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Additional Description of the Vent Scale Worm *Thermopolynoe branchiata* (Polychaeta: Polynoidae) from the North Fiji Basin

Won-Kyung Lee[1,2], Geon Hyeok Lee[3], Se-Jong Ju[4,5], Se-Joo Kim[1,*]

[1]Division of Biomedical Resesarch, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea
[2]Division of EcoScience, Ewha Womans University, Seoul 03760, Korea
[3]Department of Biological Sciences, Inha University, Incheon 22212, Korea
[4]Management Division, Korea Institute of Ocean Science & Technology, Busan 49111, Korea
[5]Marine Biology Major, University of Science & Technology, Daejeon 34113, Korea

ABSTRACT

Thermopolynoe Miura, 1994 is a monotypic genus in Lepidonotopodinae that comprises species endemic to chemosynthesis-based ecosystems. Here, we examined *T. branchiata* collected from the hydrothermal vents in the North Fiji Basin. For the first time in *Thermopolynoe*, we report sexual dimorphism detecting nephridial papillae on segments 11−13 in males, additionally describe the morphology on elytra with round to conical microtubercles on the surface, and distinguish presence of small neuropodial lobes on segments 3−26. We also revised couple of errors and ambiguities in the original description: incongruence between the description and figure and existence of individual variation in ratio of tentacular cirri and palps. In addition, three *COI* sequences of *T. branchiata* specimens from the North Fiji Basin were newly obtained and sequences divergence with other Lepidonotopodinae species were determined. These results would contribute to the taxonomy of polynoids in a chemosynthesis-based ecosystem.

Keywords: polynoids, southwestern Pacific Ocean, deep-sea, elytra, *COI*

INTRODUCTION

The polynoid subfamily Lepidonotopodinae Pettibone, 1983 is a monophyletic taxon with 48 species in eight genera, consisting of species endemic to chemosynthesis-based ecosystems (Bonifácio and Menot, 2019; Hatch et al., 2020). These species tend to have robust body and tough elytra instead of fragile body and delicate elytra in other polynoids (Bonifácio and Menot, 2019). The sexual dimorphism presented by varying numbers of pairs of nephridial papillae was first described in Lepidonotopodinae by Van Dover et al. (1999) and since then sexual dimorphism has been reported in 25 species in this subfamily (Glover et al., 2005; Zhou et al., 2018; Hatch et al., 2020).

Thermopolynoe Miura, 1994 is a monotypic genus with *T. branchiata* Miura, 1994, endemic to hydrothermal vent ecosystems (Miura, 1994). In the original description of this species, the information on sexually dimorphic character and individual variation in ratio of tentacular cirri and palp was missing, segment number in the figure do not coincide with the text in the description section, and figure and its figure legend do not correspond. In this study, we collected *T. branchiata* specimens from hydrothermal vents in the North Fiji Basin and examined their morphological characters, including sexual dimorphism and individual variation, with its molecular analysis based on cytochrome oxidase subunit I (*COI*).

MATERIALS AND METHODS

The specimens were collected from hydrothermal vent field in the North Fiji Basin on 23 November and 4 December 2016, using a suction sampler and scoop mounted on the remotely operated vehicle (ROPOS, Canadian Scientific Submersible Facility, BC, Canada). All individuals were frozen immediately upon sampling. Later on land, an elytron of each specimen was dissected and preserved in 70% ethanol for molecular analysis and the rest of the body was preserved in

*To whom correspondence should be addressed
Tel: 82-42-879-8545
E-mail: biosejoo@kribb.re.kr

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10% formalin for morphological observation. The Ministry of Land and Natural Resources, Republic of Fiji granted KIOST Minerals Limited, Korea Institute of Ocean Science and Technology permission for sampling in Fiji's Exclusive Economic Zone.

For morphological observations, all specimens were visualized under a stereomicroscope (Stemi 508; Carl Zeiss, Jena, Germany). Photograph of main morphological features of specimens were taken with DSLR camera (EOS 5D Mark IV; Canon, Tokyo, Japan) and a color camera (Axiocam 208 color; Carl Zeiss, Oberkochen, Germany) equipped on stereomicroscope. Images were processed using ZEN 3.3 blue edition (Carl Zeiss, Jena, Germany) and Helicon Focus software (version 7; Helicon Soft Ltd., http://www.heliconsoft.com), and edited using Adobe Photoshop 2022 (Adobe, San Jose, CA, USA). The identification of the specimens were examined based on work of Miura (1994).

Total genomic DNA was extracted using a QIAamp Fast DNA Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Partial *COI* sequences (616 bp) were obtained from three individuals using primers LCO1490 and HCO2198 (Folmer et al., 1994). Polymerase chain reaction was conducted in 20 μL volumes containing 1 μL of genomic DNA, 2 μL of 10× Ex Taq Buffer (Mg^{2+} plus), 2 μL of dNTP mixture (2.5 mM each), 1 μL of each primer (10 pmol), 0.2 μL of Takara Ex Taq (5 U/μL) (Takara Bio, Tokyo, Japan), and distilled water to the final volume under the following conditions: initial denaturation at 94°C for 2 min followed by 35 cycles of 95°C for 10 s, 48°C for 30 s, and 72°C for 1 min, with a 5-min final extension at 72°C. The *COI* sequences of 40 Lepidonotopodinae were retrieved from GenBank (Table 1) and total of 554 bp were aligned with the new sequences using Geneious Prime v2022.2.1 (Biomatters, Auckland, New Zealand). Sequence divergence was calculated using the *p*-distance (Nei and Kumar, 2000) in MEGA-X (Kumar et al., 2018). All specimens used in this study are deposited at the Korea Research Institute of Bioscience and Biotechnology.

RESULTS AND DISCUSSION

Order Phyllodocida Dales, 1962
Family Polynoidae Kinberg, 1856
Subfamily Lepidonotopodinae Pettibone, 1983
[1]*Genus *Thermopolynoe* Miura, 1994

[2]****Thermopolynoe branchiata* Miura, 1994 (Table 2, Figs. 1–3)**

Thermopolynoe branchiata Miura, 1994: 532, figs. 1–4; Desbruyères et al., 2006: 249–250, Pl. 1–12.

Table 1. List of *COI* sequences and information of nephridial papillae on male of Lepidonotopodinae species used in this study

Species (segment # with nephridial papillae)	GenBank accession No.	Species (segment # with nephridial papillae)	GenBank accession No.
Bathykurila guaymasensis (11)	MH233265	*Branchipolynoe pettiboneae* (11–12)	MG799393
Branchinotogluma bipapillata (12–13)	MT067561	*Branchipolynoe seepensis* (11)	MK591274
Branchinotogluma hessleri (unknown)	KY684713	*Branchipolynoe symmytilida* (12)	MH369875
Branchinotogluma japonicus (12)	MG799391	*Branchipolynoe tjiasmantoi* (12)	MH369947
Branchinotogluma marianus (unknown)	MW646931	*Lepidonotopodium fimbriatum* (unknown)	KY684717
Branchinotogluma nikkoensis (12)	ON255504	*Lepidonotopodium okinawae* (11–13)	MG799382
Branchinotogluma ovata (12)	MK357896	*Lepidonotopodium piscesae* (unknown)	MW646939
Branchinotogluma sagamiensis (12)	ON255503	*Lepidonotopodium* sp. (unknown)	KY684715
Branchinotogluma sandersi (unknown)	JN852923	*Lepidonotopodium williamsae* (unknown)	MW646938
Branchinotogluma segonzaci (12)	MK357906	*Levensteiniella intermedia* (11)	MW646937
Branchinotogluma trifurcus (12)	MW646933	*Levensteiniella iris* (11)	KY753827
Branchinotogluma tunnicliffeae (unknown)	MW646935	*Levensteiniella kincaidi* (11–12)	MZ197620
Branchinotogluma elytropapillata (12)	MG799386	*Levensteiniella undomarginata* (11–12)	MG799385
Branchinotogluma pettiboneae (12–15)	MK357904	*Peinaleopolynoe elvisi* (unknown)	MN431777
Branchiplicatus cupreus (unknown)	KY684706	*Peinaleopolynoe goffrediae* (unknown)	MN431783
Branchipolynoe eliseae (unknown)	MH369878	*Peinaleopolynoe mineoi* (unknown)	MN431776
Branchipolynoe halliseyae (12)	MH369858	*Peinaleopolynoe orphanage* (unknown)	MH115406
Branchipolynoe kajsae (12)	MH369859	*Peinaleopolynoe santacatalina* (unknown)	MH115412
Branchipolynoe longqiensis (11–12)	KY753826	*Peinaleopolynoe sillardi* (unknown)	MH115414
Branchipolynoe meridae (unknown)	MH369884	*Thermopolynoe branchiata* (11–13)	MN431772

Korean name: [1]*열수비늘갯지렁이 (신칭), [2]*다발아가미열수비늘갯지렁이 (신칭)

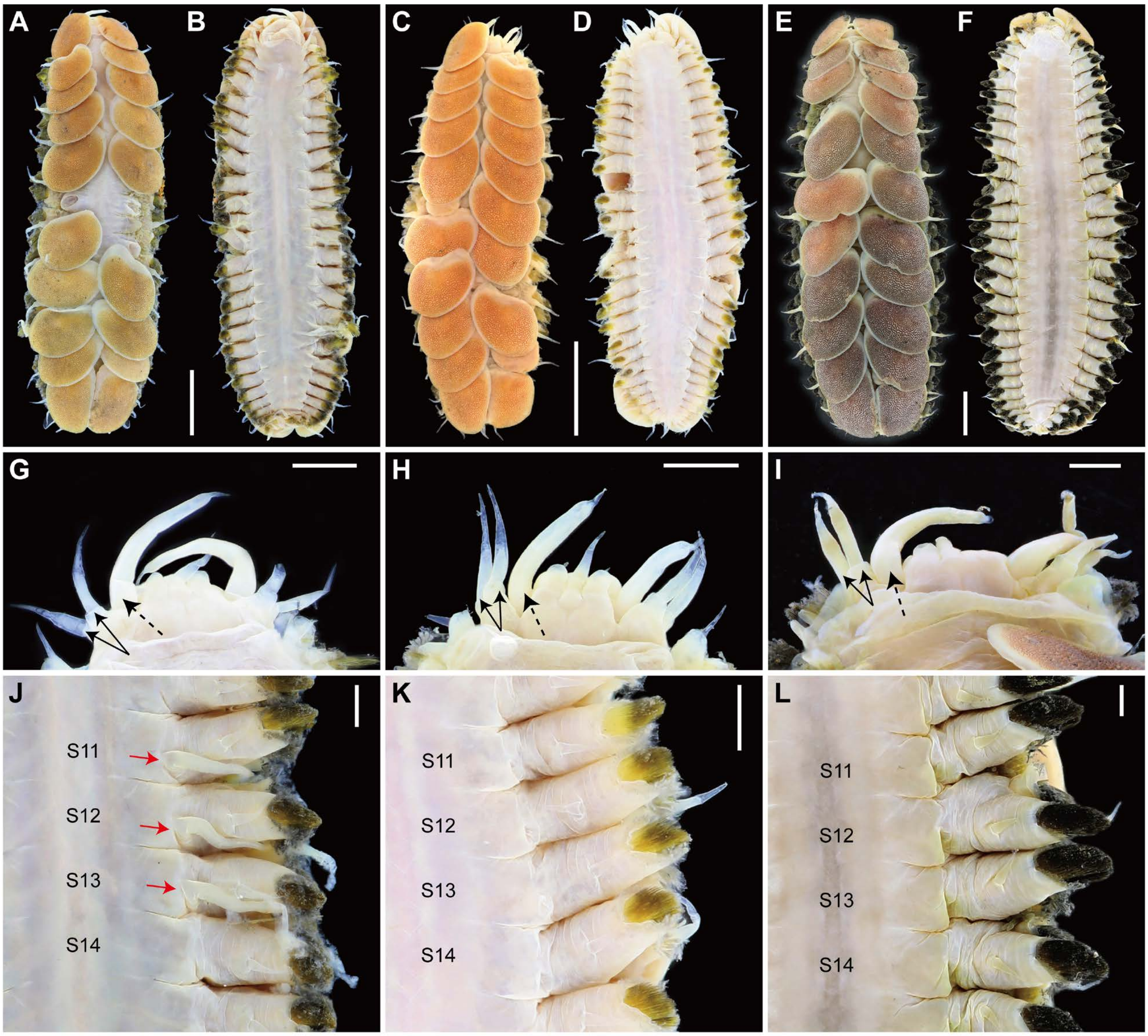

Fig. 1. Individual variation of key characters in *Thermopolynoe branchiata* Miura, 1994 from the North Fiji Basin. A, B, G, J, Male (KRIBB313011); C, D, H, K, Female (KRIBB313012); E, F, I, L, Female (KRIBB313013). A, C, E, Whole body, dorsal view; B, D, F, Whole body, ventral view; G–I, Head and anterior segments, dorsal view (solid arrows indicate tentacular cirri and dotted arrows indicate palps); J–L, Left halves of segments 11–14, ventral view (red arrows indicate nephridial papillae on male specimen). Scale bars: A–F=5 mm, G–L=1 mm.

Material examined. Three specimens (1 ind., 19°3.10′S, 173°28.89′E, 2,717 m depth, 23 Nov 2016, KRIBB313013; 2 inds., 17°6.82′S, 173°52.40′E, 2,248 m depth, 4 Dec 2016, KRIBB313011–KRIBB313012) from hydrothermal vent fields in the North Fiji Basin, collected by Lee WK.

Diagnosis. Body 27 segments. Elytra 11 pairs; surface covered with round to cone-shaped microtubercles. Dorsal cirri on non-elytragerous segments. Branchiae arborescent. Parapodia biramous; notopodia with well-developed bracts, neuropodia with fimbriated edge; small neuropodial lobe positioned on dorsal side of segments 3–26. Sexual dimorphism present; male with three pairs of nephridial papillae on segments 11–13, female without nephridial papillae.

Description. All three specimens were well-preserved. Body 27 segments, 23–50 mm long and 10–21 mm wide, flattened and fusiform in shape (Table 2, Fig. 1A–F). Elytra and elytrophores each in pair, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, and 21, size increases from anterior to posterior segments; first elytra pair oval, second and third reniform, fourth to tenth sub-reniform, eleventh longer than wide (Fig. 2A). Ely-

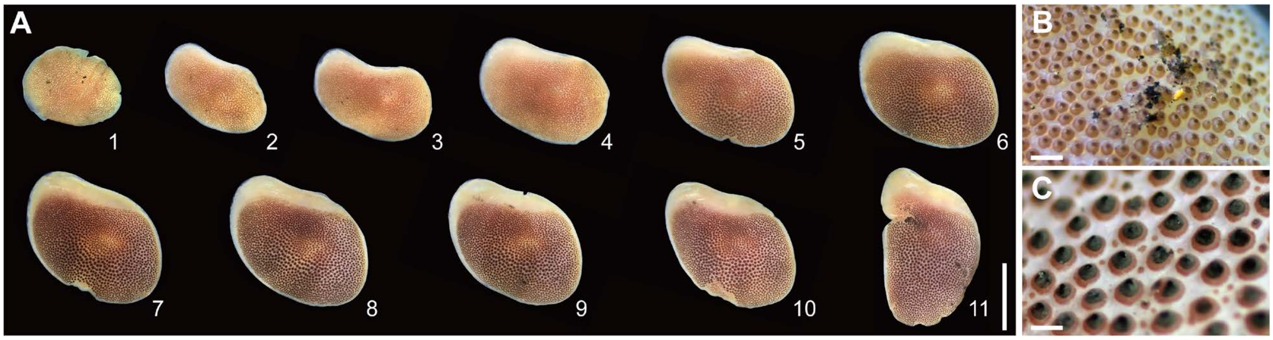

Fig. 2. Elytra of *Thermopolynoe branchiata* Miura, 1994, female (KRIBB313013). A, Right elytra from segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, and 21; B, Elytral surface of the first elytron on segment 2; C, Elytral surface of the eleventh elytron on segment 21. Scale bars: A=2 mm, B, C=0.1 mm.

Table 2. Characteristics of the *Thermopolynoe branchiata* specimens

Region	Specimen ID	Preservation method	Body length (mm)	Body width (mm)	No. of segments	Sex (NP position)	Segments with branchiae	Form of elytral microtubercles	GenBank accession No.
North Fiji Basin	Original description (22 inds.)	N/A	8.1–55	4.4–20	27	N/A (11–13 in Fig. 2H vs. 12–14 in description)[a]	3–27	Round	N/A
	KRIBB313011	Formalin	32	14	27	Male (11–13)	3–25	Round	OP435583
	KRIBB313012	Ethanol	23	10	27	Female (absent)	3–24	Round	OP435584
	KRIBB313013	Formalin	53	21	27	Female (absent)	3–27	Conical	OP435582

NP, nephridial papillae.
[a]In half of the described specimens.

tral surface light to dark brown, covered with less developed round microtubercles in small individuals, but fully developed conical microtubercles with central black pigmentation in large individual (Fig. 2B, C). Dorsal cirri on segments 3, 6, 8, 10, 12, 14, 16, 18, 20, 22–27, extending beyond the tips of neurochaetae. Branchiae arborescent, starting from segment 3, ending segments unsure (Table 2), surrounding bases of dorsal tubercles and elytrophores.

Prostomium bilobed; anterior lobes cylindrical, frontal filaments short and slender. Median antennae on anterior notch; ceratophore cylindrical, style subulate extending toward the tip of palp. Palps thick and smooth, tips subulate (Fig. 1G–I). Lateral antennae and eyes absent. Tentacular segment fused to prostomium; tentacules in pair, positioned on both lateral sides, tentacular cirri slender, length variable between individuals, from halfway to tip of the palp (Fig. 1G–I).

First segment not distinct dorsally, fused to prostomium. Second segment with first pair of elytrophores, biramous parapodia, and buccal cirri. Third segment with first pair of branchiae and dorsal and ventral cirri. Fourth to last segments with biramous parapodia, bearing a pair of ventral cirri. Notopodia subconical, with well-developed flaring bracts. Neuropodia diagonally truncate, with fimbriated edge, deeply cleft dorsally; small neuropodial lobe positioned on dorsal side of neuropodia on segments 3–26, round, flat, prominent on segments 5–21, inconspicuous on segments 3–4 and 22–26 (Fig. 3A–F).

Sexual dimorphism present; in male, nephridial papillae in pairs, positioned on segments 11–13, extending near the tip of neurochaetae, in female, nephridial papillae absent.

Habitats and distribution. Hydrothermal vents of the Manus Basin, North Fiji Basin, and Lau Basin in the southwestern Pacific Ocean.

DNA barcoding analysis. Partial *COI* barcodes (616 bp) of three *T. branchiata* specimens are newly deposited in GenBank (accession Nos. OP435582–OP435584). The intraspe-

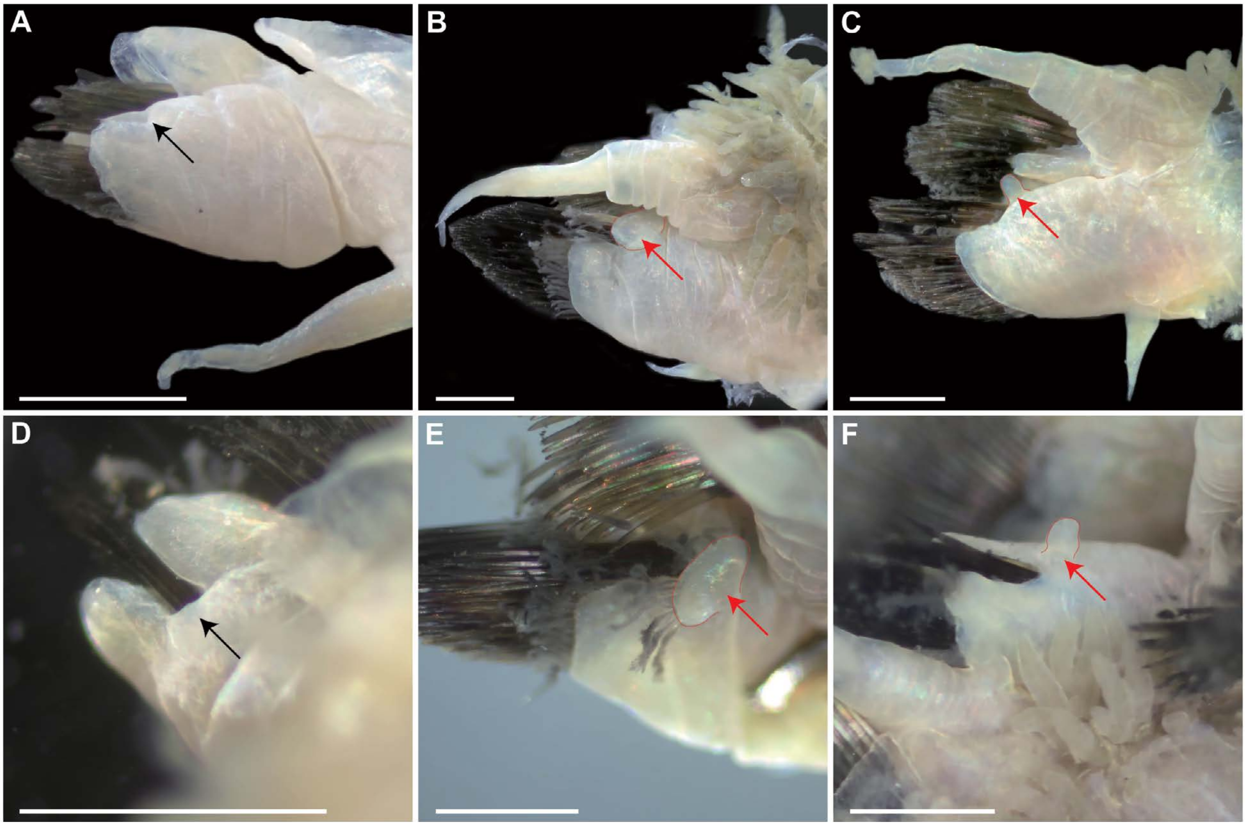

Fig. 3. Parapodia and small neuropodial lobes of *Thermopolynoe branchiata* Miura, 1994, female (KRIBB313013). A–C, Left parapodia in posterior view; D–F, Small neuropodial lobes in dorsal view. Black arrows indicate the absence of a small neuropodial lobe on segment 2 (A, D) and red arrows denote small neuropodial lobes on segments 8 (B, E) and 24 (C, F). Scale bars: 0.5 mm.

Table 3. Average sequence divergence of mitochondrial *COI* genes (554 bp) from Lepidonotopodinae species

		1	2	3	4	5	6	7	8
1	*Thermopolynoe* (1[a], N/A[b])								
2	*Lepidonotopodium* (5, 25.19)	25.01							
3	*Bathykurila* (1, N/A)	25.87	26.86						
4	*Branchinotogluma* (13, 22.88)	27.52	26.33	24.70					
5	*Branchipolynoe* (9, 14.79)	27.73	27.28	22.22	24.21				
6	*Branchiplicatus* (1, N/A)	28.03	27.67	25.95	26.20	26.09			
7	*Peinaleopolynoe* (6, 19.79)	28.10	28.09	29.36	25.40	23.56	25.70		
8	*Levensteiniella* (4, 22.01)	28.13	27.99	29.26	29.83	28.50	29.60	29.54	

Pairwise nucleotide sequence variation (%) was calculated using the *p*-distance method in Mega-X.
[a]Number of species.
[b]Interspecific mean variation (%).

cific variation among four *T. branchiata* individuals from the Lau and North Fiji Basins was 0.16–0.32%, with three non-identical bases.

The mean interspecific variation within five genera of Lepidonotopodinae was 14.79–25.19%. The intergeneric variation among eight genera of Lepidonotopodinae was 22.22–29.83% (Table 3). The variation between *Thermopolynoe* and other genera ranged from 25.01% (*Lepidonotopodium*) to 28.13% (*Levensteiniella*). Most species presented smaller interspecific variation within genera compared to the intergeneric variation. However, *Lepidonotopodium* showed larger interspecific variation (25.19%) than intergeneric variation with *Thermopolynoe* (25.01%). This is unsurprising as previous phylogenetic studies have also questioned the placement of *Lepidonotopodium* species having *L. fimbriatum* closest to the *T. branchiata* (Hatch et al., 2020). However, for now, they remain unresolved until additional sampling of other species.

Remarks. *Thermopolynoe branchiata* specimens from the North Fiji Basin generally agreed with the original description by having 27 segments, 11 pairs of elytra and well-developed arborescent branchiae (Miura, 1994). In this study, we additionally described the variation in color and form of microtubercles covering the elytral surface (Fig. 2B, C), and the existence of small neuropodial lobes on segments 3–26 (Fig. 3A–F). Meanwhile, we revised couple of errors in the original description: the incongruence in position of nephridial papillae between the description section (segments 12–14) and Fig. 2 (segments 11–13) adjusted with our results (segments 11–13); the ratio between tentacular cirri and palps confirmed as individual variation (Fig. 1G–I).

In some *Branchinotogluma* species of Lepidonotopodinae, the presence-absence and the position of nephridial papillae had been recognized as significant features for distinguishing two different species, but currently these are confirmed as sexual dimorphism (Desbruyères et al., 2006; Lee et al., 2021). Based on previous work, the position of nephridial papillae of male in Lepidonotopodinae seems to be related with their phylogenetic relationship (Hatch et al., 2020) (Tables 1, 2). In case of males with nephridial papillae starting from segement 11, such is *Lepidonotopodium* species and *T. branchiata*, they form a single clade. Therefore, additional information on nephridial papillae of males in species which it is not known is necessary.

ORCID

Won-Kyung Lee: http://orcid.org/0000-0001-7283-298X
Geon Hyeok Lee: https://orcid.org/0000-0001-7517-3086
Se-Jong Ju: https://orcid.org/0000-0003-3479-6652
Se-Joo Kim: http://orcid.org/0000-0003-1653-072X

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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