamentous (Figure 8(a)). Palps smooth, stout, tapered with fine tips. Lateral antennae and eyes absent. Tentacular segment fused to prostomium, achaetous, only visible ventrally; two pairs of tentacular cirri inserted laterally to prostomium, tentaculophores cylindrical, styles slender with long terminal
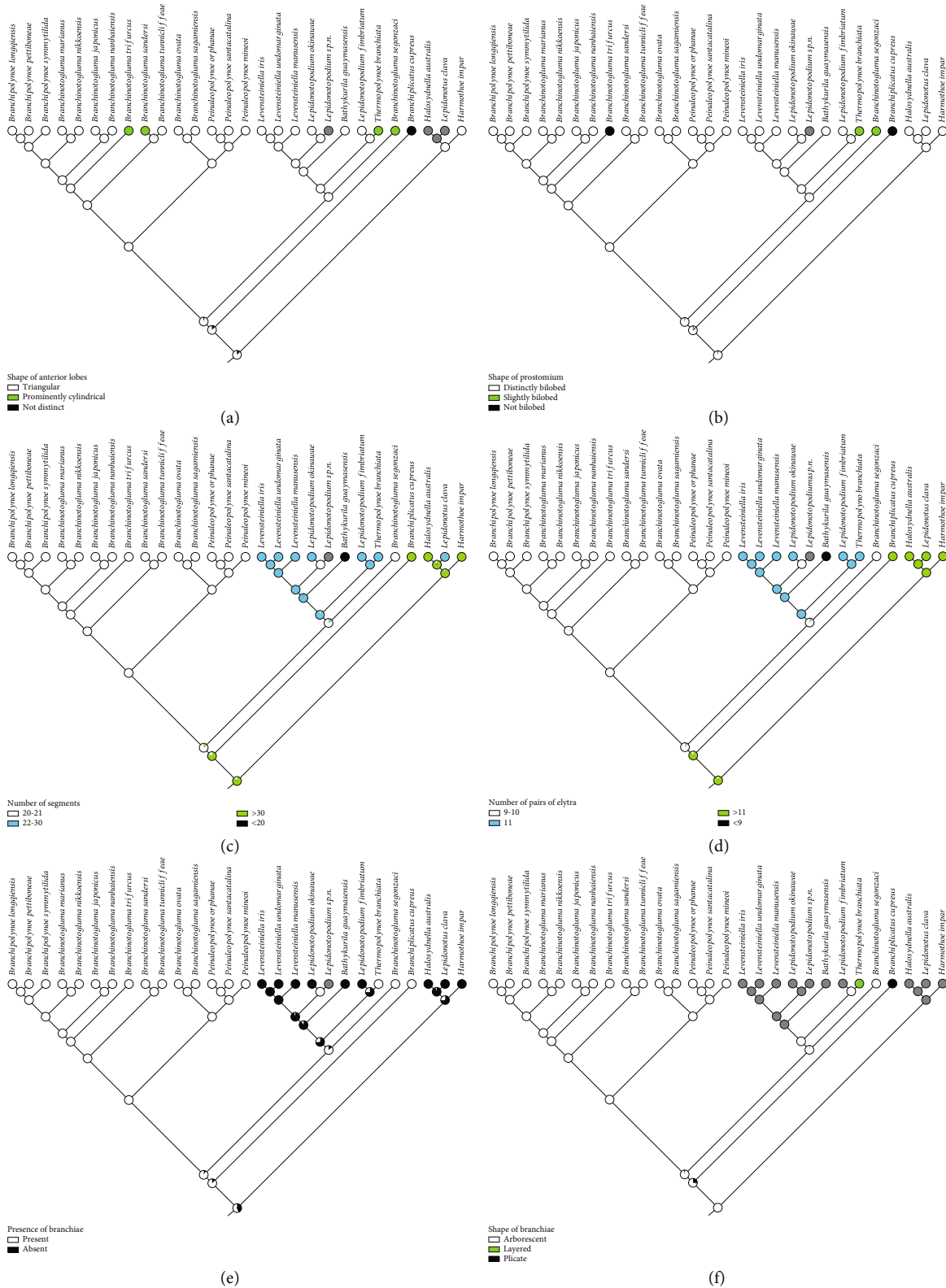
FIGURE 5: Bayesian inference (BI) tree topology from concatenated data, with transformation for (a) shape of anterior lobes, (b) shape of prostomium, (c) number of segments, (d) number of pairs of elytra, (e) presence of branchiae, and (f) shape of branchiae. Pie charts at the nodes represent probabilities for related states.
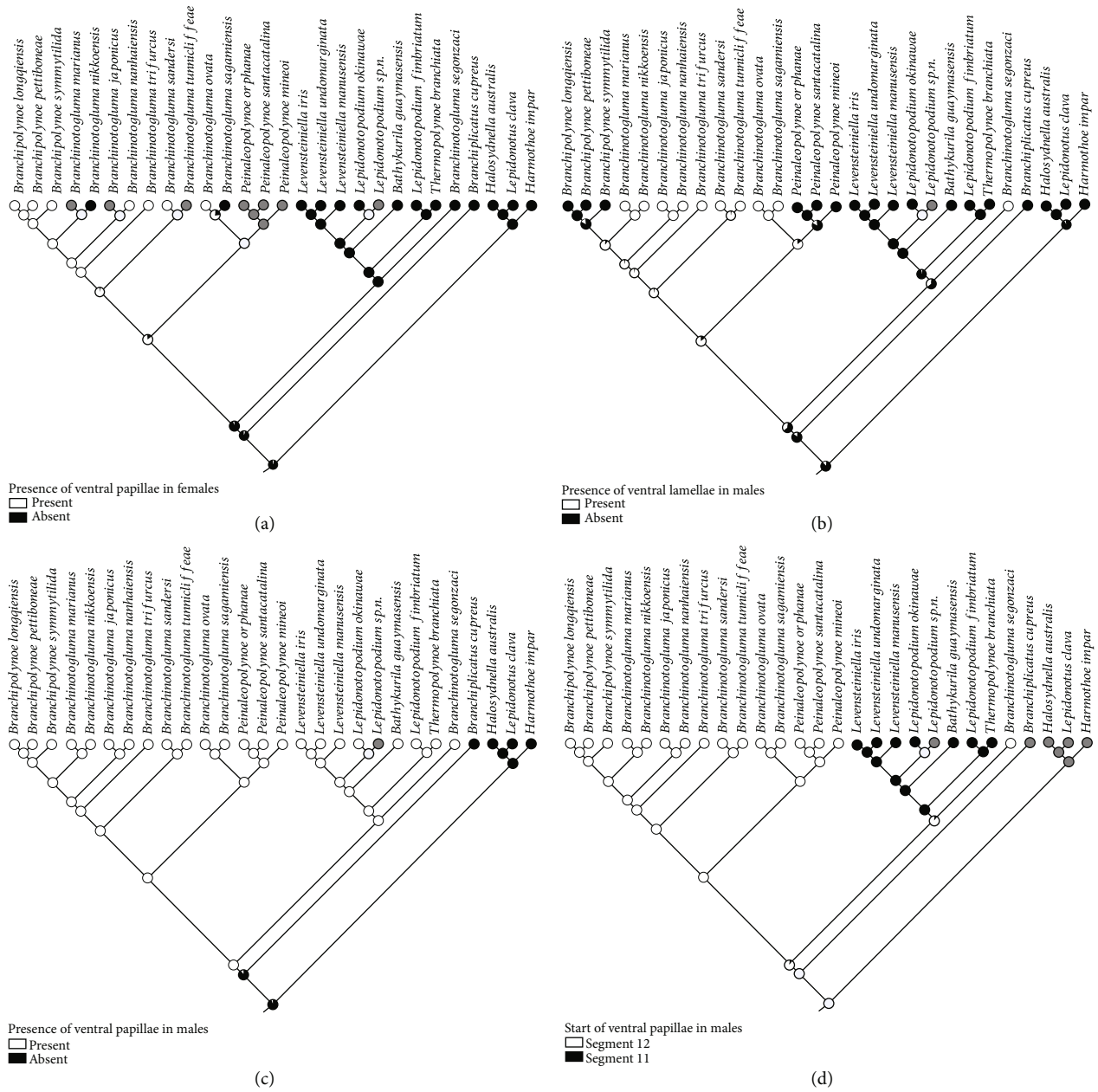
Figure 6: Bayesian inference (BI) tree topology from concatenated data, with transformation for (a) presence of ventral papillae in females; (b) presence of ventral lamellae in males; (c) presence of ventral papillae in males; (d) start of ventral papillae in males. Pie charts at the nodes represent probabilities for related states.

filaments (Figure 8(a)). Pharynx soft, slender, with two pairs of small terminal jaws, without teeth (Figure 8(e)), two clusters of small papillae in lateral sides (each with 4–5 papillae), and two processes at lateral bases (Figure 8(f)).

All parapodia biramous. Second segment bearing first pair of elytrophores; notopodia as long as neuropodia, and buccal cirri similar to ventral tentacular cirri, much thicker and longer than subsequent ventral cirri (Figures 9(a)–9(f)); notopodium conical with bract fused with acicular lobe, posteriorly forming a pocket surrounding a small bundle of notochaetae (Figure 9(a)); neuropo-

dium with a long, conical prechaetal lobe, and a short, sub-triangular postchaetal lobe (Figure 9(a)).

Following segments with notopodia shorter than neuropodia (Figures 9(b)–9(d)). Notopodium with a projecting acicular lobe on lower side and numerous notochaetae forming a radiating bundle (Figures 9(b)–9(d), 9(g), and 9(h)). Notochaetae shorter and stouter than neurochaetae, straight with rows of transverse striation (Figures 9(i) and 9(j)). Neuropodium including a long, conical prechaetal lobe with a projecting acicular lobe, and a short, subtriangular postchaetal lobe with a small projecting lobe on dorsal base
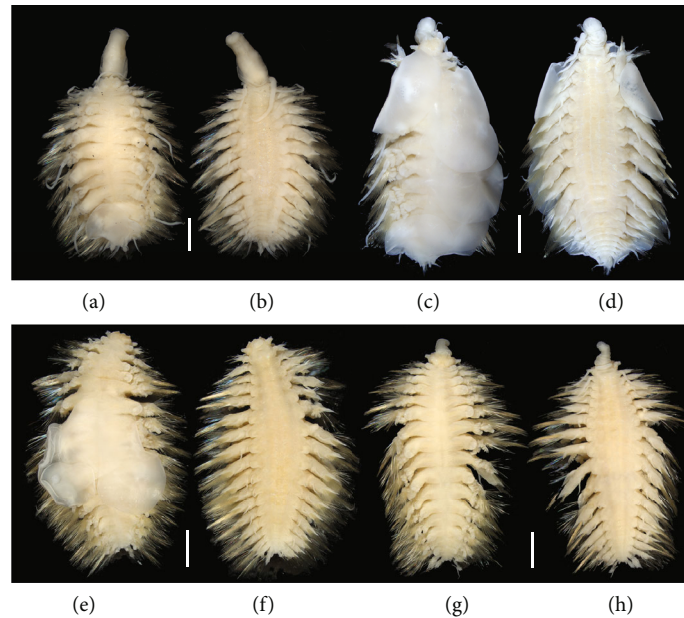
FIGURE 7: *Branchinotogluma nanhaiensis* sp. nov., holotype ((a, b) MBM286795, male) and paratypes ((c, d) MBM286797, male; (e, f) MBM286797, female; (g, h) MBM286797, another female). (a–h) Four specimens in dorsal (a, c, e, and g) and ventral view (b, d, f, and h). Scale bars: 2 mm (a–h).

(Figures 9(b)–9(d)). Neurochaetae numerous, spinous, forming a fan-shaped bundle (Figure 9(k)). Neurochaetae slightly curved, proximal region spinous with rows of transverse teeth, distal region spinous, knife-like with two rows of spines bordering a longitudinal spoon-like furrow (Figures 9(l)–9(n)); spines short, close-set distally, becoming longer and widely spaced proximally (Figure 9(n)). Ventral cirri short, tapered, attached on ventral bases of neuropodia (Figures 9(b)–9(f)). Pygidium rectangular with two slender anal cirri, inserted in posterior end (Figure 8(c)).

Males with parapodia of last two segments slightly modified, without branchiae (Figures 9(g)–9(h)). On segment 20, notopodium reduced without notochaetae, acicular lobes fused with dorsal cirrophores; neuropodium expanded basally, distally with a lamellar expansion (Figure 9(e)). Neurochaetae more delicate than in unmodified parapodia, distally spinous with two rows of spines; spines becoming indistinct in lowermost neurochaetae (Figures 9(o) and 9(p)). On segment 21, notopodium reduced, with a stout acicula, neuropodium cylindrical with small distal lobes. Notopodial aciculae smooth on surface, tapering with curved tip (Figure 9(r)); neurochaetae more delicate than on segment 20, capillary with indistinct spines (Figure 9(q)).

Males with 21 segments and a pair of prominent ventral papillae on ventral side of segment 12 (Figure 8(d)), followed by five pairs of thin, semiovate ventral lamellae along segments 13–17. Females with 20 segments and two pairs of concave ventral papillae on segments 11 and 12 (Figure 8(c)).

(4) Type Locality. Deep-sea cold seeps of the South China Sea (1118.6–1120.4 m depth).

(5) Remarks. Within *Branchinotogluma*, all known species, except *B. elytropapillata* [8, 17] and *B. ovata* [10] which possess 20 segments, have 21 segments in both males and females, and none has concave ventral papillae in females. *Branchinotogluma nanhaiensis* sp. nov. is distinguished from the congeners by the different numbers of segments in males and females (21 segments in males and 20 in females) and two pairs of concave ventral papillae in females.

Branchinotogluma nanhaiensis sp. nov. resembles *B. elytropapillata*, *B. hessleri* [11], *B. japonicus* [9], *B. nikkoensis* [6], *B. ovata*, *B. segonzaci* [7], and *B. trifurcus* [7] in having a pair of ventral papillae and five pairs of ventral lamellae in males. Among these species, *B. nanhainensis* sp. nov. is similar to *B. hessleri*, *B. japonicus,* and *B. nikkoensis* in having prominent and thick ventral papillae, which are short and tapered in the other species. *Branchinotogluma nanhainensis* sp. nov. also resembles *B. japonicus* in having similar body size and shape, slender pharynx with two bundles of lateral tapered papillae and soft jaws without teeth. *B. nanhainensis* sp. nov. differs from *B. japonicus* in having 20 segments in females (vs. 21 segments) and presence of concave ventral papillae (vs. absent).

3.3.3. Branchinotogluma robusta sp. nov. (Figures 10 and 11). ZooBank LSID: http://zoobank.org.act.8DF340AA-100F-47D5-982F-404CBADCE38B.

(1) Material Examined. Holotype. MBM286801 (LQ16028), male, Dive 81, 119°17′08.278″E, 22°06′55.443″N, 1120.4 m, 2 July 2016.

Paratypes. MBM286802 (LQ16028), 2 specimens (1♂1♀), same collection information as above; MBM286803 (LQ16116), 2 specimens, Dive 108, 119°17′08.278″E, 22°06′55.443″N, 1120.4 m, 30 August 2016. MBM286804 (LQ16069), 2
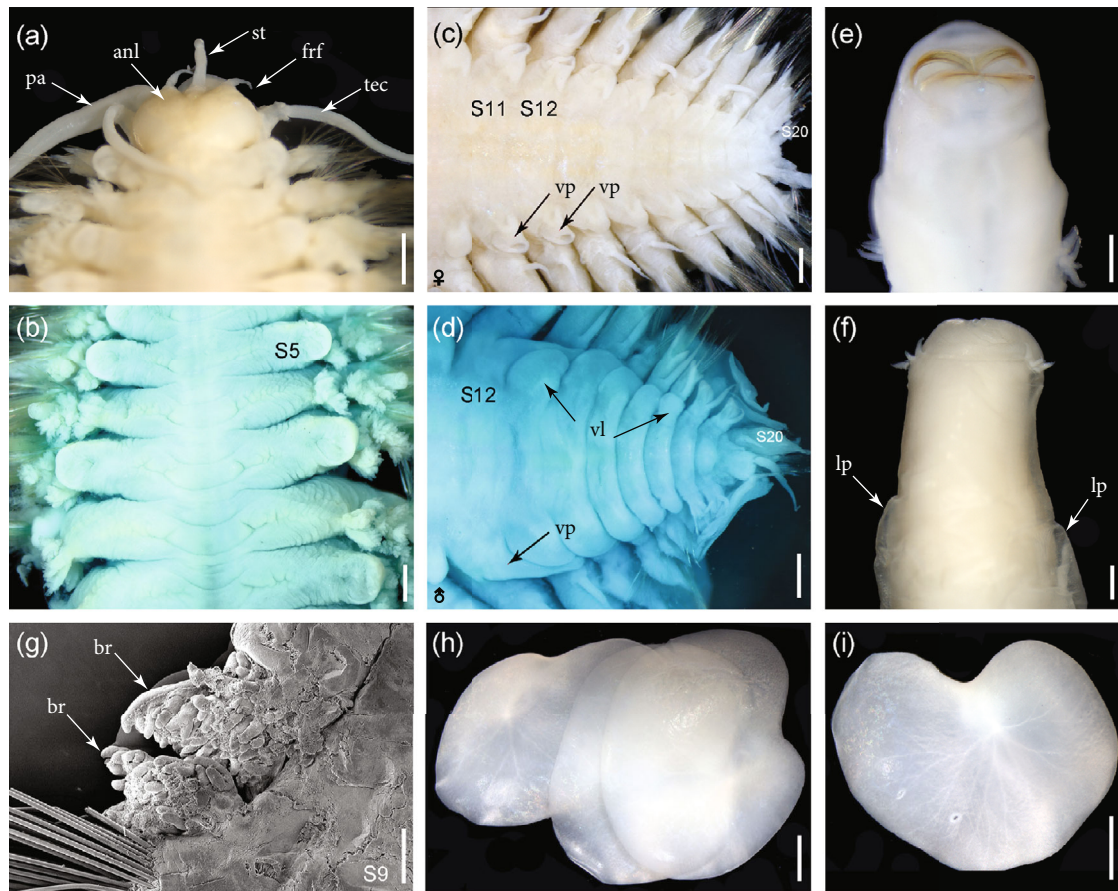
FIGURE 8: *Branchinotogluma nanhaiensis* sp. nov., paratypes ((a–e, h, and i) MBM286797; (g) MBM286800) and holotype ((f) MBM286795). (a) Head and anterior segments in dorsal view; (b) segments 4–9 in dorsal view; (c) female posterior segments in ventral view; (d) male posterior segments in ventral view; (e) fully extended pharynx in ventral view; (f) incompletely extended pharynx in dorsal view; (g) branchiae from right parapodium on segment 9; (h, i) elytra of anterior and median segments. anl: anterior lobe; br: branchiae; frf: frontal filament; lp: lateral process; pa: palp; S5: segment 5; st: style of median antenna; tec: tentacular cirri; vl: ventral lamellae; vp: ventral papilla. Scale bars: 0.5 mm (a–d, and f), 200 μm (e, g), and 1 mm (h, i).

specimens, Dive 97, 119°17′08.122″E, 22°06′55.375″N, 1119.4 m, 20 July 2016. MBM286805 (L067), 5 specimen, Dive 51, 119°17′08″E, 22°06′55″N, 1118.6 m, 17 July 2015. MBM286806 (LQ16064), 1 specimen, Dive 96, 119°17′08″E, 22°06′55″N, 1121.8 m, 20 July 2016.

(2) Etymology. The specific name is derived from the Latin adjective *robustus* (robust), referring to its robust pharynx and multidentate jaws.

(3) Description. Type specimens with 21 segments, length of 12.8–19.1 mm, and width of 7.2–9.3 mm (including chaetae) and 5.7–7.7 mm (excluding chaetae).

Body fusiform and depressed (Figures 10(a)–10(h)). Ten pairs of elytra, attached to prominent elytrophores on segments 2, 4, 5, 7, and alternate to 19, completely covering dorsum; elytra rounded to ovate, smooth (Figure 10(n)). Dorsal cirri present on segments without elytra, cirrophores long, cylindrical, styles slender with long filamentous tips,

and easily detached (Figures 11(b) and 11(d)–11(f)). Branchiae arborescent, small, filaments papillate, and attached to dorsal bases of notopodia; starting from segment 3 as two small groups (Figure 11(b)), slightly larger on middle segments (Figures 11(c) and 11(d)), and reduced on posterior segments (Figures 11(e)–11(g)).

Prostomium bilobed, wider than long; anterior lobes triangular with frontal filaments (Figure 10(i)). Median antenna with cylindrical ceratophore inserted in anterior notch, style subulate with a filamentous tip (Figure 10(i)). Palps stout, smooth, and tapered with fine tips. Lateral antennae and eyes absent. Tentacular segment fused to prostomium, achaetous, and only visible ventrally; two pairs of tentacular cirri inserted laterally to prostomium, tentaculophores cylindrical, styles long, and slender (Figures 10(b), 10(d), 10(f), and 10(h)). Pharynx robust, with a pair of processes at lateral bases, with nine distal conical papillae (five dorsal and four ventral), and two pairs of jaws, each with ca. 30 teeth (Figure 10(l)).

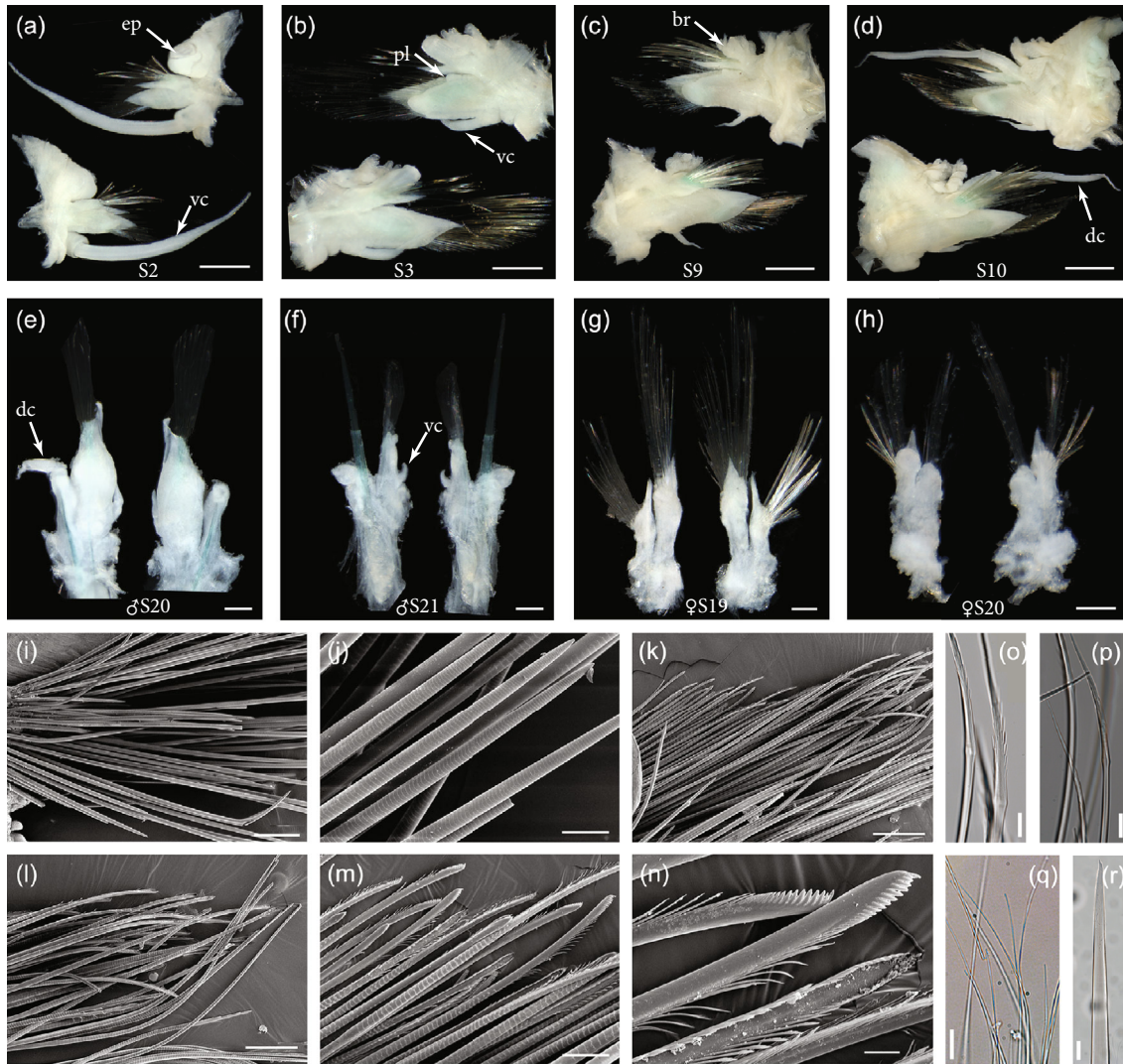All parapodia biramous. Second segment bearing first pair of elytrophores, notopodia much shorter than

FIGURE 9: Parapodia and chaetae of *Branchinotogluma nanhaiensis* sp. nov., paratypes (a–f, and o–r) MBM286797, male; (g, h) MBM286797, female; (i–n) MBM286800, female, right parapodium on segment 9. (a, g, and h) Three right parapodia in anterior (upper/right positions) and posterior (lower/left positions) view, and dorsal and ventral cirrus are detached in (g, h); (b–f) five left parapodia in anterior (lower/left positions) and posterior (upper/right positions) view, dorsal cirrus are detached in (b, f); (i) notochaetae; (j) upper notochaetae, showing details of distal regions; (k) neurochaetae; (l) supra-acicular neurochaetae in upper position; (m) subacicular neurochaetae in lower position; (n) the same, showing details of distal spinous regions; (o) neurochaetae in median position from (e); (p) neurochaetae in lower position from (e); (q) neurochaetae from (f); (r) notopodial acicula from (f). br: branchiae; dc: dorsal cirrus; ep: elytrophore; pl: projecting lobe; S2: segment 2; vc: ventral cirrus. Scale bars: 0.5 mm (a, b), 1 mm (c, d), 200 μm (e–i, k, and l), 50 μm (j, m, r), 20 μm (o, p), and 10 μm (n, q).

neuropodia, and buccal cirri similar to ventral tentacular cirri, much thicker and longer than subsequent ventral cirri (Figure 11(a)); notopodium conical with bract fused with acicular lobe, posteriorly forming a pocket surrounding a small bundle of notochaetae; neuropodium with a long conical prechaetal lobe and a short subtriangular postchaetal lobe (Figure 11(a)).

Following segments with notopodia shorter than neuropodia on anterior segments (Figures 11(b)–11(d)), becoming as long as neuropodia on posterior segments (Figures 11(e) and 11(f)). Notopodium with a projecting acicular lobe on lower side and numerous notochaetae forming a radiating

bundle. Notochaetae much stouter than neurochaetae, straight or slightly curved with blunt bare tips, smooth in upper and middle fascicle, and serrated with two rows of widely spaced spines in lower positions (Figures 11(h)–11(j)). Neuropodium including a long, conical prechaetal lobe with a projecting acicular lobe, and a short subtriangular postchaetal lobe with a small projecting lobe on dorsal base (Figures 11(b)–11(d)). Neurochaetae numerous forming a fan-shaped bundle. Neurochaetae straight, tips hooked; spinous region extending to tips, with two rows of spines along a longitudinal furrow; spines short and close-set on distal part, becoming longer and widely spaced proximally
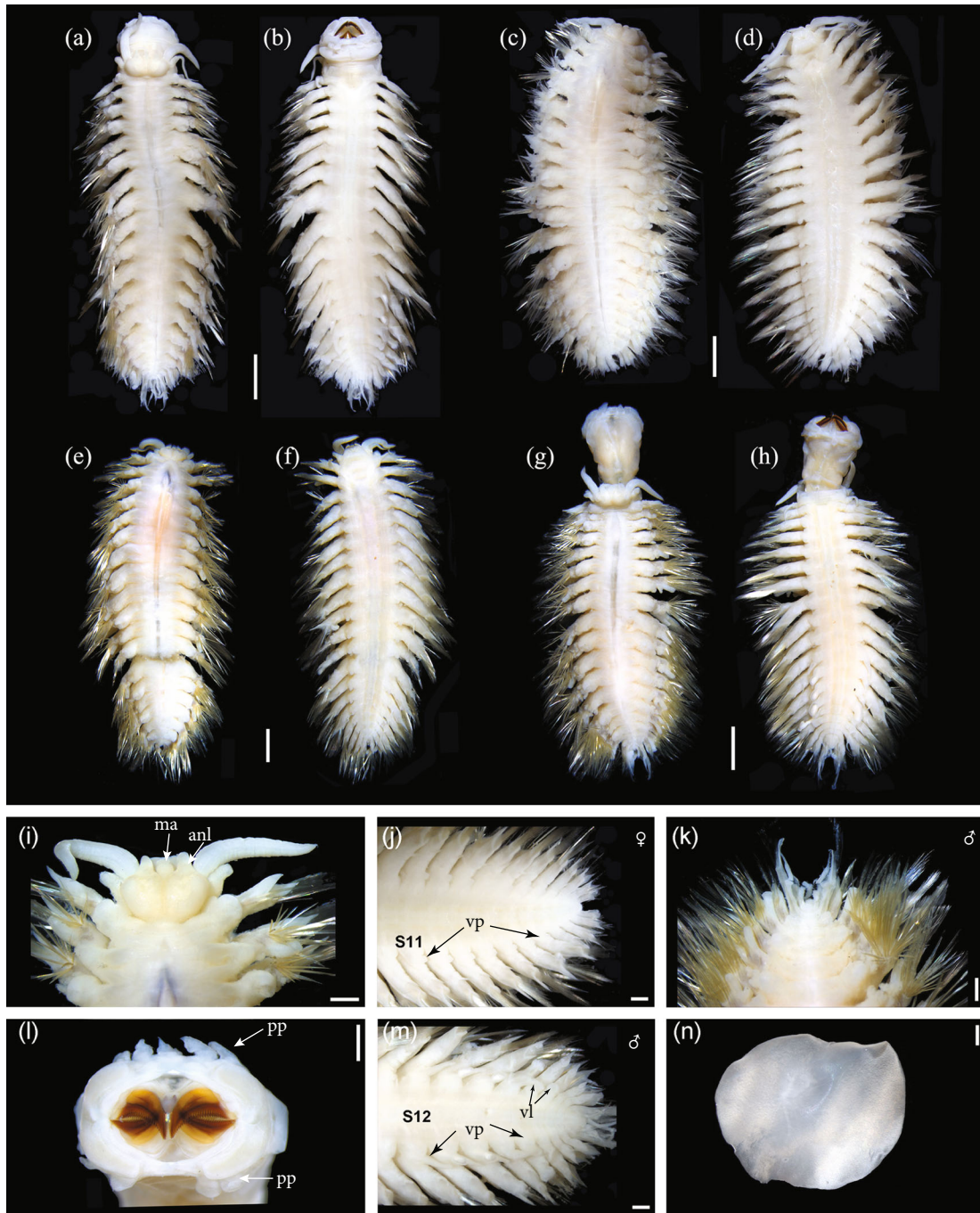
FIGURE 10: *Branchinotogluma robusta* sp. nov., holotype ((a, b, and m) MBM286801, male) and paratypes ((c, d, and j) MBM286803, female; (e, f, i, and n) MBM286805, female; (g, h, k, and l) MBM286805, male). (a–h) Four specimens in dorsal and ventral view; (i) head and anterior segments in dorsal view; (j) female posterior segments in ventral view, and anal cirri are detached; (k) male posterior segments in dorsal view; (l) extended pharynx in anterior view; (m) male posterior segments in ventral view; (n) elytra of median segments. anl: anterior lobe; ma: median antenna; pp: pharynx papilla; S11: segment 11; vl: ventral lamellae; vp: ventral papillae. Scale bars: 2 mm (a–h) and 0.5 mm (i–n).

(Figures 11(k)–11(m)). Ventral cirri short, tapered, and attached on ventral bases of neuropodia (Figures 11(b)–11(d) and 11(f)). Pygidium small, with two terminal anal cirri (Figures 10(j), 10(k), and 10(m)).

Males with parapodia of last two segments slightly modified, without branchiae (Figures 10(k), 11(e), and 11(g)). On segments 20 and 21, notopodia reduced without notochaetae, fused with cirrophores of dorsal cirri, and embedded with notoaciculae (Figures 11(e) and 11(g)); neuropodia elongate, with prechaetal lobes digitiform and postchaetal lobes conical, shorter than prechaetal lobe (Figures 11(e) and 11(g)). Neurochaetae more delicate than on unmodified
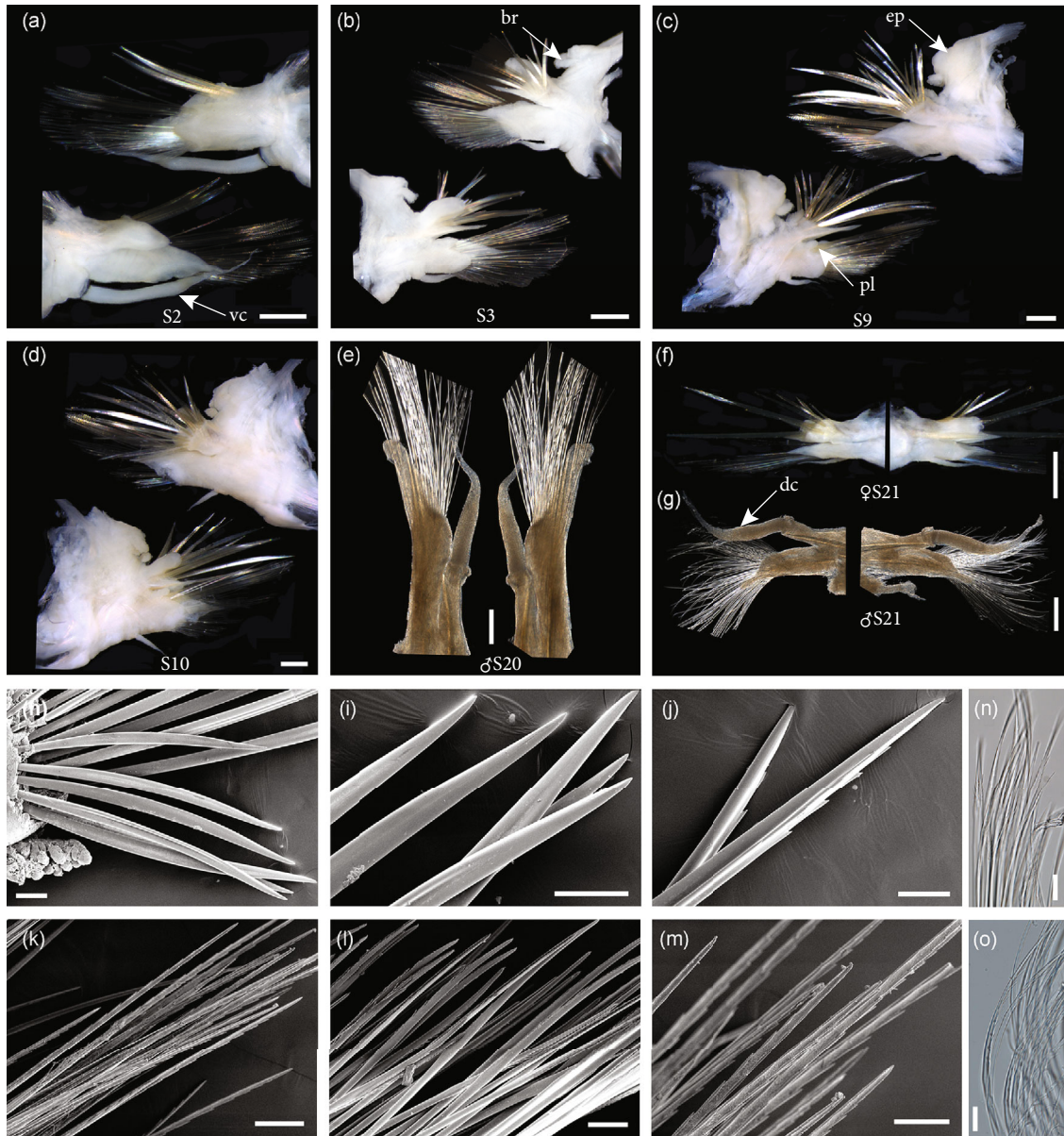
FIGURE 11: Parapodia and chaetae of *Branchinotogluma robusta* sp. nov., paratypes ((a, c, e, and f) MBM286805, female; (b, e, g, n, and o) MBM286805, male; (h–m) MBM286805, another female, right parapodium on segment 9). (a, e, and g) Three left parapodium in anterior (lower/right position) and posterior (upper/left position) view; (b–d, and f) four right parapodia in anterior (upper/left positions) and posterior (lower/right positions) view, dorsal cirrus detached in (b, d, and f), ventral cirrus are detached in (e); (h) upper notochaetae; (i) notochaetae in median position; (j) lower notochaetae; (k, m) supra-acicular neurochaetae; (l) subacicular neurochaetae; (n) neurochaetae in median position from (e); (o) neurochaetae from (g). br: branchiae; dc: dorsal cirrus; ep: elytrophore; pl: projecting lobe; S2: segment 2; vc: ventral cirrus. Scale bars: 0.5 mm (a–d, f), 200 μm (e, g), 100 μm (h–k), 50 μm (l–n) and 20 μm (o).

parapodia, spinous with two rows of spines, and distally with bare slender tips; spines long and widely spaced (Figures 11(n) and 11(o)).

Males with four pairs of prominent ventral papillae on ventral side of segments 12–15 (Figure 10(n)), followed by two pairs of short, rounded lamellae on segments 16 and 17; ventral papillae basally swollen with slender distal part; females with seven pairs of short ventral papillae on segments 11–17 (Figure 10(j)).

(4) Type Locality. Deep-sea cold seeps of the South China Sea (1118.6–1121.8 m depth).

(5) Remarks. Branchinotogluma robusta sp. nov. highly resembles *B. pettiboneae* [10] in most main features, such as body size and shape, pharynx, ventral papillae, and parapodium. However, *B. robusta* sp. nov. can be distinguished from *B. pettiboneae* in having neurochaetae with hooked tips (vs. straight spinous tips on the neurochaetae).

Branchinotogluma robusta sp. nov. also resembles *B. sandersi* [11] and *B. tunnicliffeae* [12] in having four pairs of long ventral papillae in males but differs from *B. sandersi* in having two (vs. three) pairs of ventral lamellae behind the long ventral papillae, and from *B. tunnicliffeae* in having a different pattern of pharynx papillae (5 + 4 vs. 3 + 2).

4. Discussion

4.1. Species Delimitation of Branchinotogluma. Up to date, most of the previously described species of *Branchinotogluma* have been described from deep-sea hydrothermal vents, and only one species (*B. sagamiensis* [6]) was recorded from cold seeps. Our examination of the polynoid specimens collected from the deep-sea cold seeps in the South China Sea revealed two undescribed species. We provided detailed descriptions and new sequences of four loci (COI, 16S, 18S, and 28S) for the two new species and other related taxa. Based on the newly generated data along with data downloaded from GenBank, we made a barcoding analysis for 16 species of *Brachinotogluma*. The interspecific genetic distances are much greater than the intraspecific distances, supporting the species delimitation which is based on morphological examination. In addition, phylogenetic analyses also support the establishment of the two new species. Both morphology and phylogenetic analysis supported the close relationship between *B. nanhaiensis* sp. nov. and *B. japonicus* [9] and that between *B. robusta* sp. nov. and *B. pettiboneae* [10]. *Branchinotogluma japonicus* and *B. pettiboneae* were found, respectively, from the hydrothermal vents of Okinawa Trough and Manus Basin. It is noteworthy that *Lepidonotopodium okinawae* [35] and *Branchipolynoe pettiboneae* [9], originally described from the vents of Okinawa Trough, also occur accompanying with the two new species (authors' examination), indicating the evolutionary links between hydrothermal vent and methane seep faunas.

4.2. Systematics and Phylogeny of Branchinotogluma. The genus *Branchinotogluma* is expanded to contain 16 valid species after the description of two new species. It was recovered as paraphyletic with seven clades scattering in two main clades. Six of them formed a well-supported clade together with *Branchipolynoe* and *Peinaleopolynoe*. *Branchinotogluma segonzaci* [7] was recovered as the seventh clade, located in the other main clade composed by *Bathykurila*, *Lepidonotopodium*, *Levensteiniella*, and *Thermopolynoe*. However, we suggest preserving the stability of all these genera rather than synonymizing them as a single genus due to their distinct morphological difference and the lack of morphological and molecular data.

Branchinotogluma trifurcus [7] was recovered as the sister group with high supports to the clade composed of *Branchipolynoe*, Clade 1, and Clade 2. The species is characterized in *Branchinotogluma* by having reduced parapodia and prechaetal and postchaetal lobes similar in shape [10], which are also characters of *Branchipolynoe*. The reduction of the parapodium in *Branchipolynoe* was considered as an ancestral character descended from *B. trifurcus*. In addition, *B. trifurcus* is characterized by a trapezoid prostomium and cylindrical anterior lobes without frontal filaments (Figure 4). However, these characters seem to be derived from the bilobed anterior lobes with frontal filaments according to our analyses.

Phylogenetic analyses showed the unusual placement of *B. segonzaci*, which was separated from the other species of *Branchinotogluma* and recovered as the sister group to the clade formed by species of *Levensteiniella*, *Lepidonotopodium*, *Thermopholynoe* and *Bathykurila*. The separation of *B. segonzaci* from the other congeners is in consistent with the morphological differences between them. *Branchinotogluma segonzaci* is characterized by the prominently cylindrical anterior lobes with digitiform frontal filaments, which highly resemble the median antenna (Figure 4(j)), while the other species have delicate or no frontal filaments (Figures 4(i) and 4(l)). Among the species of *Branchinotogluma*, three species, viz. *B. sandersi* [11], *B. burkensis* [5], and *B. trifurcus*, have cylindrical anterior lobes. However, the frontal filaments are slender in *B. sandersi* and *B. burkensis* and absent in *B. trifurcus* (Figure 4(k)).

In addition, *B. segonzaci* is characterized for the lack of ventral papillae in females (Figure 4(g)), while almost all species of *Branchinotogluma* have various numbers of ventral papillae in females (Figures 4(a), 4(b), 4(g), and 4(h)). The females of *B. marianus* [5] and *B. tunnicliffeae* [12] have not been described so their ventral papillae in females are unknown (Figures 3 and 5). The absence of ventral papillae in females of *B. japonicus* [9] described by Zhang et al. [8, 17] is uncertain because only a single female specimen with poor preservation was examined (Figures 3 and 5). It is interesting that the ventral papillae in females only occur in *Branchipolynoe* and *Branchinotogluma*. In contrast, all species in Clade B including *B. segonzaci* have no ventral papillae in females, which is a reason to explain why *B. segonzaci* is phylogenetically placed in the clade composed of *Bathykurila*, *Lepidonotopodium*, *Levensteiniella*, and *Thermopolynoe* (Figure 2). However, *B. segonzaci* differs distinctly from these related genera in the numbers of segments and elytra, presence of modified segments and ventral lamellae, and ventral papillae in males starting at segment 12.

4.3. Character Evolution. The polynoids inhabiting the deep-sea hydrothermal vents and cold seeps exhibit diverse morphological characters. Most of them have bilobed prostomium with triangular anterior cirri. Only the species *Branchinotogluma trifurcus* [7] and *Branchiplicatus cupreus* [11] possess fused prostomium, which seems to be a derived feature. The cylindrical anterior cirri are also inferred to be derived from triangular ones. The minute frontal filaments on the anterior cirri were considered as lateral antennae by Hatch et al. [16]. As the lateral antennae have been used as a very important character in classifying different genera and subfamilies among the *Polynoidae*, we would suggest keeping the original term unless we have anatomical evidence to support the proposal.

The numbers of segments and elytra are also key characters for polynoids. Species of Clade A have stable numbers of segments (20–21, mostly 21) and elytra (9–10, mostly 10 pairs), while most species of Clade B have more segments

(22–30) with a stable number of elytra (11 pairs). The exceptions are *Bathykurila guaymasensis* [5] and *Branchinotogluma segonzaci* [7], which have 15 segments with 7 pairs of elytra and 21 segments with 10 pairs of elytra, respectively. The characters of 20–21 segments and 9–10 pairs of elytra are considered as ancestral characters for the big clade composed of Clade A and Clade B. Almost all species have large elytra covering the whole dorsum, except the species of *Branchipolynoe*. The reduction of elytra in *Branchipolynoe* is a derived character, which is speculated to be an adaption to the parasitic lifestyle.

The branchiae are unusual in *Polynoidae* [11] and only occur in polynoids inhabiting deep-sea chemosynthetic environments. Clade A was composed of three genera with arborescent branchiae (Figure 4(m)). Clade B mainly consisted of nonbranchiate polynoids, including *Bathykurila*, *Lepidonotopodium*, and *Levensteiniella*, except for two branchiate species. *Branchinotogluma segonzaci* have developed arborescent branchiae, while *Thermopolynoe branchiata* [19] have layered branchiae (Figure 4(n)). The basal species *Branchiplicatus cupreus* has unique plicate branchiae (Figure 4(o)). Our phylogenetic analysis indicates that layered branchiae and plicate branchiae are possibly derived from arborescent ones, and the nonbranchiate polynoids in Clade B lost the branchiae in their evolution.

Polynoids inhabiting deep-sea chemosynthetic environments often exhibit sexual dimorphism, with different types of ventral papillae. Elongated ventral papillae are present in males of almost all the chemosynthesis-based polynoids and are inferred as a common character for the big clade composed of Clade A and Clade B (Figure 6(c)). Ventral papillae in Clade A start from segment 12 in males and segment 11 in females, while in Clade B, they usually start from segment 11 (except *B. segonzaci* with segment 12) in males and are absent in females. Ventral papillae starting from segment 12 in males is inferred to be an ancestral state for the big clade composed of Clade A and Clade B.

Data Availability

The specimens described in this study are available at the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS) at Institute of Oceanology, Qingdao, China. Voucher IDs: *Branchinotogluma nanhaiensis* sp. nov., MBM286795–MBM286800; *B. robusta* sp. nov., MBM286801–MBM286806. The new sequences of COI, 16S, 18S, and 28S that support the findings of this study have been deposited in NCBI GenBank (Table 1). The registration of *B. nanhaiensis* sp. nov. and *B. robusta* sp. nov. in ZooBank are as follows: urn:lsid:zoobank.org:act:5B366731-583F-4522-AF62-BFCE4430138C and urn:lsid:zoobank.org:act:8D-F340AA-100F-47D5-982F-404CBADCE38B, respectively. The publication LSID: urn:lsid:zoobank.org:pub:3419E1F3-8099-43FA-8F09-F8FCFBABB4E4.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

Authors' Contributions

Xuwen Wu and Wenquan Zhen contributed equally to this work.

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Supplementary Materials

Additional supporting information may be found online in the Supporting Information section. Figure S1: Bayesian inference (BI) tree reconstructed inferred from concatenated sequences (COI, 16S, 18S, and 28S) of *Branchinotogluma* and related genera. Figure S2: maximum likelihood (ML) tree reconstructed inferred from concatenated sequences (COI, 16S, 18S, and 28S) of *Branchinotogluma* and related genera. Table S1: details of primers used for PCR and sequencing with original reference. Table S2: mean interspecific pairwise distances for *Branchinotogluma* COI data based on the Kimura 2-parameter model. The intraspecific COI distances are shown on the diagonal. Table S3: morphological dataset for the analyzed polynoids in this study. *(Supplementary Materials)*

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