

Additional Records of the Hydrothermal Vent Scale Worm *Branchinotogluma segonzaci* (Polynoidae: Lepidonotopodinae) from the North Fiji Basin and Tonga Arc

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ABSTRACT

Branchinotogluma segonzaci (Miura and Desbruyères, 1995) occurs in hydrothermal vent fields of the southwestern Pacific Ocean. We morphologically compared *B. segonzaci* from the North Fiji Basin with the original description from the Lau Basin and a subsequent study of specimens from the Manus Basin. The main characteristics of all *B. segonzaci* populations were similar having 21 segments, 10 pairs of elytra, cylindrical-shaped anterior lobes, and ventral papillae on segment 12 and ventral lamellae on segments 13–17 in males. However, the specimens from the North Fiji Basin had rounded to sub-reniform elytra rather than oval in the original description. Additionally, we newly obtained 11 cytochrome oxidase subunit I (*COI*) DNA barcodes from the North Fiji Basin and Tonga Arc populations and compared them with known *COI* DNA barcodes of *Branchinotogluma* species. Thirteen sequences of *B. segonzaci* showed 0.0–1.07% intraspecific variation and formed two clades in the *COI* neighbor-joining tree, whereas the interspecific variation among *Branchinotogluma* species was 8.19–22.4%. The results of this study contribute to biogeographic studies of *B. segonzaci* and the evolution of polynoid scale worms in chemosynthesis-based ecosystems.

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

INTRODUCTION

Polynoid polychaetes or scale worms are distributed globally from tropical to polar regions and from shallow to deep-sea waters. Approximately 900 species in 18 subfamilies are known (Zhang et al., 2018). The subfamily Lepidonotopodinae Pettibone, 1983, a monophyletic taxon including 46 species in 8 genera, is restricted to deep-sea chemosynthetic environments, such as vents and seeps (Bonifácio and Menot, 2019; Hatch et al., 2020). The genus *Branchinotogluma* Pettibone, 1985 contains 12 species living in hydrothermal vent fields and is the largest taxon in subfamily Lepidonotopodinae (Wu et al., 2019). Most of them were found in the eastern and western Pacific Ocean, although *Branchinotogluma bipapillata* Zhou, Wang, Zhang and Wang, 2018 was

found in the Indian Ocean (Wu et al., 2019). According to Zhang et al. (2018), *Branchinotogluma* species show sexual dimorphism distinct from other deep-sea polynoids. The males have modified posterior segments with merged and/or unbranched (non-biramous) parapodia on segments 18–21 while the females have unmodified posterior segments. Additionally, on ventral side, papillae are present starting from segment 12 in males while absent in females. Some previous records overlooked the sexual dimorphism on *Branchinotogluma* species and the males and females were misidentified as two different genera, *Branchinotogluma* Pettibone, 1985 and *Opisthotrochopodus* Pettibone, 1985 (Pettibone, 1985) (Table 1). Subsequently, all were synonymized in the genus *Branchinotogluma* by Desbruyères et al. (2006), which was re-confirmed by Zhang et al. (2018) with molecular taxonomy

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Table 1. Current taxonomic status of *Branchinotogluma* species

Original description	Sex	Revised taxonomic status	COI barcode accession No. ^a	Reference
<i>Branchinotogluma hessleri</i>	Female	<i>Branchinotogluma hessleri</i>	KY684713	
<i>Opisthotrochopodus alvinus</i>	Male	<i>Branchinotogluma hessleri</i>		
<i>Branchinotogluma grasslei</i>	Female	<i>Branchinotogluma sandersi</i>	MH115399–MH115405, JN852923, KY684716	
<i>Branchinotogluma sandersi</i>	Male	<i>Branchinotogluma sandersi</i>		
<i>Opisthotrochopodus tunnicliffeae</i>	Female & Male	<i>Branchinotogluma tunnicliffeae</i>	N/A	Desbruyères et al. (2006)
<i>Branchinotogluma burkensis</i>	Female & Male	<i>Branchinotogluma burkensis</i>	N/A	
<i>Opisthotrochopodus marianus</i>	Female & Male	<i>Branchinotogluma marianus</i>	N/A	
<i>Opisthotrochopodus japonicus</i>	Female & Male	<i>Branchinotogluma japonicus</i>	KY753824, <u>MG799390–MG799392</u>	
<i>Opisthotrochopodus segonzaci</i>	Female & Male	<i>Branchinotogluma segonzaci</i>	<u>MK694792</u> , <u>MK357906</u> , <u>MZ604325–MZ604335</u>	
<i>Opisthotrochopodus trifurcus</i>	Female & Male	<i>Branchinotogluma trifurcus</i>	MK357905	
<i>Branchinotogluma elytrapapillata</i>	Female & Male	–	MG799386–MG799389	Zhang et al. (2018)
<i>Branchinotogluma bipapillata</i>	Female & Male	–	KY211996, MH202755, <u>MH712481–MH712483</u>	Zhou et al. (2018)
<i>Branchinotogluma ovata</i>	Female & Male	–	MK357895–MK357900	Wu et al. (2019)
<i>Branchinotogluma pettiboneae</i>	Female & Male	–	MK357901–MK357904	Wu et al. (2019)

^aNewly obtained sequences in this study are in bold; sequences determined for both male and female individuals are underlined.

based on cytochrome oxidase subunit I (*COI*) sequences.

Branchinotogluma segonzaci (Miura and Desbruyères, 1995) is the largest in size among the *Branchinotogluma* species and widely distribute in hydrothermal vent fields of the southwestern Pacific Ocean, ranging from the Manus Basin to the Lau Basin (Miura and Desbruyères, 1995; Desbruyères et al., 2006). However, the origin, migration, adaptation, and genetic structure of this species in the southwestern Pacific Ocean are unclear. Here, we identified *Branchinotogluma* specimens from the North Fiji Basin and Tonga Arc, and compared their morphological characters with previous morphological descriptions. We then performed *COI* DNA barcoding and discuss their genetic divergence with their relatives.

MATERIALS AND METHODS

Sample collection. *Branchinotogluma* specimens were collected from hydrothermal vents in the North Fiji Basin in December 2016 and Tonga Arc in February 2012, using the suction sampler of a remotely operated vehicle (ROPOS, Canadian Scientific Submersible Facility). All individuals were frozen immediately upon sampling, and later preserved in 10% formalin or 70% ethanol on land.

Morphological taxonomy procedures. For morphological observations, all samples were visualized under a stereomicroscope (MZ125; Leica, Jena, Germany) and photographed with a digital camera (EOS 5Ds; Canon, Tokyo, Japan). Images were blended using Helicon Focus software and edited using Adobe Photoshop 2020. The key characteristics of *Branchinotogluma* species were examined following Zhang et al. (2018) and Wu et al. (2019).

Molecular taxonomy procedures. Genomic DNA was extracted using a QIAamp Fast DNA Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. Universal primers LCO1490 and HCO2198 (Folmer et al., 1994) were used to obtain partial *COI* sequences. Polymerase chain reaction (PCR) was conducted in a total volume of 50 µL containing 1 µL of genomic DNA, 5 µL of 10× Ex Taq Buffer (Mg²⁺ plus), 4 µL of a dNTP mixture (2.5 mM each), 1 µL of each primer (10 pmol), 1.25 U of Takara Ex Taq DNA Polymerase (Takara Biotechnology, 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 denaturation at 95°C for 10 s, annealing at 48°C for 30 s, and extension at 72°C for 1 min, with a final 5-min extension at 72°C. The *COI* sequences of *B. segonzaci* from the Manus Basin and other *Branchinotogluma* were retrieved from GenBank and aligned with the newly obtained sequences using Geneious Prime v2020.0.4 (Biomatters, Auckland, New

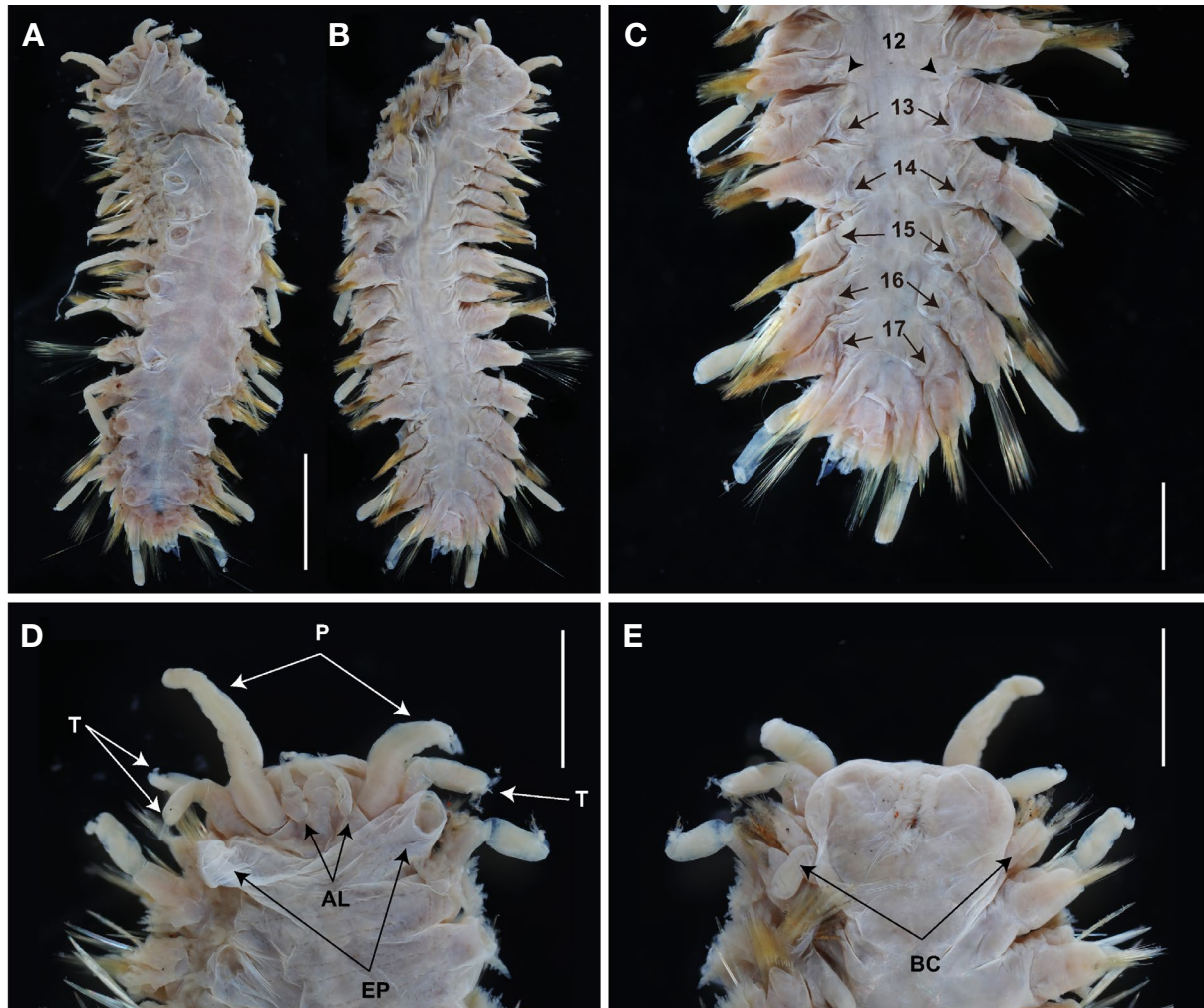


Fig. 1. *Branchinotogluma segonzaci* (Miura & Desbruyères, 1995) from a hydrothermal vent field in the North Fiji Basin, male (A–D, KRIBB310001). A, Dorsal view with elytra removed; B, Ventral view; C, Ventral posterior view showing ventral papillae (arrowheads on segment 12) and lamellae (arrows on segments 13–17); D, Segments 1–4, dorsal anterior view, anterior lobes (AL), elyrophore (EP), palp (P), and tentacles (T); E, Segments 1–4, ventral anterior view, buccal cirri (BC). Scale bars: A, B=5 mm, C–E=2 mm.

Zealand). Intra- and inter-specific variations were calculated using the p-distance (Nei and Kumar, 2000), and a neighbor-joining tree was constructed using MEGA-X (Kumar et al., 2018). All specimens and genomic DNA extracts used in this study were deposited in the Korea Research Institute of Bioscience and Biotechnology.

RESULTS AND DISCUSSION

Phylum Annelida Lamarck, 1809
Class Polychaeta Grube, 1850
Order Phyllodocida Dales, 1962

Family Polynoidae Kinberg, 1856

¹*Genus *Branchinotogluma* Pettibone, 1985

²**Branchinotogluma segonzaci*

(Miura and Desbruyères, 1995) (Table 2, Figs. 1, 2)

Opisthotrochopodus segonzaci Miura and Desbruyères, 1995:
584–589, figs. 1–4.

Branchinotogluma segonzaci Desbruyères et al., 2006: 225,
226, Pl. 1–8; Wu et al., 2019: 149, figs. 1, 2.

Material examined. Five specimens (KRIBB310001–KRI
BB310005) from hydrothermal vent fields in the North Fiji
Basin (17°6.8178'S, 173°52.4003'E; 2,248 m depth; 4 Dec

Korean name: ¹*등다리싸개비늘갯지렁이 (신칭), ²*세곤작등다리싸개비늘갯지렁이 (신칭)

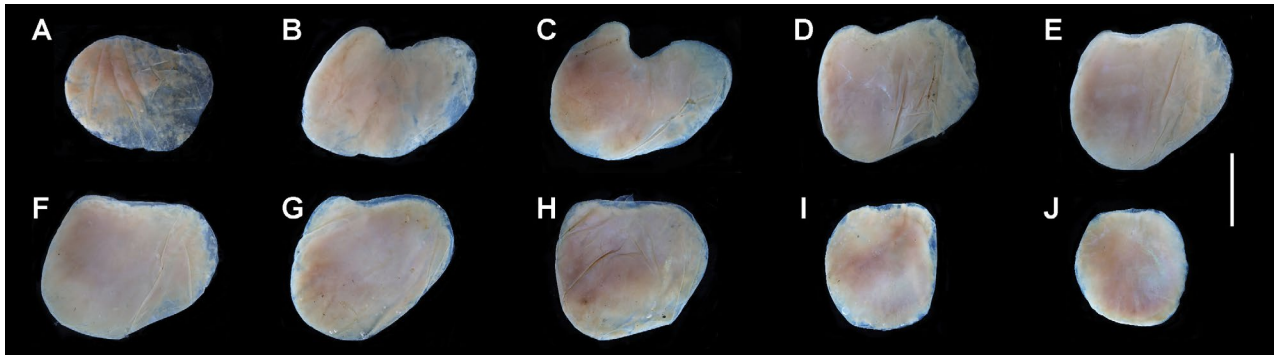


Fig. 2. Left elytra of *Branchinotogluma segonzaci* (Miura & Desbruyères, 1995), female (A–J, KRIBB310003). A–J, Elytra from segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19, respectively. Scale bar=5 mm.

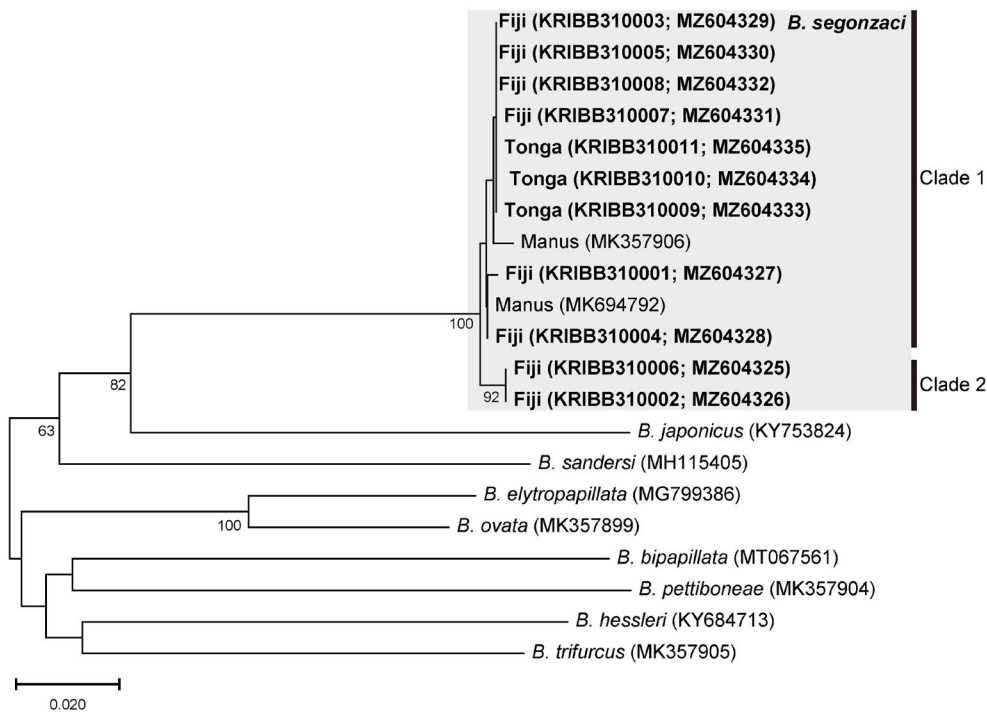


Fig. 3. Neighbor-joining tree of *Branchinotogluma* species based on the *COI* dataset (562 bp). The gray box contains the sequences of *B. segonzaci* obtained from specimens from the North Fiji Basin and Tonga Arc in this study (bold letters) and the sequences of the Manus Basin specimens retrieved from GenBank. Bootstrap values >60% are shown next to the nodes.

2016). Three specimens (KRIBB310009–KRIBB310011) from vent fields in the Tonga Arc (24°35.322'S, 176°56.784' W; ~1,040–1,070 m depth; 4 Feb 2012).

Description. Of the eight specimens, three from the North Fiji Basin well-preserved, with 21 segments, 16–55 mm long and 6–20 mm wide, body flattened and fusiform in shape (Fig. 1A, B). Elytra and elytraphores each in pairs, on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, and 19; first elytra pair, relatively small and oval; second to eighth pairs, larger and sub-reniform; ninth and tenth pairs, smaller and round (Fig. 2). Dorsal

cirri on segments 3, 6, 8, 10, 12, 14, 16, 18, and 20.

Prostomium bilobed, only visible dorsally. Median antennae on anterior notch, ceratophore cylindrical in shape, style short; anterior lobes in pair, cylindrical in shape, frontal filaments slender; palps thick and smooth, tips round (Fig. 1A, D). Tentacular segment fused to prostomium; tentacules in pair, on both lateral sides; tentaculophore achaetous; tentacular cirri stout, as long as tentaculophore (Fig. 1D).

First segment not distinct, fused to prostomium. Second segment bearing first pair of elytraphores, with biramous para-

podia and buccal cirri (Fig. 1D, E). Third segment bearing first pair of branchiae, with biramous parapodia, dorsal cirri, and ventral cirri. Fourth to nineteenth segments bearing pairs of branchiae, with biramous parapodia; notopodia positioned on anterodorsal side of neuropodia.

Sexual dimorphism present; in male, ventral papillae in pairs, positioned only on segment 12, approximately 300 µm in length; ventral lamellae present in pairs, positioned on segments 13–17, semi-oval in shape; notochaetae lacking on segments 20–21, neurochaetae lacking on segment 21 (Fig. 1C). In female, both papillae and lamellae absent; notochaetae and neurochaetae present on segments 20–21.

Distribution. Deep-sea hydrothermal vents of the Manus Basin, North Fiji Basin, Lau Basin, and Tonga Arc in the southwestern Pacific Ocean.

DNA barcoding analysis. Eleven *COI* barcodes (562 bp) of *B. segonzaci* specimens are newly deposited in GenBank (accession nos. MZ604325–MZ604335). Intraspecific variations among *B. segonzaci* individuals from three regions were 0.0–1.07%, with the maximum variation between the samples from the Manus and North Fiji Basins (Table 3). The intraspecific variation of other *Branchinotogluma* species ranged from 0–3.5%, with the maximum in *B. sandersi*. Interspecific variation among *Branchinotogluma* species was 8.19–22.4%, with the least between *B. elytrapapillata* and *B. ovata* and the most between *B. japonicus* and *B. pettiboneae*. These results indicate that *COI* barcodes are suitable for distinguishing *Branchinotogluma* species, in accordance with previous studies (Wu et al., 2019).

On the neighbor-joining tree constructed using *COI* barcodes, all *B. segonzaci* individuals were monophyletic, but they were clearly separated into two clades (Fig. 3). Clade 1 comprised *B. segonzaci* from three regions, whereas Clade 2 contained only the individuals from the North Fiji Basin. These results infer the existence of population-level genetic differentiation among *B. segonzaci* individuals, although we were not able to include sufficient individuals per site to understand their population structure fully. Further analysis of genetic structure, mitogenome, and biogeography is required to deepen our understanding of the broad distribution of *B. segonzaci* in the southwestern Pacific.

Remarks. *Branchinotogluma segonzaci* specimens from the North Fiji Basin agreed with the original description of *B. segonzaci* (Miura and Desbruyères, 1995) having 21 segments, 10 pairs of elytra, cylindrical-shaped anterior lobes, and ventral papillae on segment 12 and ventral lamellae on segments 13–17 in males (Table 2, Fig. 1A, B). The shape of elytra on ours resembles the Manus population of Wu et al. (2019) but minorly differs from those of original description (rounded to sub-reniform vs. oval).

Table 2. Morphological comparison of *Branchinotogluma segonzaci* in the present and previous studies

Region	Specimen ID	Preservation method	Body length (mm)	Body width (including chaetae; mm)	No. of segments	VP+VL ^a	Shape of elytra	Reference
Manus Basin	MBM286042	Ethanol	50	22	21	1+5	Round to subreniform	Wu et al. (2019)
	MBM286043	Ethanol	15	8.8	21	0+0	Round to subreniform	Wu et al. (2019)
North Fiji Basin	KRIBB310001	Ethanol	24	10	21	1+5	Round to subreniform	This study
	KRIBB310002	Formalin	16	6	21	0+0	Round to subreniform	This study
	KRIBB310003	Formalin	55	20	21	0+0	Round to subreniform	This study
	KRIBB310004	Ethanol	39	13	Over 18 ^b	N/A ^a	Round to subreniform	This study
	KRIBB310005	Ethanol	26	9	Over 15 ^b	N/A ^a	Round to subreniform	This study
Lau Basin	MNHN UD 852	Not mentioned	29	14	21	1+5	Oval	Miura and Desbruyères (1995)
	MNHN UD 853	Not mentioned	49	19	21	0+0	Oval	Miura and Desbruyères (1995)

^aNumber of pairs of ventral papillae starting from segment 12 (VP) + pairs of ventral lamellae following ventral papillae (VL).

^bSample condition not suitable for complete observation.

Table 3. Sequence divergence among partial sequences of the mitochondrial *COI* gene from *Branchinotogluma* species

	1	2	3	4	5	6	7	8	9
1	<i>B. segonzaci</i> ^a (13 ^b , 0–1.07 ^c)								
2	<i>B. japonicus</i> (KY753824)	16.8							
3	<i>B. ovata</i> (MK357899)	17.7	21.0						
4	<i>B. elytrapapillata</i> (MG799386)	17.7	22.1	8.19					
5	<i>B. sandersi</i> (MH115405)	17.8	21.2	18.5	19.6				
6	<i>B. trifurcus</i> (MK357905)	18.9	21.9	19.4	20.5	20.3			
7	<i>B. hessleri</i> (KY684713)	20.1	20.5	20.1	17.6	19.6	18.3		
8	<i>B. bipapillata</i> (MT067561)	21.0	20.1	20.5	19.2	21.0	19.4	21.9	
9	<i>B. pettiboneae</i> (MK357904)	21.3	22.4	19.2	19.0	21.0	20.1	20.6	21.5

Pairwise nucleotide sequence variation (%) was calculated using the p-distance method in Mega-X.

^a*B. segonzaci* accession nos. are MZ604325–MZ604335, MK357906, and MK694792.

^bNumber of individuals.

^cIntraspecific variation (%) range.

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CONFLICTS OF INTEREST

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

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