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Identification of *Lepidapedon oregonense* as the current world's deepest trematode

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Short Communication

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Abstract

The deepest recorded depth for trematodes currently stands at approximately 6200 m. This depth record was achieved solely through sequence datasets of *Lepidapedon* sp. obtained from a gastropod. Given that trematodes of this genus typically use fish as definitive hosts, the origin of the trematode sequence was thought to be larval stages. However, the specific species remained unclear owing to the absence of reported adult-stage sequences. In the present study, we definitively identified the deepest trematode as *Lepidapedon oregonense* by comparing 28S ribosomal DNA sequences from adult worms from the macrourid fish *Coelorinchus gilberti* with data from the gastropod in the previous study.

Introduction

The world's deepest recorded depth for trematodes is approximately 6200 m, as reported by Takano *et al.* (2023). They identified 28S ribosomal DNA (rDNA) of a trematode from the genus *Lepidapedon* in a DNA extract obtained from a deep-sea gastropod of the family Velutinidae, sampled via a trawl in depth from 6185 to 6221 m in the Kuril–Kamchatka Trench in the Northwest Pacific. As *Lepidapedon* species use fish as definitive hosts (eg, McCauley, 1968), the trematode found in the gastropod was presumed to be in the larval stage, although its development stage remains uncertain. Furthermore, the specific species of the trematode remained unknown because adult-stage sequences had not been reported. In the present study, we obtained an adult *Lepidapedon oregonense* from the macrourid fish *Coelorinchus gilberti* in Japan. Based on a comparison of 28S rDNA sequences, we determined that *L. oregonense* is the trematode species reported by Takano *et al.* (2023). Overall, the objective of the present study was to identify the species of the trematode recorded at the deepest depth.

Materials and methods

On February 18 and April 1, 2023, one individual *Coelorinchus gilberti* was sampled through angling at 1000 and 1000 – 1100 m depths, respectively, in Sagami Bay, North Pacific, Japan. The collected fishes were dissected immediately, and their internal organs were transported to the laboratory. These organs were examined under a stereomicroscope, and 4 trematodes were detected. Three of the 4 trematode individuals were damaged. The trematodes were mounted on slides, gently pressed between the slides and coverslips, and fixed in 70% ethanol for several days. Subsequently, a piece of body tissue was cut from each trematode for polymerase chain reaction (PCR), as described later. For morphological observation, the remaining tissues of the trematodes were stained with iron-hematoxylin, dehydrated with ethanol series, cleared with xylene, and mounted on slides with Canada balsam. The intact trematode specimen was identified as *L. oregonense*, as described later, although the remaining 3 species could not be identified morphologically because of the extent of damage. The trematodes were measured using a light microscope (BX53, Olympus) equipped with a digital camera unit (AdvanCam-U3II, Advan vision). All measurements are in micrometers.

Alkaline lysates of the trematode tissues were individually made, as previously reported by Waki *et al.* (2022), and used as templates for PCR. Genes for nuclear 28S rDNA, mitochondrial cytochrome *c* oxidase subunit 1 (COI), and mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 (nad1) were selected. Primers, PCR amplification, subsequent DNA sequencing, and alignment data preparation were performed as described by Pérez-del-Olmo *et al.* (2019) and Waki *et al.* (2022). For the 28S rDNA (794 bp), Bayesian inference analyses employing sequences of related species from GenBank were conducted using MrBayes v.3.2.7 (Ronquist et al., 2012), including Markov chain Monte Carlo searches on simultaneous runs for 800,000 generations, with trees sampled every 1000 generations under the Hasegawa–Kishono–Yano + G model (Hasegawa et al. 1985). In this analysis, *Gorgocephalus* spp. (MW353882 and MW353889), closely related to the family Lepidapedidae, was selected for the outgroup. Pairwise

comparison of divergence values was performed using MEGA X (Kumar *et al.* 2018) for both 28S rDNA and nad1 datasets to identify the species of our trematode, setting the *p*-distance. The determined DNA sequences were deposited in the DDBJ/ENA/GenBank databases under the accession numbers.

Results and discussion

Molecular analysis

We amplified 4 DNA fragments (1205 – 1257 bp) of 28S rDNA from 4 trematode individuals. Two sequences, including that from the trematode identified as *L. oregonense*, were identical. By comparing these sequences (796 bp) with those from previous studies, we found that the divergence values for intraspecific and interspecific variations within the family Lepidapedidae were 0 – 0.0013 and 0.0026 – 0.0771, respectively. Our 2 identical sequences matched with *Lepidapedon* sp. TT-2023 reported in Takano *et al.* (2023) (LC767449) with a divergence value of zero, indicating that they belonged to the same species: *L. oregonense.* Regarding nad1 analysis, intraspecific divergence values within the genus *Lepidapedon* were 0 – 0.0467. Both sequences from our *L. oregonense* adults were identical (divergence values of zero), confirming their identity as the same species. The remaining 2 broken adults were phylogenetically classified as members of the genus *Lepidapedon*, as detailed below; however, they could not be identified at the species level through molecular analysis because of the absence of closely matching sequences in the online database (divergence values between the unidentified adults and *Lepidapedon* spp. from the database: 0.0151 – 0.0465 [28S rDNA] and 0.1729 – 0.2664 [nad1]). The 28S rDNA tree, based on 794 bp, showed that our 4 trematode individuals belonged to the genus *Lepidapedon* because they formed a clade with other species of this genus supported by a high posterior probability value (0.99) (Fig. 1A). Therefore, the 2 unidentified trematodes were labeled "*Lepidapedon* sp. TK1" and "*Lepidapedon* sp. TK2." Determining the DNA sequences of 28S

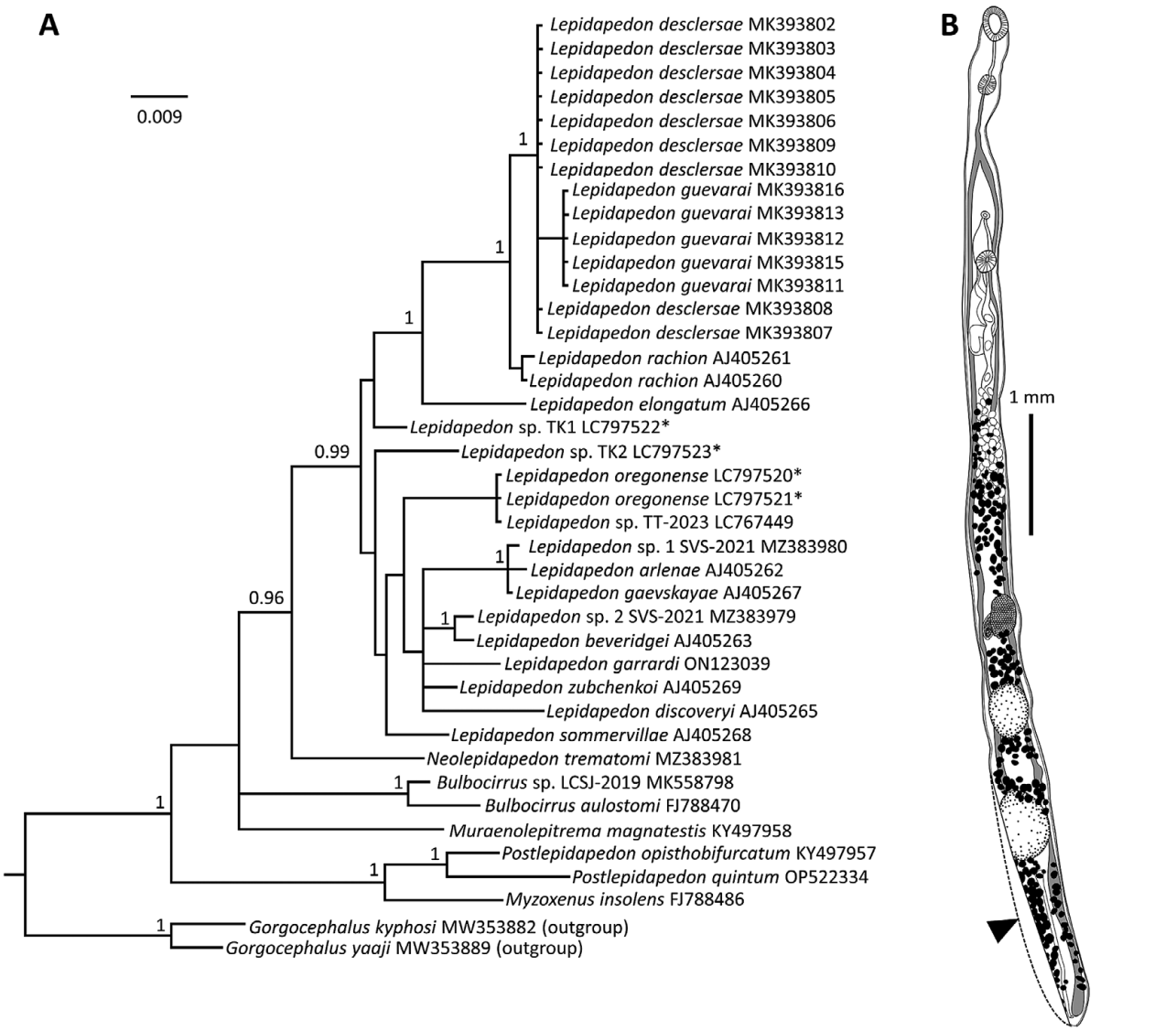

Figure 1. Bayesian inference phylogram based on trematodes inferred from partial nucleotide sequences of nuclear 28S ribosomal DNA (794 bp) (A) and *Lepidapedon oregonense* sampled in the present study (B). In A, posterior probability values lower than 0.95 are omitted. Database accession numbers are shown after the names of the sequences. Asterisks represent the sequences of the present study (LC797520-797523). The outgroups are *Gorgocephalus* spp. The scale bar indicates the number of substitutions per site. In B, the dotted line shows the outline of tissue excised for use in polymerase chain reaction (arrowhead).

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rDNA and nad1 for these 2 species will aid in identifying their species once adults that can be identified by morphology are obtained and their DNA sequences established.

Morphologic descriptions of *L. oregonense*

This study used a gravid adult with no damage for morphologic description. The specimen was observed ventrally. All measurements, unless indicated otherwise, are in micrometers.

Family Lepidapedidae Yamaguti, 1958

Genus *Lepidapedon* Stafford, 1904

Species *Lepidapedon oregonense* McCauley, 1968

Descriptions (Figure 1B, Table 1): Body narrow and long, subcylindrical. Body 7.1 mm in length and 340 in greatest width. Oral sucker situated anterior terminal of body, 223 in length and 159 in width. Prepharynx long, 280 in length. Pharynx large and clearly observed, 116 in length and 92 in width. Esophagus long, 299 in length. Caeca bifurcation middle between pharynx and ventral sucker. Ventral sucker at about an anterior quarter of body length, 153 in length and 130 in width. Distance between caeca bifurcation and ventral sucker 569. Testis oval and slightly elongated, width close to body width. Distance between two testes 436. Anterior testis 330 in length and 272 in width. Posterior testis 334 in length. External seminal vesicle 512 in length and 164 in width. Cirrus sac 280 in length and 138 in width. Genital opening situated posterior to caeca bifurcation. Ovary anterior two-thirds of body, slightly elongated oval with irregular outline, 250 in length and 114 in width. Distance between ovary and anterior testis 294. Anterior margin of vitelline not reaching ventral sucker. Eggs oval, 64 — 73 in length and 45 — 50 in width. Excretory vesicle reaching close to the posterior margin of the posterior testis.

Remarks: The genus *Lepidapedon* currently includes 47 species (WoRMS 2024). In our specimen, the vitellarium does not extend to the ventral sucker, and the excretory vesicle is close to the posterior margin of the posterior testis. These morphologic features characterize our specimen as a member of the "Desclersae subgroup," which includes 10 species, such as *L. blairi* (Bray *et al.*, 2013). In this subgroup, our specimen resembles *L. filiforme* and *L. oregonense* because of the narrow body. Among them, we identified our specimen as *L. oregonense* based on specific morphologic features, such as ovaries with a slightly irregular outline (vs spherical in *L. filiforme*), slightly elongated testes (vs oval almost circular), and small eggs (egg length 72 — 85 μm in *L. oregonense* and 95 — 108 μm in *L. filiforme*).

Hosts and trematode stages: *Coelorinchus gilberti* (adult, this study). *Coryphaenoides filifer* (adult, McCauley 1968), *Coryphaenoides leptolepis* (adult, McCauley, 1968). Velutinidae gen. sp. (larval stage, Takano *et al.*, 2023)

Sampling locations: North Pacific Ocean. Sagami Bay (1000 – 1100 m depth) (this study). 166 – 165 km off Oregon, USA (2800 – 2865 m depth) (McCauley, 1968). The Kuril–Kamchatka Trench (6185–6221 m depth) (Takano *et al.*, 2023).

Date of collection: April 1, 2023

DNA markers: 28S rDNA, LC797520 – 797521. COI, LC797524. Nad1, LC810999–LC811000.

Table 1. Morphological comparisons between the adult trematode in the present study and related *Lepidapedon* species

Checkpoints	Lepidapedon oregonense	Lepidapedon oregonense	Lepidapedon filiforme
Body (size)	7.1 x 0.34 mm	6.05 (3.80 – 7.10 mm) x 0.43 mm (0.32 – 0.43 mm)	6.5 (4.95 – 7.90 mm) x 0.50 mm (0.43 – 0.60 mm)
Oral sucker (size)	222 x 159	180 (150 – 200) x 170 (150 – 190)	210 (210 – 230) x 240 (230 – 270)
Prepharynx (size)	280	Longer than oral sucker	Approximately 3x of pharynx length
Pharynx (size)	116 x 92	100 (100 – 120) x 90 (60 – 90)	100 (100 – 130) x 100 (80 – 120)
Esophagus (size)	299	–	–
Ventral sucker (size)	153 x 130	150 (150 – 230) x 160 (150 – 240)	200 (170 – 230) x 200 (170 – 230)
Cirrus pouch (size)	280 x 138	–	–
Testis	Oval and slightly elongated	Oval and slightly elongated	Oval close to circular
Anterior testis (size)	330x272	400 (300 – 500) x 220 (220 – 300)	340 (310 – 450) x 320 (320 – 390)
Posterior testis (size)	334*	400 (350 – 420) x 270 (260 – 320)	400 (350 – 430) x 350 (300 – 400)
Ovary	Slightly elongated oval with irregular outline	More or less irregular in outline but not lobed	Spherical
Ovary (size)	250x114	270 (170 – 270) x 200 (200 – 310)	220 (220 – 300) in diameter
External seminal vesicle (size)	512x164	–	–
Eggs (size)	68 (64 – 73) x 48 (45 – 50)	72 – 85 x 40 – 55	95 – 108 x 40 – 60
Host	*Coelorinchus gilberti*	*Coryphaenoides filifer, Coryphaenoides leptolepis*	*Coryphaenoides filifer*
Locality	Sagami Bay, Japan, North Pacific (1000 – 1100 m depth)	166 – 165 km off Oregon, USA, North Pacific (2800 – 2865 m depth)	166 – 165 km off Oregon, USA, North Pacific (2800 – 2865 m depth)
Citation	The present study	McCauley (1968)	McCauley (1968)

All measurements, unless indicated otherwise, are in micrometers. Dashes indicate no description in the cited reference.
[a]The width could not be measured because part of the testis was lost when the tissue was cut out for polymerase chain reaction.

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Japanese name: Sokodara shinkai kyuchu (trematode of macrourid fish in deep sea)

Lepidapedon oregonense distributed across a wide range of depths and a broad area of the North Pacific Ocean (McCauley, 1968; Takano *et al*., 2023). Given the presence of *L. oregonense* in 2 genera of fish belonging to the family Macrouridae, this trematode species possibly infects various species of macrourid fish. The intermediate host gastropod Velutinidae gen. sp. has only been reported at depths of approximately 6200 m (Fukumori *et al*., 2019; Takano *et al*., 2023). Considering the extensive depth range of *L. oregonense*, it is plausible that other mollusc species inhabiting shallower waters may also serve as intermediate hosts of this trematode.

Morphologic descriptions of the remaining 2 species

Gravid adult of each species, referred to as *Lepidapedon* sp. TK1 and TK2 were used for morphologic descriptions.

Lepidapedon sp. TK1

Descriptions (Supplementary Fig. 1A) Body long, anterior and posterior sides of body damaged. Body 1.2 mm in maximum width. Pharynx slightly damaged, 218 in length and 202 in width. Esophagus long, 567 in length. Ventral sucker small, 216 in length and 193 in width. Testis oblong and its width one-third of body width. Anterior testis 642 in length and 415 in width. Posterior testis 687 in length and 411 in length. External seminal vesicle large. Cirrus sac 440 in length and 274 in width. Ovary damaged. Anterior margin of vitelline not reaching ventral sucker. Eggs oval, 57 – 62 in length and 24 – 28 in width. Excretory vesicle reaching close to the posterior margin of the posterior testis.

Remarks: This species differs from *L. oregonense* in body size (maximum width 1.2 mm vs 340μm in *L. oregonense*) and testes size (ca. half to one-third of body width vs close to body width in *L. oregonense*). This specimen's vitellarium does not reach the ventral sucker, and the excretory vesicle reaches the posterior margin of anterior testis; therefore, it is classified to "elongatum subgroup" in Bray (2013). *Lepidapedon* sp. TK1 shares similarities with *L. arlenae*, *L. mariannae,* and *L. sereti* given its elongated body and small testes. However, *Lepidapedon* sp. TK1 can be distinguished from *L. arlenae* by divergence values of 0.0289 for 28S rDNA and 0.1752 for nad1, which are all outside of the ranges of intraspecific values in this genus.

DNA markers: 28S rDNA, LC797522. Nad1, LC810997.

Lepidapedon sp. TK2

Descriptions (Supplementary Fig. 1B) Anterior and posterior sides of body damaged. Body 1.9 mm in maximum width. Testis round, width ca. half of body width. Anterior testis 850 in length and 773 in width. Posterior testis 785 in length and 855 in length. Ovary oval, 470 in length and 405 in width. Eggs oval, 61 – 69 in length and 32–38 in width. Excretory vesicle reaching close to the posterior margin of the posterior testis.

Remarks: This species differs from *L. oregonense* in body size (maximum width 1.9 mm vs 340 μm in *L. oregonense*) and testes shape and size (round and approximately half of the body width vs oval and slightly elongated with a width close to that of the body in *L. oregonense*). Due to substantial damage or loss of many organs in *Lepidapedon* sp. TK2, finding species with similar morphologic characters is exceedingly challenging.

DNA markers: 28S rDNA, LC797523. Nad1, LC810998.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X24000269.

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Competing interest. The author(s) declare none.

Ethical standard. No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated fish and invertebrate species.

References

Bray RA, Faliex E, Allienne JF and Mouahid G (2013) *Lepidapedon sereti* n. sp. (Digenea: Lepidapedidae) in *Coelorinchus sereti* (Gadiformes: Macrouridae) from deep waters off Vanuatu. *Parasitology Research* **112**, 3981–3989.

Fukumori H, Takano T, Hasegawa K and Kano Y (2019) Deepest known gastropod fauna: species composition and distribution in the Kuril–Kamchatka Trench. *Progress in Oceanography* **178**, 102176. https://doi.org/10.1016/j.pocean.2019.102176

Hasegawa M, Kishino H and Yano T (1985) Dating the human-ape split by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160–174. https://doi.org/10.1007/BF02101694

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

McCauley JE (1968) Six species of *Lepidapedon* Stafford, 1904 (Trematoda: Lepocreadiidae) from deep-sea fishes. *Journal of Parasitology* **54**, 496–505.

Pérez-del-Olmo A, Dallarés S, Georgieva S, Constenla M, Kostadinova A and Carrassón M (2019) Species of *Lepidapedon* Stafford, 1904 (Digenea: Lepidapedidae) from deep-sea fishes in the Western Mediterranean: molecular and morphological evidence. *Systematic Parasitology* **96**(**2**), 149–169. https://doi.org/10.1007/s11230-019-09845-z

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, … Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542. https://doi.org/10.1093/sysbio/sys029

Takano T, Fukumori H, Kuramochi T and Kano Y (2023) Deepest digenean parasite: Molecular evidence of infection in a lower abyssal gastropod at 6,200 m. *Deep Sea Research Part I: Oceanographic Research Papers* **198**, 104078. https://doi.org/10.1016/j.dsr.2023.104078

Waki T, Nakao M, Sasaki M, Ikezawa H, Inoue K, Ohari Y, … Miyazaki S (2022) *Brachylaima phaedusae* n. sp. (Trematoda: Brachylaimidae) from door snails in Japan. *Parasitology International* **86**, 102469. https://doi.org/10.1016/j.parint.2021.102469

WoRMS (2024) Lepidapedon *Stafford,* 1904. Available at https://www.marinespecies.org/aphia.php?p=taxdetails&id=108573 (accessed January 27, 2024).