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A new species of free-living marine nematode, *Fotolaimus cavus* sp. nov. (Nematoda, Oncholaimida, Oncholaimidae), isolated from a submarine anchialine cave in the Ryukyu Islands, southwestern Japan

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Abstract

Fotolaimus cavus sp. nov. was described from a submarine anchialine cave called Akuma-no-yakata on the Shimoji Island, Miyako Island Group, Ryukyu Islands, southwestern Japan. This is the first free-living marine nematode isolated from a submarine cave in Japan, and the third species of the genus *Fotolaimus*. This new species differs from its congeners by its small body size, wide amphids, long buccal cavity, long conico-cylindrical tail, and proximally curved gubernaculum. We provide amended dichotomous keys to genera in the subfamily Oncholaiminae and species in *Fotolaimus*. We also analyzed partial DNA sequences encoding ribosomal small subunit RNA and cytochrome *c* oxidase subunit I from *Fotolaimus cavus* sp. nov. and six other species of Oncholaimidae collected from Japanese waters. The phylogenetic tree based on the ribosomal small subunit RNA sequences using maximum likelihood analysis suggested a close relationship between *Fotolaimus* and *Wiesoncholaimus* as well as *Oncholaimus*. The topology of the tree was similar to those from previous studies; however, it suggested a new phylogenetic position of *Adoncholaimus* as a sister clade for *Viscosia* and *Oncholaimus*.

Key Words

cave scuba diving, Enoplea, meiofauna, Miyako Island Group, molecular phylogeny, Oncholaiminae

Introduction

Free-living nematodes are the most abundant taxon in the marine environment and the dominant organisms in the meiobenthos of submarine caves, including anchialine caves (D'Addabbo et al. 2008; Arunimaand and Mohan 2021). However, surveys of meiofauna, including nematodes, in submarine caves have only been conducted in the Canary Islands (García-Valdecasas Huelín 1985; Riera et al. 2018), Hong Kong (Zhou and Zhang 2003, 2008), Italy (Wieser 1954; Ape et al. 2015; Onorato and Belmonte 2017), and Cuba (Pérez-García et al. 2018). Thus, our taxonomic and ecological knowledge of nematodes from submarine caves is insufficient.

In the present study, we describe a new species of the free-living marine nematode genus *Fotolaimus* Belogurova & Belogurov, 1974 collected from an anchialine cave called Akuma-no-yakata (= Devil's Palace) and located on the Shimoji Island (or Shimojijima Island), Miyako Island Group, Ryukyu Islands, southwestern Japan. Osawa and Fujita (2019) have provided a detailed description of this cave, and faunal surveys conducted in Akuma-no-yakata in recent years identified the following organisms in the cave: crustaceans (Fujita et al. 2013, 2017; Anker and Fujita 2014; Osawa and Fujita 2016, 2019; Kakui and Fujita 2018, 2020; Saito and Fujita 2022), ophiuroids (Okanishi and Fujita 2018, 2019), annelids (Worsaae et al. 2021), molluscs (Mizuyama et

al. 2022), sponges (Ise et al. 2023), and pycnogonids (Kakui and Fujita 2023).

Belogurova and Belogurov (1974) established the genus *Fotolaimus* in Oncholaimidae Filipjev, 1916. Currently, this genus contains two species, i.e., *F. marinus* Belogurova & Belogurov, 1974 (type species) and *F. apostematus* (Wieser, 1959) Belogurova & Belogurov, 1974. *Fotolaimus* belongs to the subfamily Oncholaiminae Filipjev, 1916, based on its left ventrosublateral tooth being larger than the other two teeth and the presence of a prodelphic ovary. Members of *Fotolaimus* resemble those of *Metoncholaimus* Filipjev, 1918 as males have a gubernaculum and females have a Demanian system, but differ from the *Metoncholaimus* species by the presence of ten or more terminal ducts and pores (whereas *Metoncholaimus* members have two ducts and two pores) (Belogurova and Belogurov 1974; Belogurov and Belogurova 1988; Smol et al. 2014). However, there are currently no molecular data supporting the phylogenetic position of *Fotolaimus*.

In a recent classification system by Hodda (2022), the family Oncholaimidae belongs to the order Oncholaimida Siddiqi, 1983 = suborder Oncholaimina De Coninck, 1965 = superfamily Oncholaimoidea Filipjev, 1916 with two other families, i.e., Enchelidiidae Filipjev, 1918 and Thalassogeneridae Orton Williams & Jairajpuri, 1984. Thalassogeneridae is a terrestrial family that includes only the type genus *Thalassogenus* Andr ssy, 1973, and several authors do not include Thalassogeneridae in Oncholaimoidea based on morphological data (Jensen 1976; Lorenzen 1981). Since molecular data do not allow any conclusion, only Oncholaimidae and Enchelidiidae are considered members of Oncholaimoidea sensu stricto. Several molecular phylogenetic analyses strongly support the monophyly of the clade composed of Oncholaimoidea sensu stricto (Meldal et al. 2007; van Megen et al. 2009; Bik et al. 2010a, b; Pereira et al. 2010; Smythe 2015; Smythe et al. 2019; Ahmed et al. 2022). However, analyses of ribosomal small subunit RNA sequences (Meldal et al. 2007; Bik et al. 2010a, b; Smythe 2015) and whole-genome/transcriptome (Smythe et al. 2019; Ahmed et al. 2022) do not support monophyly of Oncholaimidae. The monophyly of the seven subfamilies comprising Oncholaimidae (for three of which there are no molecular data) is also not supported by molecular analyses (Bik et al. 2010a, b; Smythe 2015), and phylogenetic relationships within Oncholaimoidea that have been supported by morphological analyses (e.g., Smol et al. 2014; Hodda 2022) are considered to require significant revision.

Materials and methods

Sampling and morphological observation

Sediment samples were collected by scuba diving from a completely dark, anchialine zone at 7–20 m depth (“second slope zone,” Osawa and Fujita 2019) in the

submarine cave Akuma-no-yakata, Shimoji Island, Miyako Island Group, Ryukyu Islands, southwestern Japan (24°49'22.5"N, 125°08'07.8"E) on 26 Oct. 2018. Thirteen nematode individuals were isolated from the sediments and preserved in DESS solution (Yoder et al. 2006). We considered that these individuals belonged to the same species in the family Oncholaimidae. Three males and four females were permanently mounted in anhydrous glycerin (Shimada et al. 2021), observed using a BX51 light microscope (Olympus, Japan) with differential interference contrast, and photographed with an PCM500 digital camera (AS ONE, Japan). One male and one female were dried using the hexamethyldisilazane method (Nation 1983), sputter-coated with gold to a thickness of 20 nm, and observed using an S-3000N scanning electron microscope (SEM; Hitachi, Japan). We edited digital photographs using GIMP ver. 2.10 (<https://www.gimp.org>) and generated measurements and drawings from digital photographs using Inkscape ver. 1.0 (<https://inkscape.org>). We deposited all specimens examined in the Invertebrate Collection of Hokkaido University Museum (ICHUM). The terminology used to describe the arrangement of morphological features such as setae follows that of Decraemer et al. (2014). The following de Man’s ratios (Hooper 1986) were used: a, ratio of body length to maximum body diameter; b, ratio of body length to pharyngeal length; c, ratio of body length to tail length; c’, ratio of tail length to body diameter at cloacal opening or anus; and V, position of vulva from anterior body end expressed as percentage of body length.

Molecular experiments

Two males and two females isolated from the Akuma-no-yakata cave were used. Additionally, we included 18 individuals of six oncholaimid species from Japanese waters, i.e., *Oncholaimus secundicollis* Shimada, Kajihara, & Mawatari, 2009, *Oncholaimus* cf. *oxyuris* Ditlevsen, 1911, *Oncholaimus* cf. *vesicarius* (Wieser, 1959), *Wiesoncholaimus jambio* Shimada & Kakui, 2021, *Adoncholaimus daikokuensis* Shimada & Kajihara, 2014, and *Adoncholaimus pseudofervidus* Shimada & Kajihara, 2014, for phylogenetic analysis (Table 1). Total DNA was extracted using an ISOHAIR extraction kit (NIPPON GENE, Japan) following the protocols described by Tanaka et al. (2012) and Iwahori (2014) with minor modifications. Each nematode was placed in 20 μ L dissolving solution containing 18.5 μ L 1% extraction buffer in TE buffer pH 8.0, 0.5 μ L lysis solution, and 1.0 μ L enzyme solution and incubated at 55 $^{\circ}$ C for 60 min (extraction buffer, lysis solution, and enzyme solution are included in the kit). Nearly full-length sequences encoding ribosomal small subunit RNA (18S) and mitochondrial cytochrome *c* oxidase subunit I (COI) were amplified by PCR using KOD One PCR Master Mix (TOYOBO, Japan). PCR primers for 18S amplification were as follows: forward EukA (AACCTGGTTGATCCTGCCAGT) (D ez et al. 2001)

Table 1. List of nematodes sequenced from Japanese waters with INSD accession numbers. *Type locality.

Species	N	Date	Locality	Accession numbers	
				18S	COI
<i>Fotolaimus cavus</i> sp. nov.	4	26 Oct. 2018	Akuma-no-yakata	LC746839–LC746842	LC746861–LC746864
<i>Wiesoncholaimus jambio</i>	4	25 Jun. 2015	*off Misaki, Sagami Bay	LC746843–LC746846	LC759639–LC759641
<i>Oncholaimus secundicollis</i>	2	13 Jul. 2014	*Akkeshi, Hokkaido	LC746847, LC746848	–
<i>Oncholaimus</i> cf. <i>oxyuris</i>	5	23 Jun. 2013	Hamanaka, Hokkaido	LC746849, LC746850	LC746865–LC746869
<i>Oncholaimus</i> cf. <i>vesicarius</i>	18	17 Mar. 2015	Akkeshi, Hokkaido	LC746851, LC746852	LC746870–LC746887
<i>Adoncholaimus daikokuensis</i>	4	19 Jun. 2012	*Daikoku Island, Hokkaido	LC746853–LC746856	LC746888–LC746891
<i>Adoncholaimus pseudofervidus</i>	4	20 May 2012	*Mukawa, Hokkaido	LC746857–LC746860	LC746892–LC746895

and reverse 26R (CATTCTTGCCAAATGCTTTCG), forward 9FX (AAGTCTGGTGCCAGCAGCCGC) and reverse 13R (GGGCATCACAGACCTGTTA), and forward 2FX (GGAAGGGCACCACCAGGAGTGG) and reverse 18P (TGATCCWKCYGCAGGTTTAC) (De Ley et al. 2002). PCR primers for COI amplification were forward F1 (CCTACTATGATTGGTGGTTTTGGTA-ATTG) and reverse R2 (GTAGCAGCAGTAAAATA-AGCACG) (Kanzaki and Futai 2002) and forward JB3 (TTTTTTGGGCATCCTGAGGTTTAT) and reverse JB5 (AGCACCTAAACTTAAAACATAATGAAAATG) (Derycke et al. 2005). The thermal cycling program consisted of 35 cycles at 98 °C for 10 s, 50 °C for 5 s, and 68 °C for 10 s. We determined the nucleotide sequences by direct sequencing using a BigDye Terminator Kit ver.3.1 (Applied Biosystems, USA) with a 3730 Genetic Analyzer (Applied Biosystems). Fragments were joined into a single sequence using MEGA X (Kumar et al. 2018). We deposited the obtained sequences (Table 1) in the International Nucleotide Sequence Database (INSD) through the DNA Data Bank of Japan.

Phylogenetic analysis

The 18S dataset (1442 bp long after alignment) used for phylogenetic analysis included 21 oncholaimid sequences obtained in the present study, and 38 oncholaimid sequences, 12 enchelidiid sequences, and three outgroup sequences (*Enoplus* Dujardin, 1845 and *Enoploides* Ssaweljev, 1912) obtained from the INSD (Table 2). All sequences were aligned according to the secondary structure predicted using RNAfold WebServer (Gruber et al. 2008; Lorenz et al. 2011). Sequences were trimmed in MEGA X and alignment-ambiguous sites were then removed using Gblocks ver. 0.91b (Castresana 2000) in NGPhylogeny.fr (Lemoine et al. 2019) and the “relaxed” parameters described in Talavera and Castresana (2007). The optimal substitution model was GTR+F+R3, determined under the corrected Akaike information criterion option in ModelFinder (Kalyaanamoorthy et al. 2017). A maximum likelihood (ML) analysis was constructed based on the 18S dataset using IQ-TREE ver. 2.1.2 (Minh et al. 2020) under the ‘bnni’ option (Hoang et al. 2018). Clade support was estimated using 1,000 replicates for both SH-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and ultrafast bootstraps

(UFBoot; Hoang et al. 2018) as a function of IQ-TREE. The ML tree was drawn using FigTree ver. 1.4.4 (<http://tree.bio.ed.ac.uk>) and processed using Illustrator CS6 (Adobe, USA).

Results

Taxonomy

Subfamily Oncholaiminae Filipjev, 1916

Type genus. *Oncholaimus* Dujardin, 1945.

Diagnosis (modified from Belogurova and Belogurov 1974; Smol et al. 2014). Oncholaimidae. Cuticle smooth. Buccal cavity barrel-shaped, with three teeth. Left ventrosublateral tooth larger than other teeth (usual) or left and right ventrosublateral teeth of same size. Spicules short or long, gubernaculum present or absent. Copulatory bursa absent. Female reproductive system monodelphic-prodelphic with an antidromously reflexed ovary. Demanian system present or absent.

Remarks. Cobb (1930) established the new genus *Oncholaimium* Cobb, 1930, which differs from *Oncholaimus* mainly by the presence of a distinct precloacal appendiculate (papilla) in males. Subsequently, Kreis (1932) established *Pseudoncholaimus* Kreis, 1932 based on the lack of Demanian system. Kreis (1934) also distinguished *Oncholaimium* from *Oncholaimus* based on the Demanian system, which is without terminal pore in *Oncholaimium* and with terminal pores in *Oncholaimus*. However, Rachor (1969) synonymized *Oncholaimium* and *Pseudoncholaimus* to *Oncholaimus*, because the presence of a Demanian system is unknown for many species of *Oncholaimus*. Belogurov and Belogurova (1988), who proposed the currently accepted taxonomic system of Oncholaimidae, treated *Oncholaimium* and *Pseudoncholaimus* as valid genera. *Pseudoncholaimus* is still considered valid by several researchers (e.g., Smol et al. 2014; Tsalolikhin 2015; Milovankina and Fadeeva 2019). However, because it is unclear for numerous species whether they rather belong to *Oncholaimus*, *Oncholaimium*, or *Pseudoncholaimus*, we included the latter two in *Oncholaimus* sensu lato as described by Rachor (1969). Thus, the subfamily Oncholaiminae comprises six genera, i.e., *Oncholaimus*, *Metoncholaimus*, *Prooncholaimus* Micoletzky, 1924, *Metaparoncholaimus* De Coninck &

Table 2. List of nematode sequences from the INSD including the phylogenetic analysis. Abbreviations: A = Atlantic Ocean side; FS = French Southern and Antarctic Lands; nd = no data; P = Pacific Ocean side. *Outgroup.

Family	Subfamily	Species		Accession number	Reference	
Oncholaimidae	Oncholaiminae	<i>Oncholaimus</i> sp. AUK23	UK	HM564402	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. AUK35	UK	HM564474	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. AUK36	UK	HM564475	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. BUS1	USA (A)	HM564404	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. BUS4	USA (A)	HM564409	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. BUS5	USA (A)	HM564410	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. BUS7	USA (A)	HM564411	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NAR4	USA (A)	HM564429	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NAR7	USA (A)	HM564432	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NAR16	USA (A)	HM564426	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NUS2	USA (A)	HM564438	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NUS5	USA (A)	HM564444	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NUS6	USA (A)	HM564445	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. NUS7	USA (A)	HM564446	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. OUS2	USA (A)	HM564450	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. SBA2	South Africa	HM564592	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. SBA3	South Africa	HM564593	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. SBA5	South Africa	HM564594	Bik et al. (2010b)	
		<i>Oncholaimus</i> sp. AS479	Japan	KR265044	Smythe (2015)	
		<i>Oncholaimus</i> sp. DS-2015	Japan	LC093124	Shimada (unpubl.)	
	<i>Pseudoncholaimus</i> sp. AS89	USA (A)	KR265048	Smythe (2015)		
		Adoncholaiminae	<i>Adoncholaimus</i> sp.	nd	AF036642	Mullin et al. (2005)
		Oncholaimellinae	<i>Viscosia</i> sp. AUK10	UK	HM564399	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL9	UK	HM564570	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL10	UK	HM564557	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL11	UK	HM564558	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL15	UK	HM564560	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL24	UK	HM564565	Bik et al. (2010b)
			<i>Viscosia</i> sp. HCL27	UK	HM564566	Bik et al. (2010b)
			<i>Viscosia</i> sp. LUK1	UK	HM564417	Bik et al. (2010b)
			<i>Viscosia</i> sp. LUK3	UK	HM564419	Bik et al. (2010b)
			<i>Viscosia</i> sp. SBN2	UK	HM564595	Bik et al. (2010b)
			<i>Viscosia</i> sp. SBN4	UK	HM564597	Bik et al. (2010b)
	<i>Viscosia dossena</i> Leduc & Zhao, 2023		New Zealand	OK317193	Leduc and Zhao (2023)	
	<i>Oncholaimellinae</i> sp. AS71		USA (A)	KR265043	Smythe (2015)	
	Pontonematinae	<i>Pontonema</i> sp. Nem.209	USA (P)	MN250102	Pereira et al. (2020)	
		<i>Pontonema</i> sp. Nem.213	USA (P)	MN250105	Pereira et al. (2020)	
Enchelidiidae		<i>Bathyeurystomina</i> sp. Cr78a	FS	HM564537	Bik et al. (2010b)	
		<i>Bathyeurystomina</i> sp. Cr80b	FS	HM564539	Bik et al. (2010b)	
		<i>Bathyeurystomina</i> sp. TCR81	USA (P)	HM564646	Bik et al. (2010b)	
		<i>Bathyeurystomina</i> sp. TCR109	USA (P)	HM564602	Bik et al. (2010b)	
		<i>Calyptonema</i> sp. 1068	the Netherlands	FJ040503	van Megen et al. (2009)	
		<i>Calyptonema</i> sp. AUK13	UK	HM564400	Bik et al. (2010b)	
		<i>Calyptonema</i> sp. LUK7	UK	HM564421	Bik et al. (2010b)	
		<i>Calyptonema</i> sp. LUK12	UK	HM564418	Bik et al. (2010b)	
		<i>Eurystomina</i> sp. AS485	Japan	KR265038	Smythe (2015)	
		<i>Pareurystomina</i> sp. BCA3	Antarctica	HM564491	Bik et al. (2010b)	
		<i>Pareurystomina</i> sp. NUS1	USA (A)	HM564435	Bik et al. (2010b)	
		<i>Symplocostoma</i> sp. AS520	Panama (A)	KR265050	Smythe (2015)	
	Enoplidae		* <i>Enoplus brevis</i> Bastian, 1865	nd	U88336	Aleshin et al. (1998)
			* <i>Enoplus meridionalis</i> Steiner, 1921	Croatia	Y16914	Kampfer et al. (1998)
	Thoracostomopsidae		* <i>Enoplodes</i> sp. 1252	the Netherlands	FJ040490	van Megen et al. (2009)

Schuermans Stekhoven, 1933, *Wiesoncholaimus* Inglis, 1966, and *Fotolaimus*. Members of the Oncholaiminae can be distinguished from these of the other six subfamilies as follows. They are distinct from Krampiinae De

Coninck, 1965, Adoncholaiminae Gerlach & Riemann, 1974, and Pontonematinae Gerlach & Riemann, 1974 as they have only one ovary (whereas the three other genera have two); from Pelagonematinae De Coninck, 1965

and Octonchinae De Coninck, 1965 as they have three distinct teeth (whereas Pelagonematinae member have minute or no tooth and Octonchinae have eight or more); and from Oncholaimellinae De Coninck, 1965 because their left ventrosublateral tooth is larger than the other two teeth (right ventrosublateral tooth is larger in Oncholaimellinae). Recent revisional works were provided by Mawson (1958) for *Metaparoncholaimus*, Yoshimura (1982) for *Metoncholaimus*, and Chen et al. (2015) for *Prooncholaimus*.

Genus *Fotolaimus* Belogurova & Belogurov, 1974

Type species. *Fotolaimus marinus* Belogurova & Belogurov, 1974.

Diagnosis (modified from Belogurova and Belogurov 1974). Oncholaiminae. Left ventrosublateral tooth larger than the two other teeth. Spicules shorter than 2.0 cloacal body diameters. Gubernaculum present. Demanian system present, posterior end of main duct forming two symmetrical sacs each with five or more terminal ducts.

Remarks. The genus *Fotolaimus* can be distinguished from *Metaparoncholaimus* and *Wiesoncholaimus* by the left ventrosublateral tooth being larger than the right ventrosublateral tooth (left and right ventrosublateral teeth are equal in size in *Metaparoncholaimus* and

Wiesoncholaimus). It also differs from *Prooncholaimus* by the absence of the large bubble-like cells in pseudocoelom (presence in *Prooncholaimus*). *Fotolaimus*, *Oncholaimus*, and *Metoncholaimus* are similar to each other and are distinguished by the morphological characters of the terminal ducts of the Demanian system. Belogurova and Belogurov (1974) characterized the Demanian system of *Fotolaimus* as follows: the posterior end of the main duct forms two symmetrical sacs, each of which is pierced by five terminal ducts ending in terminal pores. On the other hand, the main ducts of *Oncholaimus* and *Metoncholaimus* do not form a sac at the posterior end, but branch separately into two or more terminal ducts (Belogurov and Belogurova 1988). Belogurov and Belogurova (1977) distinguished *Metoncholaimus* from *Oncholaimus* in that the terminal ducts are covered with moniliform glands. However, some *Metoncholaimus* species do not mention the shape of the terminal duct in their description (e.g., Mawson 1958; Salma et al. 2017). For convenience, a species may be considered belonging to *Oncholaimus* if it has short (equal to cloacal body diameter) spicules and without gubernaculum, and belonging to *Metoncholaimus* if it has longer spicules (with or without gubernaculum) or short spicules with gubernaculum (cf. Platt and Warwick 1983). The distinction between *Oncholaimus* and *Metoncholaimus* will need to be reexamined in the future.

Key to genera in Oncholaiminae (cf. Belogurov and Belogurova 1988; Smol et al. 2014).

- 1 Right and left ventrosublateral teeth of same size..... 2
- Left ventrosublateral tooth larger than the other teeth..... 3
- 2 Spicule length almost equal to cloacal body diameter..... *Metaparoncholaimus*
- Spicules longer than 3.0 cloacal body diameters *Wiesoncholaimus*
- 3 Large bubble-like cells present in pseudocoelom..... *Prooncholaimus*
- Large bubble-like cells absent..... 4
- 4 Demanian system including two posterior sacs..... *Fotolaimus*
- Demanian system absent, or present but without posterior sacs 5
- 5 Terminal ducts covered with moniliform glands..... *Metoncholaimus*
- Terminal ducts not covered with moniliform glands..... *Oncholaimus*

***Fotolaimus cavus* sp. nov.**

<https://zoobank.org/A0509360-77DD-4387-9FAD-92B14758952F>

Figs 1–4, Table 3

Material examined. Holotype. JAPAN • male (permanent whole mount in glycerin); Ryukyu Islands, Miyako Island Group, Shimoji Island, a submarine cave called Akuma-no-yakata; 24°49'22.5"N, 125°08'07.8"E; 26 Oct. 2018; anchialine zone, depth 7 m, collected by Yoshihisa Fujita; ICHUM 8474.

Paratypes. JAPAN • two males (permanent whole mounts in glycerin); same collection data as for holotype; 26 Oct. 2018; anchialine zone, depth 20 m, collected by Yoshihisa Fujita; ICHUM 8475 and 8476 • four females (permanent whole mounts in glycerin); same collection data as for preceding; ICHUM 8477–8480.

Other material. JAPAN • one male (SEM specimen); same collection data as for paratypes; • one female (SEM specimen); same collection data as for preceding.

Etymology. The specific name *cavus* (cave) is a Latin noun in apposition derived from the type locality.

Description. Body (Fig. 1A, B) elongated, almost cylindrical but gradually tapering toward both extremities. Cuticle smooth throughout body besides oblique striations (Fig. 2A; cf. Leduc 2013) crossing at angle of ca. 120° between amphids and anteriormost cervical setae visualized using SEM. Somatic sensilla arranged in eight longitudinal rows: setiform in anterior half of cervical (Figs 1C, 2B) and caudal (Fig. 2C) regions; papilliform or very short setiform in rest of body (Fig. 2D), difficult to observe without SEM. Cephalic region (Figs 1D–F, 2E–G, 3A–D) rounded at anterior end, with six lips, slightly

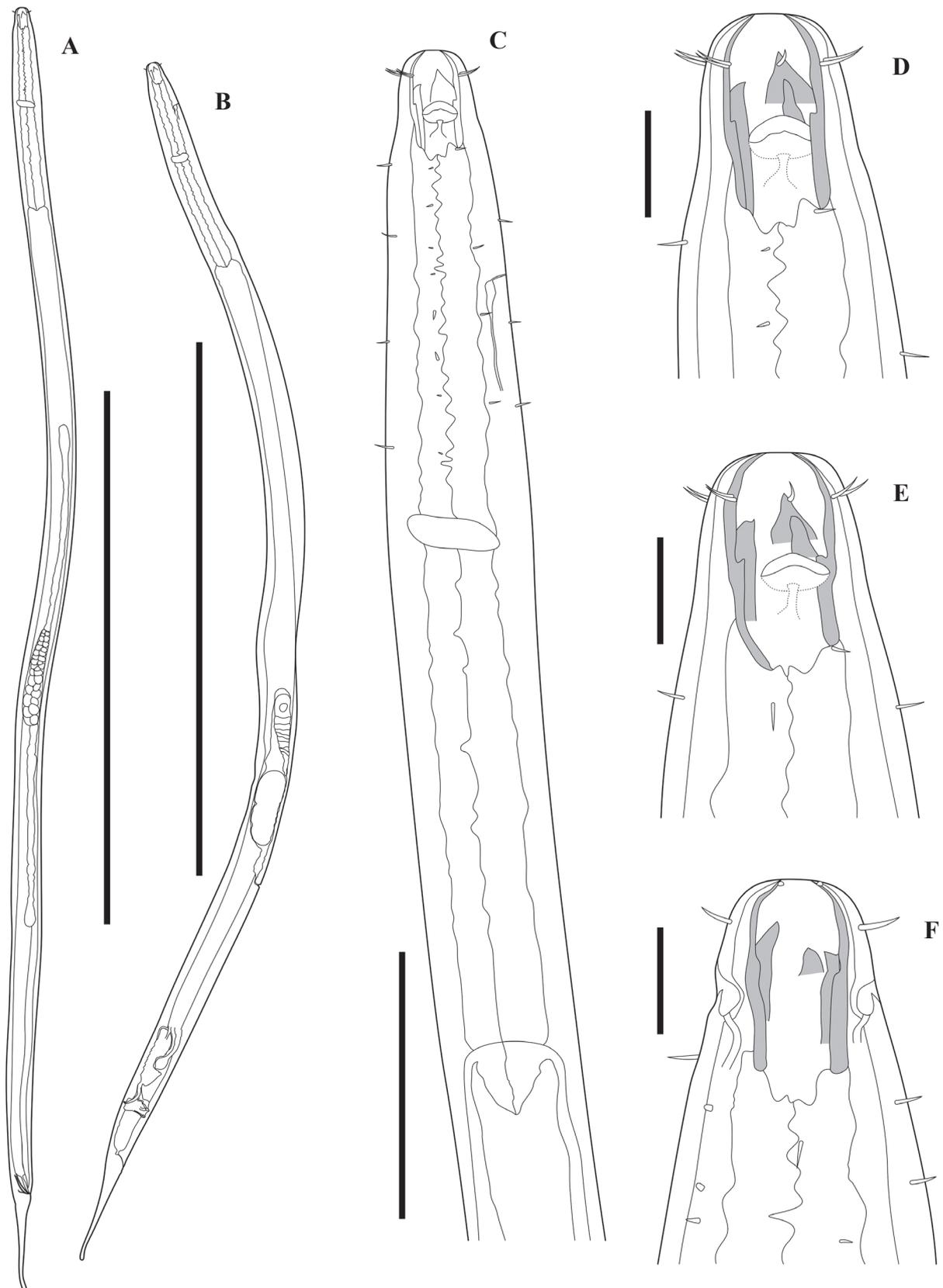


Figure 1. Line drawings of *Fotolaimus cavus* sp. nov. **A, C, D.** Holotype (ICHUM 8474); **B, E.** Paratype (ICHUM 8477); **F.** Paratype (ICHUM 8479). **A.** Male body, right lateral view; **B.** Female body, right lateral view; **C.** Male cervical region, right lateral view; **D.** Male cephalic region, right lateral view; **E.** Female cephalic region, right lateral view; **F.** Female cephalic region, dorsal view. Scale bars: 1 mm (**A, B**); 100 μ m (**C**); 20 μ m (**D–F**).

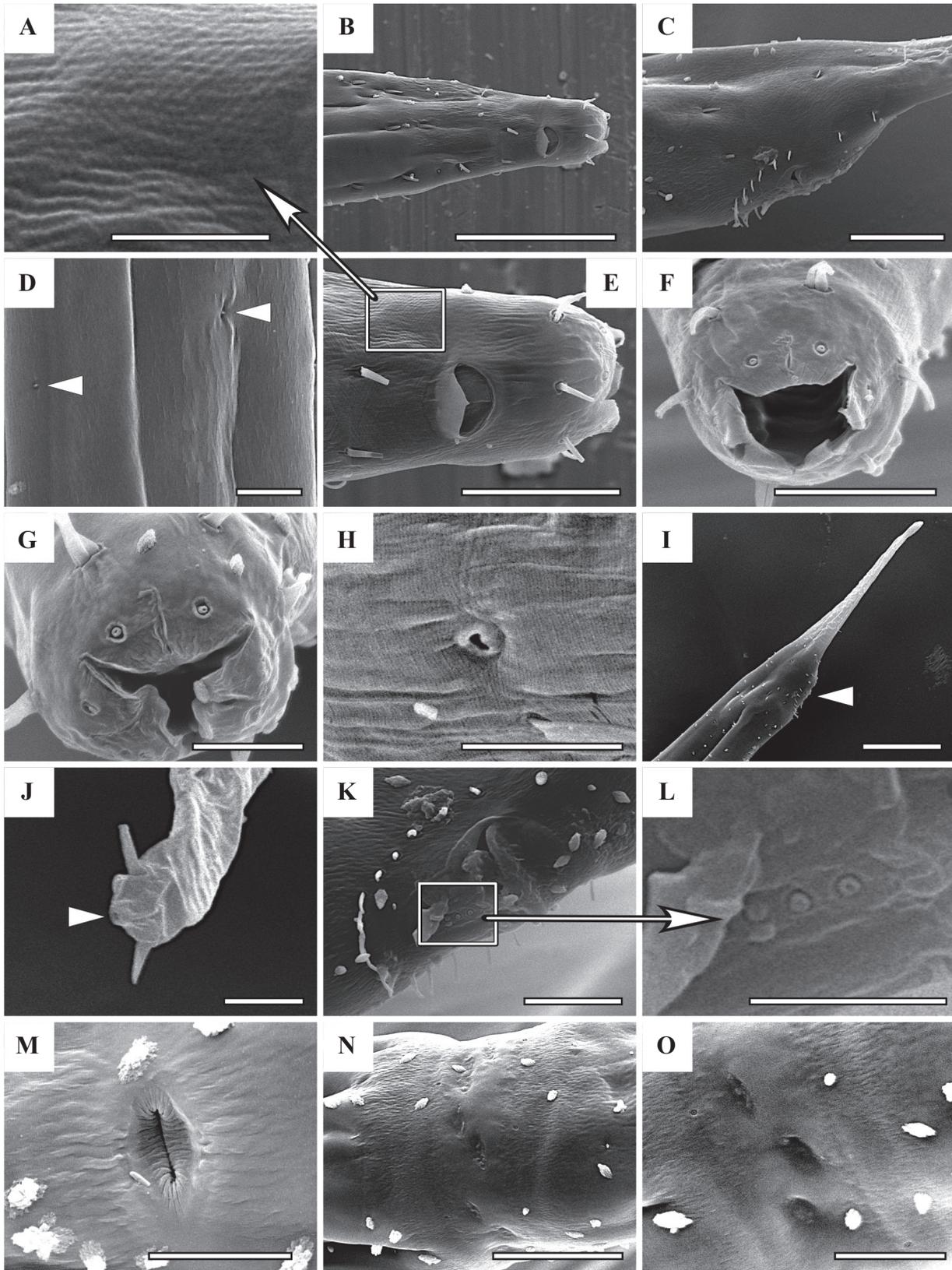


Figure 2. SEM photographs of *Fotolaimus cavus* sp. nov. **A, B, D–F, M–O.** Non-type female; **C, G–L.** Non-type male. **A.** Oblique striations on cuticle; **B.** Anterior region; **C.** Male cloacal region; **D.** Papilliform somatic sensilla (arrowheads); **E.** Female cephalic region, lateral view; **F.** Female cephalic region, anterior view; **G.** Male cephalic region, anterior view; **H.** SE-pore; **I.** Male posterior region with cloacal opening (arrowhead); **J.** Tail tip with spinneret (arrowhead); **K.** Cloacal region, subventral view; **L.** Ventral papillae; **M.** Vulva; **N.** Circle of terminal pores; **O.** Terminal pores. Scale bars: 5 μm (**A, H, J, L**); 50 μm (**B, D**); 20 μm (**C, E, N**); 10 μm (**D, F, G, K, M, O**).

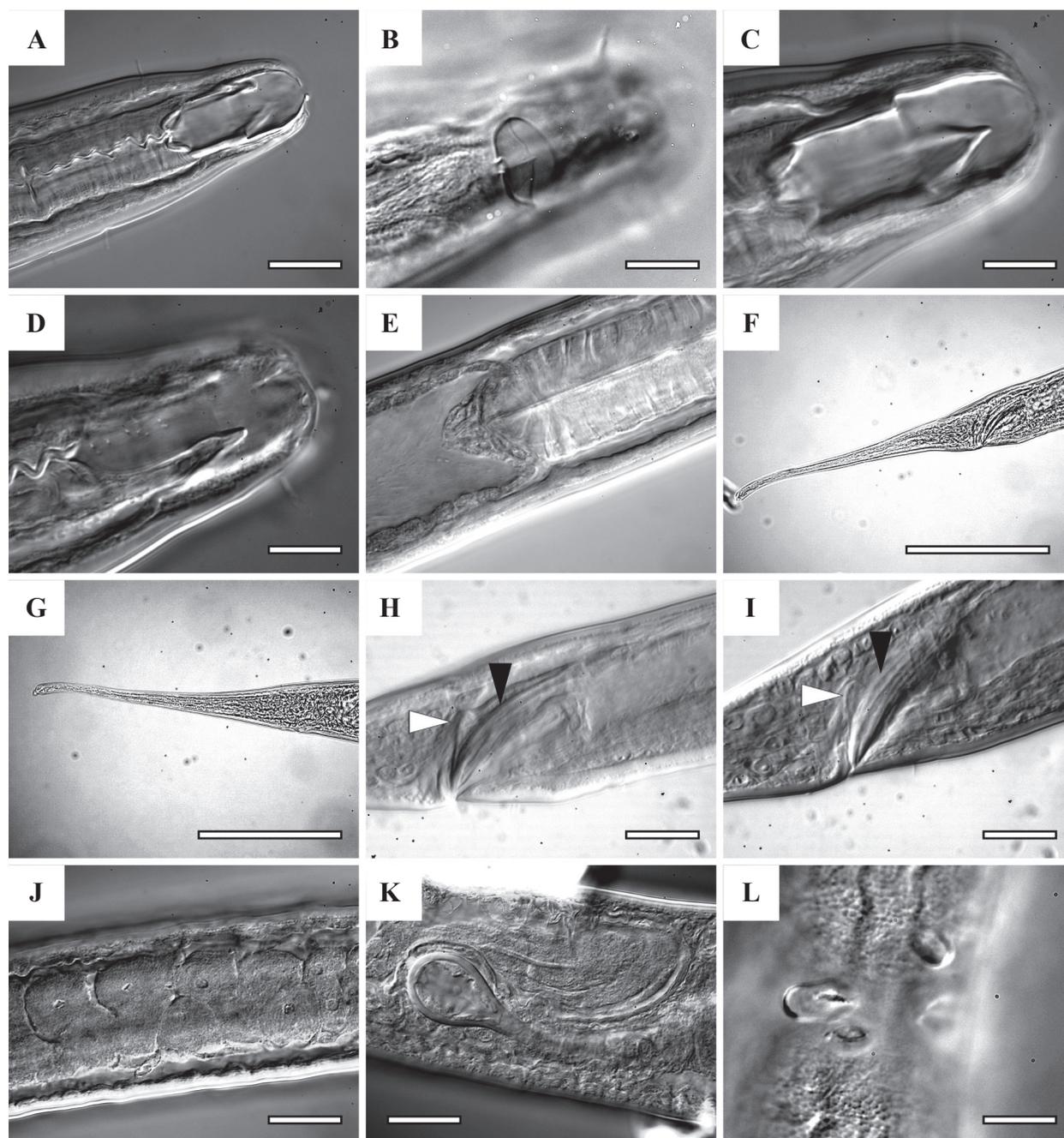


Figure 3. Light micrographs of *Fotolaimus cavus* sp. nov. **A–F, H, J, L.** Holotype (ICHUM 8474); **G, K.** Paratype (ICHUM 8477); **I.** Paratype (ICHUM 8475); **L.** Paratype (ICHUM 8479). **A.** Anterior region; **B.** Amphid; **C.** Buccal cavity with left ventrosublateral tooth; **D.** Buccal cavity with right ventrosublateral tooth; **E.** Posterior end of pharynx with cardia; **F.** Male tail; **G.** Female tail; **H, I.** Spicule (black arrowhead) and gubernaculum (white arrowhead); **J.** Sperms in seminal vesicle; **K.** Uvette and ductus entericus; **L.** Terminal pores. Scale bars: 20 μm (**A, E, H–K**); 10 μm (**B–D, L**); 100 μm (**F, G**).

constricted posterior to amphids, as wide as 0.3–0.4 maximum body diameters at cephalic sensilla level. Six inner labial sensilla papilliform. Six outer labial and four cephalic sensilla setiform, arranged in single circle, 5–8 μm or 0.20–0.30 corresponding body diameters long in males and 6–9 μm or 0.25–0.35 corresponding body diameters long in females. Amphids (Figs 1D, E, 2E, 3B) pocket-like, with elliptical aperture and cup-shaped fovea, 0.40–0.45 corresponding body diameters wide in males and 0.35–0.40 corresponding body diameters wide in females,

anterior margin located at 0.4–0.5 buccal cavity lengths from anterior body end. Buccal cavity (Figs 1D–F, 3C, D) barrel-shaped, length/width = 2.5–3.0, surrounded by pharyngeal tissue in posterior 15%–25%. Three well-developed teeth: left ventrosublateral tooth largest, 4–6 μm longer than right and dorsal teeth. Tip of left ventrosublateral tooth at 0.2 buccal cavity lengths from anterior body end (i.e., level of cephalic sensilla). Pharynx (Figs 1C, 3E) cylindrical, evenly muscular, gradually widened toward posterior end. Cardia surrounded by intestine. Rectum

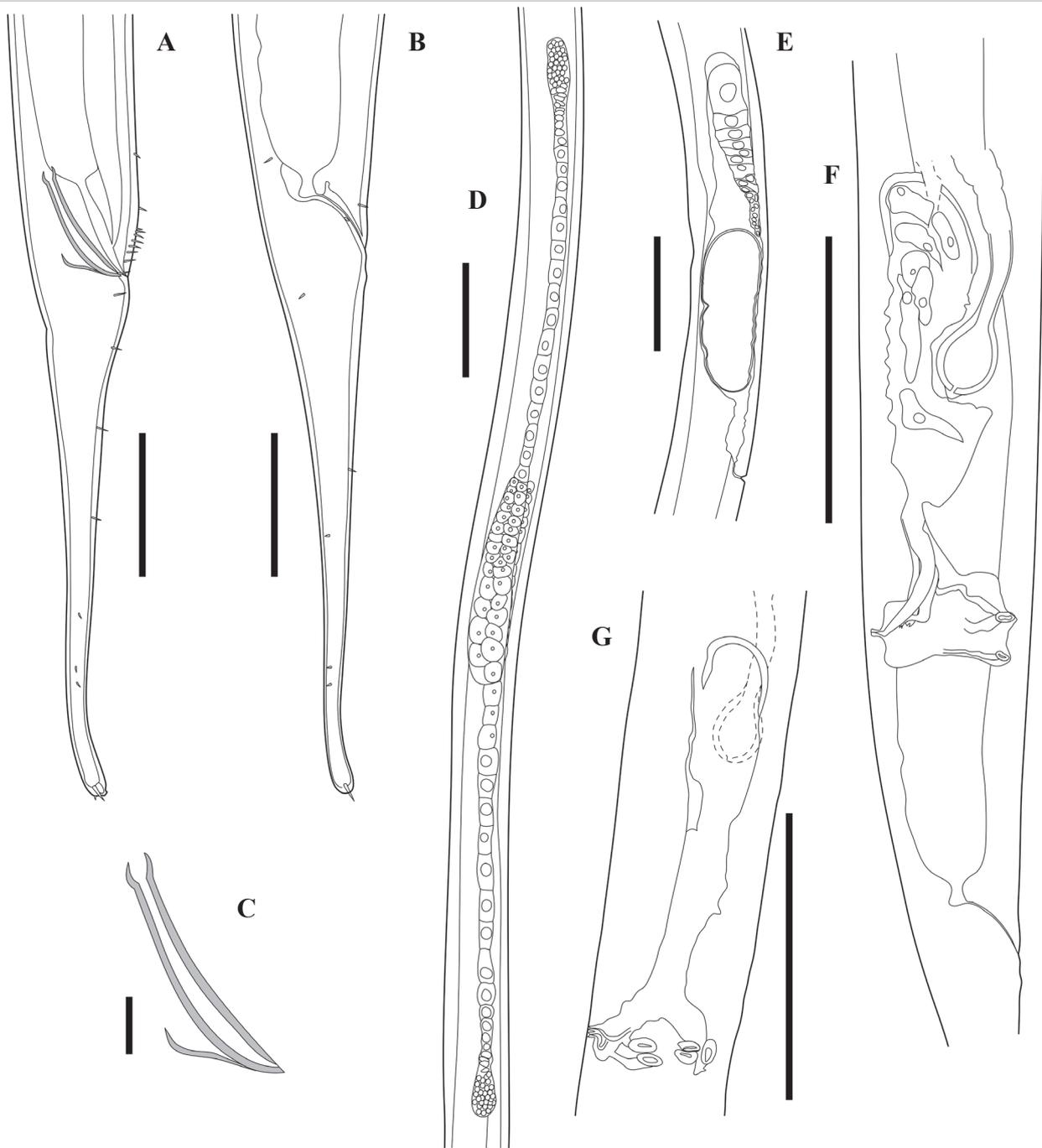


Figure 4. Line drawings of *Fotolaimus cavus* sp. nov. **A, C, D.** Holotype (ICHUM 8474); **B, E, F.** Paratype (ICHUM 8477); **G.** Paratype (ICHUM 8479). **A.** Male posterior region, right lateral view; **B.** Female posterior region, right lateral view; **C.** Spicule and gubernaculum, right lateral view; **D.** Male reproductive system, right lateral view; **E.** Female reproductive system, right lateral view; **F.** Demanian system, right lateral view; **G.** Demanian system, dorsal view. Scale bars: 50 μm (**A, B**); 10 μm (**C**); 100 μm (**D–G**).

(Fig. 4A, B) slightly longer than cloacal body diameter. Pore of secretory-excretory system (Figs 1C, 2H; SE-pore) with ampulla, at 2.0–2.2 buccal cavity lengths or 0.2 pharyngeal lengths from anterior body end. Renette cell not observed. Nerve ring (Fig. 1C) located at 0.45–0.50 pharyngeal lengths from anterior body end. Tail sexually dimorphic: male tail (Figs 2I, J, 3F, 4A) strongly tapering in anterior 1/5, gradually tapering in next 2/5, and almost cylindrical in posterior 2/5, as long as 3.9–5.7 cloacal

body diameters; female tail (Figs 3G, 4B) more gradually tapering throughout its length, as long as 5.5–6.1 anal body diameters. Tail tip slightly expanded, with spinneret and one pair of subterminal setae in both sexes. Body diameter at level of cloacal opening or anus equal to 0.5 maximum body diameters, at cylindrical portion of tail equal to 0.20–0.25 cloacal body diameters. Caudal glands inconspicuous. Caudal setae arranged in longitudinal rows, number and location varying from individuals.

Table 3. Morphometrics of *Fotolaimus cavus* sp. nov. All measurements are in μm and in the form: mean \pm s.d. (range). *Distance from the anterior body end.

	Male		Female
	Holotype	Paratype	Paratype
n	–	2	4
Body length	2417	2169 \pm 109 (2060–2277)	2481 \pm 284 (2266–2970)
a	39.6	31 \pm 1.2 (29.9–32.1)	34.2 \pm 4.0 (30.7–40.7)
b	6.2	6.1 \pm 0.1 (6.1–6.2)	6.2 \pm 0.6 (5.8–7.2)
c	13.3	13.5 \pm 1.7 (11.8–15.1)	12.7 \pm 0.8 (11.9–13.7)
V	–	–	66.0 \pm 1.1 (64.9–67.5)
Body diameter at cephalic sensilla	25	24.5 \pm 0.5 (24–25)	25.8 \pm 0.5 (25–26)
Body diameter at amphids	29	30	31 \pm 1.6 (29–33)
Maximum body diameter	61	70.0 \pm 1.0 (69–71)	72.8 \pm 2.3 (69–75)
Body diameter at vulva	–	–	68.3 \pm 2.8 (65–71)
Body diameter at cloacal opening or anus	32	35	35 \pm 0.9 (34–36)
Outer labial and cephalic setae length	5.0–7.5	6.4 \pm 0.8 (5.0–7.4)	6.9 \pm 1.0 (5.5–9.1)
Amphid position*	20	18 \pm 1.0 (17–19)	20 \pm 1.9 (18–23)
Amphid width	13	12.5 \pm 0.5 (12–13)	12
Buccal cavity length	40	41 \pm 1.0 (40–42)	42 \pm 0.8 (41–43)
Buccal cavity width	16	15.5 \pm 0.5 (15–16)	15.5 \pm 0.5 (15–16)
Pharyngeal length	389	356 \pm 13 (343–369)	403 \pm 11 (386–416)
SE-pore*	85	81	88
Nerve ring*	175	165 \pm 8 (157–173)	180 \pm 5 (172–185)
Tail length on arc	182	165 \pm 28 (137–193)	199 \pm 13 (189–218)
Spicule length on arc	47	48 \pm 2.5 (45–50)	–
Gubernaculum length on arc	17	20 \pm 3.0 (17–23)	–
Anterior gonad*	785	760 \pm 55 (705–814)	1302 \pm 94 (1214–1460)
End of posterior gonad*	1732	1546 \pm 60 (1486–1606)	–
Vulva*	–	–	1638 \pm 200 (1473–1975)
Uvette*	–	–	2067 \pm 278 (1839–2541)
Terminal pores*	–	–	2163 \pm 268 (1961–2622)

Spicules (Figs 3H, I, 4A, C) paired, arcuate, distally acute with subterminal opening, proximally with capitulum, as long as 1.3–1.5 cloacal body diameters or 0.25–0.35 tail lengths. Gubernaculum (Figs 3H, I, 4A, C) plate-like, distally associated with spicules, proximally curved, shape similar to that of gubernaculum from *Admirandus belogurovi* Tchesunov, Mokievsky & Nguyen, 2010 (Tchesunov et al. 2010), as long as 0.5–0.7 cloacal body diameters or 0.3–0.5 spicule lengths. No precloacal supplement. Circumcloacal setae (Fig. 2C, K) arranged in single circle, 9–12 setae in each body side. Three ventral papillae (Fig. 2L) present just anterior to cloacal opening, arranged in single longitudinal row, observed only with SEM. Male reproductive system (Fig. 4D) diorchic. Two opposed and outstretched telogonic testes, both situated on right side of intestine: germinal zone filled with small cells not arranged in rows; growth zone with larger cells arranged in single row. Blind end of anterior testis at 30%–40% of body length from anterior end; blind end of posterior testis at 70% of body length from anterior end. Seminal vesicle including two (dorsal and ventral) rows of sperms (Fig. 3J). Vas deference located on ventral side of intestine, anteriorly filled by single row of sperms.

Female reproductive system (Fig. 4E) monodelphic-prodelphic. Telogonic ovary antidromously reflexed, situated on right side of intestine, as long as 6%–10% of body length; anterior end connecting with oviduct at 50%–55% of body length from anterior end; blind end located

at 60%–65% of body length from anterior end; germinal zone with small cells not arranged in rows; growth zone with gradually growing cells arranged in two (right and left) rows; ripening zone with grown oocytes arranged in single row. Oviduct situated on left side of ovary (between intestine and ovary). Uterus well developed in two specimens (ICHUM 8477 and 8478), ca. 200 μm in length, each containing one egg (131–141 μm long and 48–62 μm wide). In other two individuals, uterus very short without egg and blind end of ovary located just anterior to vulva. However, the size and degree of maturity of the ovaries similar to those of egg-bearing specimens. Vulva (Figs 2M, 4E) transverse slit with thick cuticular walls, located at 2/3 from anterior body end. Vagina weakly sclerotized. Demanian system (Figs 3K, 4F, G) oncholaimoid type variant D (cf. Belogurov and Belogurova 1988). Ductus uterinus anteriorly inconspicuous. Uvette well-developed, spherical with thick sclerotized wall, situated on right side of intestine at 80%–85% of body lengths, connected to main duct with posterior tip. Main duct on dorsal side of intestine, anteriorly forming one short sac with thick sclerotized wall and filled with sperms, posteriorly forming two sacs on both sides. Ductus entericus very short, situated subterminally on the left side of anterior sac of main duct. Osmosium inconspicuous, may be simple pore. Terminal ducts branching off from posterior sacs of main duct, five or more in number on both sides. Terminal pores (Figs 2N, O, 3L, 4F, G) arranged in single circle surrounding body, 3–4 anal body diameters anterior to anus.

Diagnosis. *Fotolaimus cavus* sp. nov. is characterized by small body size (2.1–3.0 mm), wide amphids (0.35–0.45 corresponding body diameters), a long buccal cavity (length/width = 2.5–3.0), a long ($c = 12–15$, $c' = 3.9–6.1$) conico-cylindrical tail, and a proximally curved gubernaculum.

Remarks. *Fotolaimus cavus* sp. nov. differs from the other two species of the genus, i.e., *F. marinus* and *F. apostematus*, by the curved shape of the gubernaculum

(not curved and smaller in *F. marinus* and *F. apostematus*). *Fotolaimus cavus* sp. nov. is also different from *F. marinus* by its shorter body (2.1–3.0 mm in *F. cavus* sp. nov. vs 5.8–6.2 mm in *F. marinus*) and longer tail ($c = 12–15$, $c' = 3.9–6.1$ in *F. cavus* sp. nov. vs $c = 39–51$, $c' = 2.3–3.8$ in *F. marinus*) with conico-cylindrical shape (vs clavate in *F. marinus*) (cf. Wieser 1959; Belogurova and Belogurov 1974).

Key to *Fotolaimus* species (cf. Wieser 1959; Belogurova and Belogurov 1974)

- 1 Tail clavate, as long as 2.0–2.5 cloacal body diameters..... *F. marinus*
- Tail conico-cylindrical, longer than 3.0 cloacal body diameters..... 2
- 2 Gubernaculum almost straight*F. apostematus*
- Gubernaculum proximally curved*F. cavus* sp. nov.

Phylogenetic analysis

We obtained 38 partial COI sequences (315–801 bp) and 22 partial 18S sequences (1221–1692 bp) for seven oncholaimid species including *F. cavus* sp. nov. (Table 1). The DNA sequences isolated from *Fotolaimus* species were deposited in the INSD for the first time.

The ML tree based on 18S sequences (Fig. 5) suggested, albeit with low support values, the presence of two major clades within the Oncholaimoidea. The first major clade was composed of two highly supported subclades: all sequences from *Adoncholaimus* Filipjev, 1918 (SH-aLRT = 100% and UFBoot = 100%), and all sequences from *Viscosia* de Man, 1890 and several sequences from *Oncholaimus*, including *Pseudoncholaimus* (SH-aLRT = 97.3% and UFBoot = 99%). *Oncholaimus* from South Africa (SBA) and the Atlantic side of the USA (BUS, NAR, NUS, and OUS) did not form clades. *Oncholaimus* from the UK (AUK) and Japan placed in another major clade. Within the genus *Viscosia*, 11 sequences from the UK (AUK, HCL, LUK, and SBN) formed one clade, which did not include *V. dossena* Leduc & Zhao, 2023 from New Zealand. The second major clade contained *Pontonema* sp. and four well- or moderately-supported subclades: (1) *Eurystomina* Filipjev, 1921 and *Pareurystomina* Micoletzky, 1930 (SH-aLRT = 95% and UFBoot = 85%); (2) *Bathyeurystomina* Lamshead & Platt, 1979 (SH-aLRT ≥ 90% and UFBoot = 100%); (3) *Calyptronema* Marion, 1870 and *Symplocostoma* Bastian, 1865 (SH-aLRT = 94.8% and UFBoot = 97%); and (4) parts of *Oncholaimus* with all *Wiesoncholaimus*, *Fotolaimus*, *Meyersia* Hopper, 1967 and *Oncholaimellinae* sp. (SH-aLRT = 90.7% and UFBoot = 90%). The support values for a second major clade were not well-supported (SH-aLRT < 70% and UFBoot < 80%).

Discussion

The 18S phylogenetic tree (Fig. 5) suggested that *Fotolaimus cavus* sp. nov. was assembled in one major clade with some members of *Oncholaimus* and all members of

Wiesoncholaimus and *Oncholaimellinae* sp. with a high level of support (SH-aLRT = 96.3% and UFBoot = 97%). *Fotolaimus* and *Wiesoncholaimus* are included, with *Oncholaimus*, in the subfamily Oncholaiminae (Hodda 2022), and they all present an oncholaimid-type Demanian system (Belogurov and Belogurova 1988). Therefore, the position of *Fotolaimus* in the Oncholaiminae based on the morphology was supported by molecular phylogeny.

Oncholaimus sp. (KR265044) from Wakayama (Pacific side of central Japan) is considered to be *O. secundicollis* because DNA sequences obtained from the former was identical to those from the topotypes of *O. secundicollis*. *Oncholaimus secundicollis* is distributed on the Pacific and Sea of Japan sides of northeastern Japan (Shimada unpubl.) and on the Sea of Japan side of South Korea (Lee et al. 2015). Wieser (1955) also reported on *O. dujardinii* de Man, 1876 from Wakayama but provided limited morphological information and no illustration. A redescription of *O. dujardinii* by the same author (Wieser 1953) mentions a gubernaculum (Zhang and Platt 1983), which should be absent in *Oncholaimus*. Therefore, *O. dujardinii* sensu Wieser (1955) may not be a true *Oncholaimus*.

Meyersia branch has been assembled (SH-aLRT = 90.7% and UFBoot = 90%) with the *Oncholaimus*–*Wiesoncholaimus*–*Fotolaimus* clade. *Meyersia* did not form the clade with *Adoncholaimus*, indicating that monophyly of Adoncholaiminae is unlikely. Members of Adoncholaiminae possess two ovaries and a well-developed Demanian system, but other morphological features distinguish *Meyersia* from the other three genera. *Adoncholaimus* (including *Metoncholaimoides* Wieser, 1954), *Admirandus* Belogurov & Belogurova, 1979, and *Kreisoncholaimus* Rachor, 1969 have larger teeth on the right side, and the terminal pores of the Demanian system are located near the anus (i.e., much posterior to both ovaries). In contrast, in *Meyersia*, right and left teeth are large, and the terminal pores of the Demanian system are located at the level of the vulva (i.e., between both ovaries).

Monophyly of Enchelidiidae was not supported by our phylogenetic tree, although previous studies by Bik et al. (2010a, b) evidenced monophyly with 95% of bootstrap

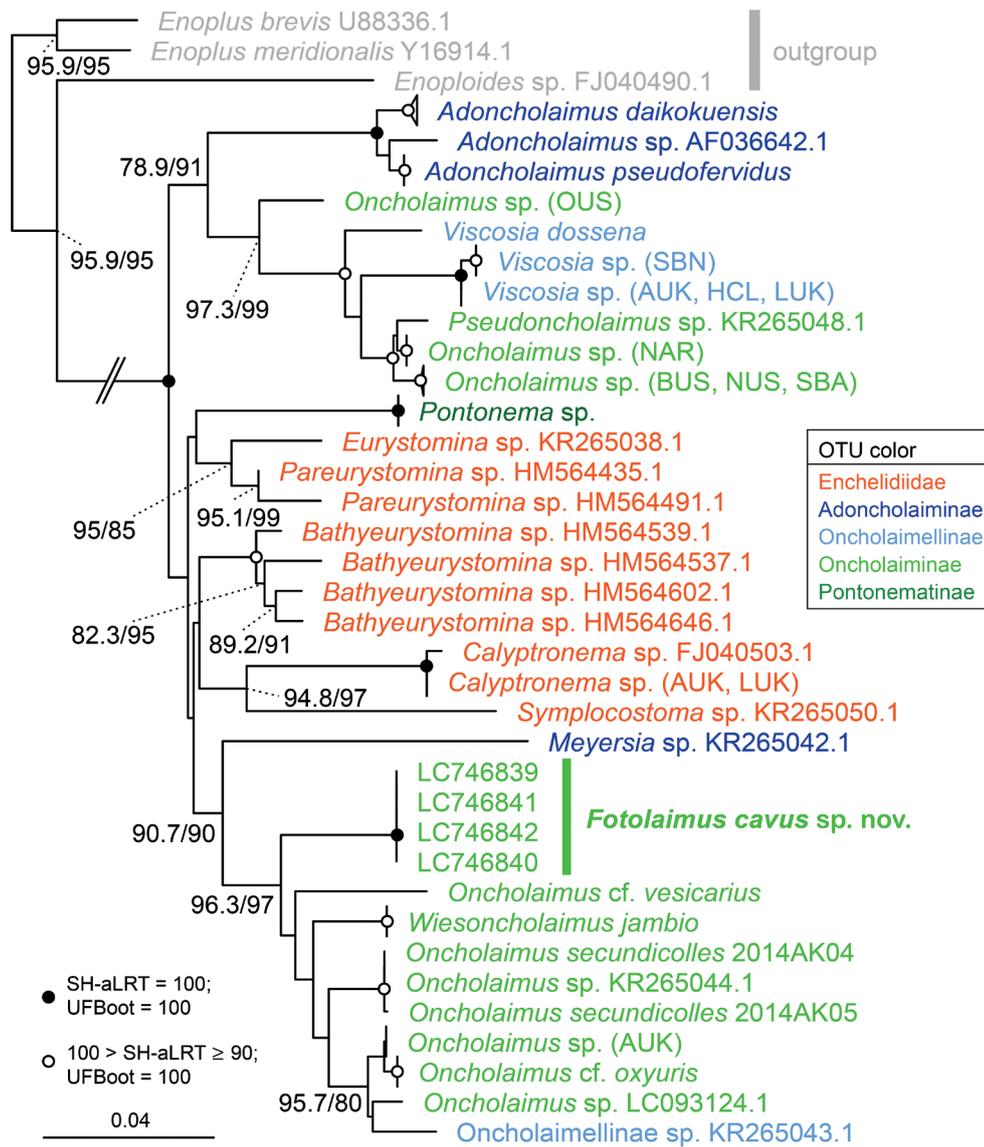


Figure 5. ML tree of Oncholaimoidea based on 18S DNA sequences. Numbers at the nodes are SH-aLRT (left) greater than 70% and UFBboot (right) greater than 80%.

values. Monophyly of the clade comprising *Calytronema* and *Symplocostoma* was well supported (SH-aLRT = 94.8% and UFBboot = 97%). Both genera present a sexually dimorphic cephalic region, elongated spicules, and papilliform (not winged) preloacal supplements, and have been considered, together with *Symplocostomella* Micoletzky, 1930, which has the same three characteristics, closely related groups within Enchelidiidae. *Pontonema* appeared to be a sister group of the clade consisting of *Eurystomina* and *Pareurystomina* Micoletzky, 1930; however, the support values are quite low (SH-aLRT < 70% and UFBboot < 80%), consequently, the position of *Pontonema* remains uncertain.

Some members of *Oncholaimus* and all members of *Pseudoncholaimus* and *Viscosia* appear to be monophyletic (SH-aLRT = 97.3% and UFBboot = 99%). As aforementioned, *Pseudoncholaimus* is considered a junior synonym of *Oncholaimus*, but both taxa can be distinguished by the presence or absence of Demanian system. Our tree strongly suggested that unidentified *Oncholaimus* spp. was not clustered in a single clade. The species of *Oncholaimus*

clustered with *Pseudoncholaimus* might not have a Demanian system. In fact, the species that doubtlessly had a Demanian system (*O. secundicollis*) belonged to a separate clade from *Pseudoncholaimus*. *Fotolaimus* and *Wiesoncholaimus*, suggested to be closely related to *O. secundicollis*, also have a Demanian system. Therefore, there was no evidence supporting the synonymization of *Pseudoncholaimus* with *Oncholaimus*. Because members of *Oncholaimus* (in which the left tooth is the largest) are included in two well-supported clades, which both contain Oncholaimellinae spp. (in which the right tooth is the largest), it is likely that the size of the left and right teeth does not reflect phylogenetic relationships. Additionally, *Wiesoncholaimus*, in which left and right teeth are large, was in the same clade as *Oncholaimus* and *Fotolaimus*, suggesting that the teeth size has evolved independently many times.

In Bik et al. (2010a, b) and Smythe (2015), the phylogenetic position of *Adoncholaimus* within Oncholaimoidea was not clear. Our phylogenetic analysis suggested that

Adoncholaimus is a sister clade of *Oncholaimus*–*Pseud-oncholaimus*–*Viscosia* clade with a certain degree of support (SH-aLRT = 78.9% and UFBoot = 91%).

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