Travisia sanrikuensis, a New Species of Traviidae (Annelida) from the Lower Bathyal Zone of the Northwestern Pacific

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A new species of the genus *Travisia* Johnson, 1840, the single genus of the family Traviidae, is described. Specimens of *Travisia sanrikuensis* sp. nov. were collected in the Sanriku region, Japan, from the lower bathyal zone (871–1684 m depth) of the northwestern Pacific. Molecular phylogenetic analysis based on partial 16S rRNA gene sequences revealed that the new species is phylogenetically close to *Travisia brevis* Moore, 1923, but differs from all congeneric species by the following morphological characters: 25 segments, 20 chaetigers, and 19–20 pairs of cirriform branchiae starting from chaetiger 2.

Key Words: 16S rRNA gene, deep sea, polychaete, Sedentaria, taxonomy.

Introduction

The family Traviidae includes vermiform annelids within a single valid genus, *Travisia* Johnson, 1840, which comprises 36 described species (Wiklund et al. 2019; Blake and Maciolek 2020; Rizzo and Salazar-Vallejo 2020). Previously, Traviidae was considered a subfamily of the family Opheliidae (Dauvin and Bellan 1994; Rouse 2001); however, subsequent molecular phylogenetic analyses indicated a close relationship between the species of Traviidae and the family Scalibregmatidae (Persson and Pleijel 2005; Paul et al. 2010; Martínez et al. 2013, 2014; Law et al. 2014; Drennan et al. 2019). More recently, the subfamily status of Traviidae has been re-evaluated and raised to the family level on the basis of morphological evidence (Blake and Maciolek 2020).

To date, at least seven described species and one subspecies of *Travisia* have been recorded from intertidal to hadal zones around the coastline of Japan: *Travisia brevis* Moore, 1923; *Travisia forbesii* Johnston, 1840; *Travisia forbesii intermedia* Annenkova, 1937; *Travisia fusus* (Chamberlin, 1919); *Travisia glandulosa* McIntosh, 1879 [as “*Kesun abyssorum*” Monroe, 1930” in Levenstein (1973)]; *Travisia japonica* Fujiwara, 1933; *Travisia profundus* Chamberlin, 1919; and *Travisia pupa* Moore, 1906 (Imajima and Hartman 1964; Levenstein 1970, 1973; Imajima 2001, 2009, 2011). During cruises undertaken as part of the Tohoku Ecosystem-associated Marine Sciences (TEAMS) research project, we collected specimens of *Travisia* from the lower bathyal zone of the northwestern Pacific off the Sanriku region (the northeastern part of Honshu Island), Japan (Fig. 1). In this study, we examined the morphology of the specimens, determined partial sequences

of the 16S rRNA gene, and conducted phylogenetic analyses of Traviidae. We describe these traviid specimens as being new to science based on the results of these analyses.

Materials and Methods

Specimens were collected during the cruises of the research vessel (R/V) *Shinsei-Maru* in the northwestern Pacific off the Sanriku region of northeastern Japan (Fig. 1). Details of sampling are noted in Results and Discussion. The map of sampling localities was produced using Generic Mapping Tools (GMT) v5.1.1 (Wessel et al. 2013). All specimens were fixed and preserved in 70% ethyl alcohol. The holotype and paratypes have been deposited in the National Museum of Nature and Science, Tsukuba (NSMT). The morphology of the specimens was examined and photographed under a stereomicroscope (Figs 2D–F, 3) and an entire specimen was photographed without the aid of a stereomicroscope (Fig. 2A–C). Furthermore, a single specimen (paratype NSMT-Pol P-831) was stained with methyl green solution to highlight the superficial morphology and characterize the staining pattern.

Portions of the body wall of the holotype, a paratype (NSMT-Pol P-828), and a non-type specimen (identifier GK627) were excised and treated with proteinase K solution (Nacalai Tesque) and then with 100 µL of a 10% solution of Chelex 100 Resin (Bio-Rad) at 100°C for more than 20 min. The resulting supernatant was used as template DNA.

Partial sequences of the mitochondrial 16S rRNA gene of the three specimens were determined by PCR using one or other of the following two reaction mixtures: (Mixture

Fig. 1. Maps showing the sampling localities of *Traviaia sanrikuensis* sp. nov. The red and yellow circles indicate the type locality and the sampling locality of a non-type specimen of *T. sanrikuensis* sp. nov., respectively.

Fig. 2. *Traviaia sanrikuensis* sp. nov. holotype (NSMT-Pol H-827). A, Dorsal view; B, ventral view; C, left lateral view; D, anterior end, ventral view; E, posterior end, ventral view; F, close-up view showing details of the lateral morphology. Arrows indicate interramal pores. Scale bars: 10 mm (A–C); 1 mm (D–F).

1) 17.5 µL of sterilized water, 0.13 µL of TaKaRa Ex Taq Hot Start Version (TaKaRa Bio), 2.5 µL of 10× Ex Taq Buffer, 2.0 µL of 2.5 µM dNTP mixture, 0.3 µL of 20 µM forward and reverse primers (Table 1), and 2.5 µL of template DNA; (Mixture 2) 4.81 µL of sterilized water, 0.25 µL of MightyAmp DNA Polymerase Ver. 2 (TaKaRa Bio), 6.25 µL of MightyAmp Buffer, 0.19 µL of 20 µM forward and reverse primers (Table 1), and 1.0 µL of template DNA. PCR amplifications were performed using the following programs: (Mixture 1) initial denaturation at 94°C for 120 s; followed by 35 cycles comprising denaturation at 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 20 s; and then a final extension at 72°C for 300 s or (Mixture 2) initial denaturation at 98°C for 120 s; followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 15 s, and extension at 68°C for 20 s. The PCR products thus obtained were purified using ExoSAP-IT (Thermo Fisher Scientific), and sequencing was performed either by using an ABI 3130xl automated DNA sequencer (Applied Biosystems,

ABI) or was outsourced to Eurofins Genomics. Prior to sequencing with the ABI sequencer, the PCR products were initially treated using a BigDye XTerminator Purification Kit (ABI) and a BigDye Terminator Cycle Sequence Kit v3.1 (ABI). The nucleotide sequences obtained have been deposited in the DNA Data Bank of Japan (DDBJ) with DDBJ/EMBL/GenBank accession numbers LC566243 (holotype; 321 bp), LC566241 (paratype, NSMT-Pol P-828; 520 bp), and LC566242 (non-type specimen, GK627; 519 bp).

Phylogenetic analysis based on the 16S rRNA gene sequences was conducted using five traviisiid and two scalibregmatid species to determine those species most closely related to the newly described species among those species registered in GenBank (Table 2). All nucleotide sequences except for those of the new species were obtained from the GenBank database. Alignment was performed using the MAFFT online service (Q-INS-i option, considering secondary structure) (Katoh et al. 2017). Ambiguous positions were deleted using Gblocks v0.91 (Castresana 2000) using default parameters, apart from the option “Allowed gap positions,” which was set to “With half.” For the purposes of this analysis, we used only a single sequence from one of our specimens (GK627), as the nucleotide sequences for all specimens were identical after trimming using Gblocks. The final dataset included 482 characters, and we selected the GTR + G model based on the Akaike information criterion (AIC) using JModelTest v2.1.10 (Darriba et al. 2012).

Molecular phylogenetic analyses using the 16S rRNA gene sequences were conducted using Bayesian inference and maximum likelihood (ML) methods. Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the setting “branch lengths unlinked.” Two parallel runs were performed for 5 million generations (with a sampling frequency of 1000), using the default value of four Markov chains. The initial 25% of samples were discarded and the subsequent 75% were accepted to ensure that the four chains reached stationary distributions, referring to the average standard deviation of split frequen-

Fig. 3. Methyl green staining pattern on the prostomium of a *Traviisia sanrikuensis* sp. nov. paratype (NSMT-Pol P-831), ventral view. Scale bar: 1 mm.

Table 1. Nucleotide sequences of the primers for 16S rRNA used in this study.

Primer	Sequence (5'-3')	Direction ¹	Reference
16SarL	CGCCTGTTTATCAAAAACAT	F	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	R	Palumbi (1996)
16Sann-f2	CCTGACYGTGCWAAGGTAGC	F	This study
16Sann-r2	CCYTAAGYCAACAYCGAGGT	R	This study

¹ Forward (F) or reverse (R).

Table 2. Species used in the phylogenetic analysis based on 16S rRNA gene sequences. The newly obtained sequence is shown in bold type.

Family	Taxon	GenBank accession No.	References
Traviisiidae	<i>Traviisia sanrikuensis</i> sp. nov. (GK627)	LC566242	This study
	<i>Traviisia brevis</i>	HM746721	Paul et al. (2010)
	<i>Traviisia pupa</i>	HM746722	Paul et al. (2010)
	<i>Traviisia</i> sp. (NHM_1244)	MN217468	Wiklund et al. (2019)
	<i>Traviisia zieglerae</i>	MN217490	Wiklund et al. (2019)
Scalibregmatidae	<i>Polyphysia crassa</i>	HM746719	Paul et al. (2010)
	<i>Scalibregma inflatum</i>	KF511816	Law et al. (2014)

Table 3. Comparison of morphological characters of *Travisia sanrikuensis* sp. nov. and morphologically similar or genetically close species.

	<i>T. sanrikuensis</i> sp. nov. ¹	<i>T. brevis</i> ²	<i>T. chiloensis</i> ³	<i>T. oksae</i> ⁴
Number of segments	25	29	27	23–27
chaetigers	20	?	24	20–25
branchiae	19–20	22	20	22
first chaetiger with branchiae	2	3 ⁵	2	2
posterior achaetous segments	5	?	3	up to 3

References: ¹ this study; ² Moore (1923); ³ Kükenthal (1887); ⁴ Hartmann-Schröder and Parker (1995); ⁵ Rizzo and Salazar-Vallejo (2020).

cies (Ronquist and Huelsenbeck 2003). ML phylogenetic analysis was conducted using IQ-tree (Nguyen et al. 2014) based on 1000 ultrafast bootstrap replicates, and the resultant tree was edited using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Taxonomy

Family **Travisiidae** Hartmann-Schröder, 1971

Genus ***Travisia*** Johnston, 1840

Type species. *Travisia forbesii* Johnston, 1840.

***Travisia sanrikuensis* sp. nov.**

(Figs 2, 3)

Type material. Holotype: (NSMT-Pol H-827), off Miyagi, Japan, the northwestern Pacific, the cruise KS-16-18 (R/V *Shinsei-Maru*), St. ON6, 11 November 2016, 871–880 m deep, 38°23'57"–22°58'N, 142°20'05"E, collected using a 3 m beam trawl. Four paratypes: (NSMT-Pol P-828–P-831), from the same sample as the holotype.

Other material examined. Non-type specimen: off Otsuchi, Japan, the northwestern Pacific, the cruise KS-17-12 (R/V *Shinsei-Maru*), St. Bent4, 3 October 2017, 1659–1684 m deep, 39°17'30"–18°18'N, 142°48'25"–49°59'E, collected using a 3 m beam trawl, identifier GK627, 1 specimen.

Description. All type specimens complete. 18.9–25.8 mm (holotype 24.6 mm) in length and 5.0–6.4 (holotype 5.8 mm) in width at widest segment, whitish in alcohol, without pigmentation (Fig. 2A–C). Epidermal papillae present, indistinctive without Methyl green staining (Fig. 3). All specimens with 25 segments; first 20 segments chaetigerous, last five achaetous. Achaetous segments less developed (Fig. 2E) except first achaetous segment in the posterior region of paratype (NSMT-Pol P-829). Prostomium conical, eyes absent, a pair of nuchal organs present as dorsolateral slits. Peristomium short restricted to the mouth; mouth ventral with folds (Fig. 2D). Parapodia biramous, no parapodial lamellae on chaetiger 1. Parapodial lappets developed from chaetiger 15 or 16 to end of body. Subsequent parapodia very small. All chaetae capillary, approximately 10–20 per ramus, most chaetae shorter than branchiae. Notopodial and neuropodial chaetal rami well separated. Transverse ridges projected on last 10–11 segments, but somewhat indistinct in relaxed specimens. Middle body triannulate, an-

Fig. 4. Bayesian phylogeny of travisiid species based on 16S rRNA gene sequences (482 characters). Numbers above branches indicate posterior probability followed by percentage maximum likelihood bootstrap values greater than 50%. *Travisia sanrikuensis* sp. nov. is shown in bold type.

teriorly and posteriorly less annulated (biannulate or not annulated). Annulation in lateral body indistinctive. Branchiae cirriform from chaetiger 2 (19 pairs) except for paratype (NSMT-Pol P-829) with 20 branchiae, the last one short. Branchiae just posterior to notochaetae, long on middle chaetigers, short on first 3–4 chaetigers and most posterior chaetigers. Interramal pores, present on all chaetigers, posterior ones indistinct (Fig. 2F). Mid-ventral groove absent. Pygidium with shallow incisions, resulting in about 8 lobes around terminal anus (Fig. 2E). Methyl green staining pattern not characteristic in entire body, except white band (non-staining) on prostomium (Fig. 3)

Type locality. Off Miyagi, the Sanriku region, Japan, 871–880 m deep.

Etymology. The specific epithet *sanrikuensis* refers to the Sanriku region, encompassing the three prefectures Aomori, Iwate, and Miyagi, in the northeastern part of Japan, from where the specimens were collected, with the Latin suffix-*ensis*.

Remarks. *Travisia sanrikuensis* sp. nov. morphologically resembles *Travisia chiloensis* Kükenthal, 1887 and *Travisia oksae* Hartmann-Schröder and Parker, 1995, based on the taxonomic key presented by Rizzo and Salazar-Vallejo (2020). However, there are certain differences between *T. sanrikuensis* sp. nov. and these two species with respect to the number of segments, chaetigers, branchiae, and achaetous segments [note that achaetous segments in the posterior region of *Travisia* are regarded as annulations of the

pygidium by Maciolek and Blake (2006)] (Table 3): 25 segments in *T. sanrikuensis* sp. nov. versus 27 in *T. chiloensis*; 20 chaetigers in *T. sanrikuensis* sp. nov. but 24 in *T. chiloensis*; 5 posterior achaetous segments in *T. sanrikuensis* sp. nov. although 3 or less in *T. oksae*; and 19 or 20 pairs of branchiae in *T. sanrikuensis* sp. nov. compared with 22 pairs in *T. oksae*. In addition, these three species show notable differences in bathymetric and/or geographic distribution: *T. sanrikuensis* sp. nov. inhabits sea beds in Japanese waters at depths from 871 to 1684 m, whereas *T. chiloensis* is described from Chile [water depth unavailable from Kükenthal (1887)] and *T. oksae* is described from South Australia at depths of up to 18 m (Kükenthal 1887; Hartmann-Schröder and Parker 1995).

The results of phylogenetic analysis based on mitochondrial 16S rRNA gene sequences indicate that *T. sanrikuensis* sp. nov. is clustered with *Travisia brevis* (posterior probability=1.0, ML bootstrap value=100%) (Fig. 4) collected from the eastern Pacific (Friday Harbor, WA) (Paul et al. 2010). However, the genetic difference between these two species is quite large (3.4–3.7%, 11–12 bp of 321 bp) compared with the variation among three specimens of *T. sanrikuensis* sp. nov. (<0.6%, ≤2 bp in 321 bp). *T. sanrikuensis* sp. nov. and *T. brevis* also differ in the following morphological characters (Table 3): the number of segments (25 in *T. sanrikuensis* sp. nov. vs. 29 in *T. brevis*) and branchiae (19 or 20 in *T. sanrikuensis* sp. nov. vs. 22 in *T. brevis*); branchiae starting from chaetiger 2 in *T. sanrikuensis* sp. nov. but from chaetiger 3 in *T. brevis* (Moore 1923; Rizzo and Salazar-Vallejo 2020). The numbers of branchiae and chaetigers have been established as key diagnostic characters for distinguishing traviid species (Rizzo and Salazar-Vallejo 2020). Moreover, *T. sanrikuensis* sp. nov. and *T. brevis* differ with respect to bathymetric distribution, with *T. sanrikuensis* sp. nov. being collected from the lower bathyal zone (871–1684 m depths), whereas *T. brevis* was collected from the sublittoral zone (50–110 fathoms, i.e., 91–201 m depths in the eastern Pacific off San Diego, California) in the original description (Moore 1923). These results indicate that *T. sanrikuensis* sp. nov. differs sufficiently from *T. brevis* to be considered new to science.

The new species is distinguishable from all known traviid species recorded from Japan by following characters: the smaller number of segments (25 in *T. sanrikuensis* sp. nov.) than *T. brevis* (29) (Moore 1923), *T. japonica* (39–40) (Fujiwara 1933), and in *T. pupa* (31–34) (Moore 1906; Imajima 2009); the numbers of chaetigers (20 in *T. sanrikuensis* sp. nov.) instead *T. forbesii* (23–26) (Dauvin and Bellan 1994), *T. forbesii intermedia* (26–29) (Annenkova 1937), *T. fusus* (28) (Chamberlin 1919), *T. profundus* (26) (Dauvin and Bellan 1994; Rizzo and Salazar-Vallejo 2020), and *T. pupa* (29) (Imajima 2009); and the number of branchiae (19–20 in *T. sanrikuensis* sp. nov.) from *T. brevis* (22) (Moore 1923), *T. fusus* (0) (Chamberlin 1919), *T. glandulosa* (0) (Dauvin and Bellan 1994), *T. japonica* (26) (Fujiwara 1933), and *T. profundus* (12) (Chamberlin 1919).

Distribution. Japan, the northwestern Pacific, in 871–1684 m deep.

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