Research Paper

Anantrum gallopintoi sp. nov. (Bothriocephalidae Blanchard, 1849), a cestode parasite of the shorthead lizardfish *Synodus scituliceps* (Synodontidae) from the Pacific coast of Costa Rica

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Abstract

A new species of bothriocephalid cestode in the genus *Anantrum* is described from the intestine of the shorthead lizardfish *Synodus scituliceps* from the north Pacific coast of Costa Rica. The new species is described based on an integrative taxonomic approach that includes the use of light and scanning electron microscopy, 28S rDNA sequencing, and phylogenetic analysis. *Anantrum gallopintoi* sp. nov. is the third known member of this genus and can be distinguished from *A. tortum* (Linton, 1905) and *A. histocephalum* Jensen & Heckmann, 1977 by a combination of morphological and ecological traits and, in particular, by having a vaginal sphincter, different number of testes, and different type host and type locality. The molecular analysis and the phylogenetic reconstructions supported its status as a new taxon placing it within a well-supported separate branch of *Anantrum* spp. subclade. According to the present finding, *S. scituliceps* represents a new host record and Costa Rica a new geographical record for *Anantrum* species, and, in general, for a bothriocephalid cestode.

Introduction

The cestode family Bothriocephalidae Blanchard, 1849 (order Bothriocephalidea) comprises 18 genera and approximately 70 valid species of intestinal parasites infecting marine and freshwater teleost fishes plus three species of *Bothriocephalus* Rudolphi, 1808, infecting salamanders (Caira & Jensen, 2017; Kuchta & Scholz, 2017; Caira *et al.*, 2024). Bothriocephalids exhibit a complex life cycle that involves one or two intermediate hosts. Definitive hosts typically become infected by the ingestion of infected copepod crustaceans that serve as intermediate hosts (Kuchta *et al.*, 2008b; Kuchta & Scholz, 2017).

Costa Rica represents an important hotspot of aquatic biodiversity with more than 2030 fish species recorded from both freshwater (Angulo, 2021) and marine ecosystems (Froese & Pauly, 2024). The Synodontidae are small teleost benthic fishes belonging to the aulopiform order which occur in marine and estuarine environments. The genus *Synodus* comprises 47 recognized species of which only four are found from the coastal waters of Costa Rica (Froese & Pauly, 2024). Among these, the shorthead lizardfish *Synodus scituliceps* Jordan & Gilbert is a common demersal predator mainly feeding on small benthic fishes (Rodríguez-Romero *et. al.*, 2019). This fish is known as a host for few cestode species including *Rhinebothrium* spp. and tetraphyllideans (Escalante *et al.*, 1987; Luque *et al.*, 1991; Alves *et al.*, 2017), whereas Synodontidae in general have been recorded as host for at least four species of bothriocephalids and two trypanorhinch species (Caira *et al.*, 2024).

According to previous literature (Rodríguez-Ortíz *et al.*, 2004; Caira & Jensen, 2017; Solano-Barquero *et al.*, 2023; Caira *et al.*, 2024), approximately 33 cestode species have been reported solely from the Costa Rican marine waters. These include adult stages of the orders Cathetocephalidea, Onchoproteocephalidea, Phyllobothriidea, Rhinebothriidea, Tetrabothriidea, "Tetraphyllidea", and Trypanorhyncha from elasmobranchs and larval stages of two Phyllobothriidea (*Clistobothrium delphini* (Bosc, 1802) Caira, Jensen, Pickering, Ruhnke & Gallegher, 2020 and *Clistobothrium grimaldii* (Moniez, 1899) Caira, Jensen, Pickering, Ruhnke & Gallegher, 2020 from cetaceans, whereas no records of Bothriocephalidea, and cestodes from teleost fishes in general, exist from Costa Rican waters.

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During a parasitological survey of fish from the Pacific coast of Costa Rica, some individuals of a bothriocephalid species were found from the intestine of *S. scituliceps.* These tapeworms proved to represent a morphologically distinct, previously unknown, species of *Anantrum* Overstreet, 1968, which is described here, based on morphological and molecular characters, and phylogenetic analysis.

Materials and methods

Sample collection

On April 7, 2023, seven individuals of the shorthead lizardfish were obtained from off Playa Cuajiniquil (Guanacaste) on the Pacific coast of Costa Rica using nets at benthic depths ranging from 5 to 10 m. They were three females and four males with total length ranging from 15.5 to 21 cm and from 15 to 25 cm, respectively. Fish were obtained under the framework of a project of the Centro de Investigación en Ciencias del Mar y Limnología of the University of Costa Rica "Proyecto BioMar - ACG" (see Cortés & Joyce, 2020; Santoro *et al.*, 2024), aimed at studying the marine biodiversity of the Pacific coast of Costa Rica (permit no. ACG 019-2023). Fish parasites were studied in the frame of a collaborative project between the Stazione Zoologica Anton Dohrn (Italy) and the University of Costa Rica (Costa Rica).

Fish were refrigerated (4 °C) and transferred to the laboratory, where they were studied within 6 h from fishing. During necropsy, the intestine of each individual fish was examined and cestodes were obtained alive under a dissecting microscope (Axio Zoom V16, Zeiss, Switzerland) using the methods described in Santoro *et al.* (2022, 2023). Cestodes were washed in physiological saline solution and, when relaxed, they were preserved in 70% ethanol for subsequent morphological and molecular analyses.

Morphological study

For light microscopy, cestodes were stained with Mayer's acid carmine, dehydrated through a graded ethanol series, cleared in methyl salicylate, and mounted in permanent slides in Canada balsam (Santoro *et al.*, 2024). In the species description, measurements (in micrometres, except where stated) are reported as range values with mean ± standard deviation in parentheses followed by the total number (n) of observations. Measurements were obtained using a compound microscope (Axio Imager M1, Zeiss) and a dissecting microscope equipped with the ZEN 3.1 imaging system (Zeiss). Drawings were made with the aid of a XP PEN Deco 02 drawing tablet (Deco, Italy) and the software Adobe Illustrator and Adobe Photoshop.

For scanning electron microscopy (SEM) analysis, two specimens were fixed overnight in 2.5% glutaraldehyde, then transferred to 40% ethanol (10 min), rinsed in 0.1 M cacodylate buffer, postfixed in 1% OsO_4 for 2 h, and dehydrated in ethanol series, critical point dried, and sputter-coated with platinum. Observations were made using a JEOL JSM 6700F SEM operating at 5.0 kV (JEOL, Japan).

Molecular and phylogenetic analyses

Genomic DNA was extracted from one specimen using the Quick-gDNA Miniprep Kit (Zymo Research, USA), according to the manufacturer protocol. The 28S rRNA segment was amplified using two sets of primer pairs allowing the amplification of two contiguous regions. The primer sets selected were ZX-1 (5'-ACCCGCTGAATTTAAGCAT-3') - ECD2 (5'-CTTGGTCC GTGTTTCAAGACGGG-3'), and LSU_300F (5'-CAAGTACCGT GAGGGAAAGTTG-3') - 1500R (5'-GCTATCCTGAGGGAAA CTTCG-3'). Polymerase chain reaction (PCR) was performed in 25-μL reactions with 2 μL of DNA sample, 0.6 μL of each primer at 10 mM and 10 μL of MyFi Mix (Bioline Ltd., United Kingdom). The thermocycling amplification program included a preliminary denaturation step at 94 °C (3 min) followed by 40 cycles of 94 °C (30 s), 54 °C (30 s), 72 °C (2 min), and a final extension step at 72 °C (10 min). Amplified products were preserved at 4 °C. Amplicons were visualized in a 1% agarose gel with GelRed (Biotium, UK) stain on a ~35 min, 95 V electrophoresis. Successful PCR products were purified using Agencourt AMPure XP (Beckman Coulter, USA), following the standard manufacturer recommended protocol. Clean PCR products were Sanger sequenced from both strands and from an additional internal primer 1090F (5'-TGAAA CACGGACCAAGG-3') using an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, USA) and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Life Technologies, USA). The obtained contiguous sequences were assembled and edited using MEGAX v. 11 (Kumar *et al.*, 2018). Sequence identity was verified using the Nucleotide Basic Local Alignment Search Tool (BLASTn) (Morgulis *et al.*, 2008).

All the available sequences representatives of the family Bothriocephalidae, according to the latest taxonomic classification (Kuchta *et al.*, 2008b, 2008a; Brabec *et al.*, 2015), were retrieved from GenBank (Table 1) and aligned, together with the sequence generated in this study, using the multiple sequence alignment package T-Coffee (Notredame *et al.*, 2000). The alignment was then submitted to the transitive consistency score (TCS) to verify the reliability of aligned positions and optimise the phylogenetic topology (Chang *et al.*, 2015). In total, 36 sequences were analysed, including the outgroup *Grillotia pristiophori* Beveridge & Campbell, 2001 (Table 1). Based on recent results indicating a good response of Bayesian inference (BI) in integrative taxonomic studies of parasites of fishes with complicated taxonomy (Kuchta *et al.*, 2012; Ailán-Choke *et al.*, 2021; Choudhury *et al.*, 2022), the phylogenetic hypotheses in the present work were inferred using this approach implemented in MrBayes v. 3.2.7 (Ronquist & Huelsenbeck, 2003). Additionally, maximum likelihood phylogenetic tree was calculated using iQtree v. 1.6.12 (Nguyen *et al.*, 2015), performing 5000 ultrafast bootstrap approximations to test the phylogenetic reliability. The best fitted evolutionary model was TIM3+I+Γ, as suggested by jModelTest v. 2.1.10 (Darriba *et al.*, 2012). Posterior probability distributions for the Bayesian analysis were generated using the Markov Chain Monte Carlo (MCMC) method. MCMC searches were run for 10 million generations on two simultaneous runs of four chains and sampled every 1000 generations; the first 25% of samples from the MCMC algorithm were discarded as burn in. The quality of the Bayesian analysis (parameter densities, effective sample size, and burn-in) and the chain convergence were examined in Tracer (Rambaut *et al.*, 2018), and trees were visualised using Figtree v. 1.4.4 (Rambaut, 2012). The genetic divergences among taxa for a subset of our dataset, which included the most closely related species to our specimen, were estimated using absolute nucleotide differences and p-distances using MEGAX v. 11 (Kumar *et al.*, 2018).

Results

Description (Figures 1–5)

ZooBank: LSID urn:lsid:zoobank.org:act:C992F0A4-0F83-4909-AD37-0F7BDB799A2B

Orden Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008

Table 1. Information about sequences used in the phylogenetic analysis obtained from GenBank (the sequence generated in this study is shown in bold).

GenBank ID	Parasite species	Host species	Host family	Geographic origin	Reference
PP756387	**Anantrum gallopintoi** sp. nov.	*Synodus scituliceps*	Synodontidae	Costa Rica	This study
KR780919	*Anantrum* sp. (PBI_609)	*Trachinocephalus myops*	Synodontidae	USA	Brabec *et al*. (2015)
AF286941	*Anantrum tortum*	*Synodus foetens*	Synodontidae	USA	Olson *et al*. (2001)
KR780883	*Anantrum tortum*	*Synodus foetens*	Synodontidae	USA	Brabec *et al*. (2015)
KR780894	*Bothriocephalidae* gen. sp. (PBI_033)	*Epinephelus coioides*	Serranidae	Indonesia	Brabec *et al*. (2015)
KR780886	*Bothriocephalus australis*	*Platycephalus aurimaculatus*	Platycephalidae	Australia	Brabec *et al*. (2015)
KR780921	*Bothriocephalus celineae*	*Cephalopholis aurantia x spiloparaea*	Serranidae	New Caledonia	Brabec *et al*. (2015)
KR780888	*Bothriocephalus carangis*	*Uraspis uraspis*	Carangidae	Indonesia	Brabec *et al*. (2015)
DQ925323	*Bothriocestus claviceps*[a]	*Anguilla anguilla*	Anguillidae	Czech Republic	Brabec *et al*. (2006)
KR780910	*Bothriocestus claviceps*[a]	*Trinectes maculatus*	Achiridae	USA	Brabec *et al*. (2015)
KR780908	*Bothriocestus cuspidatus*[a]	*Sander vitreus*	Percidae	USA	Brabec *et al*. (2015)
KR780887	*Bothriocephalus manubriformis*	*Istiophorus platypterus*	Istiophoridae	Maldives	Brabec *et al*. (2015)
KY909259	*Bothriocephalus scorpii*	*Peltorhamphus novaezeelandiae*	Pleuronectidae	New Zealand	Anglade & Randhawa (2018)
KR780905	*Bothriocephalus* sp. (PBI_485)	*Micropterus dolomieu*	Centrarchidae	USA	Brabec *et al*. (2015)
KR780906	*Bothriocestus kupermani*[a]	*Lepomis gibbosus*	Centrarchidae	USA	Brabec *et al*. (2015)
KR780907	*Bothriocephalus* sp. (PBI_525)	*Lepisosteus oculatus*	Lepisosteidae	USA	Brabec *et al*. (2015)
KR780885	*Bothriocephalus timii*	*Cottoperca gobio*	Bovichtidae	Argentina	Brabec *et al*. (2015)
KR780912	*Bothriocephalus travassosi*	*Anguilla marmorata*	Anguillidae	China	Brabec *et al*. (2015)
KR780884	*Clestobothrium crassiceps*	*Merluccius merluccius*	Merlucciidae	UK	Brabec *et al*. (2015)
KR780901	*Clestobothrium cristinae*	*Merluccius hubbsi*	Merlucciidae	Argentina	Brabec *et al*. (2015)
KR780920	*Clestobothrium splendidum*	*Merluccius australis*	Merlucciidae	Argentina	Brabec *et al*. (2015)
KR780902	*Ichthybothrium* sp. (PBI_427)	*Mesoporous crocodilus*	Distichodontidae	Sudan	Brabec et al. (2015)
JQ811838	*Kirstenella gordoni*	*Heterobranchus bidorsalis*	Clariidae	Ethiopia	Kuchta *et al*. (2012)
KR780893	*Oncodiscus sauridae*	*Saurida tumbil*	Synodontidae	Indonesia	Brabec et al. (2015)
KR780892	*Penetrocephalus ganapattii*	*Saurida tumbil*	Synodontidae	Indonesia	Brabec *et al*. (2015)
JQ811836	*Polyonchobothrium polypteri*	*Polypterus senegalus*	Polypteridae	Sudan	Kuchta *et al*. (2012)
DQ925333	*Ptychobothrium belones*	*Strongylura leiura*	Belonidae	Pacific Ocean	Brabec *et al*. (2006)
MW092753	*Ptychobothrium belones*	*Tylosurus imperialis*	Belonidae	Tunisia	Châari & Neifar (2022)
KY969627	*Regobothrium microhamulinum*	*Ageneiosus ucayalensis*	Auchenipteridae	Brazil	Scholz *et al*. (2017)
KR780889	*Schyzocotyle acheilognathi*	*Cyprinus carpio*	Cyprinidae	Czech Republic	Brabec *et al*. (2015)
KR780922	*Schyzocotyle nayarensis*	*Barilius* sp.	Danionidae	India	Brabec *et al*. (2015)
KR780891	*Senga lucknowensis*	*Mastacembelus armatus*	Mastacembelidae	Vietnam	Brabec *et al*. (2015)
KR780913	*Senga magna*	*Siniperca chuatsi*	Percichthyidae	Russia	Brabec *et al*. (2015)
KR780890	*Senga visakhapatnamensis*	*Channa punctata*	Channidae	India	Brabec *et al*. (2015)
JQ811835	*Tetracampos ciliotheca*	*Clarias gariepinus*	Clariidae	Ethiopia	Kuchta *et al*. (2012)
DQ642763	*Grillotia pristiophori*[b]	*Pristiophorus nudipinnis*	Pristiophoridae	Australia	Olson *et al*. (2010)

[a]Reassigned to this genus (see Caira *et al.*, 2024).
[b]Outgroup.

Family Bothriocephalidae Blanchard, 1849

Anantrum gallopintoi Santoro, López-Verdejo & Occhibove, 2024 sp. nov.

Based on six specimens as whole mounts and two observed with SEM. Medium-sized worms, flattened dorsoventrally, 119–153 (135.8 ± 14.6; n = 6) mm long, 1603–2522 (1962 ± 383.4; n = 6) maximum wide. Scolex unarmed, elongate, slightly spatulate, without bothria and apical disc (Figures 1a, 3a, 5a), 1445–2655 (2016 ± 432.1; n = 6) long, 550–951 (689 ± 155.2; n = 6) maximum wide; scolex length: width ratio 1:2.2–3.5 (1:2.9 ± 0.5; *n* = 6). Neck absent
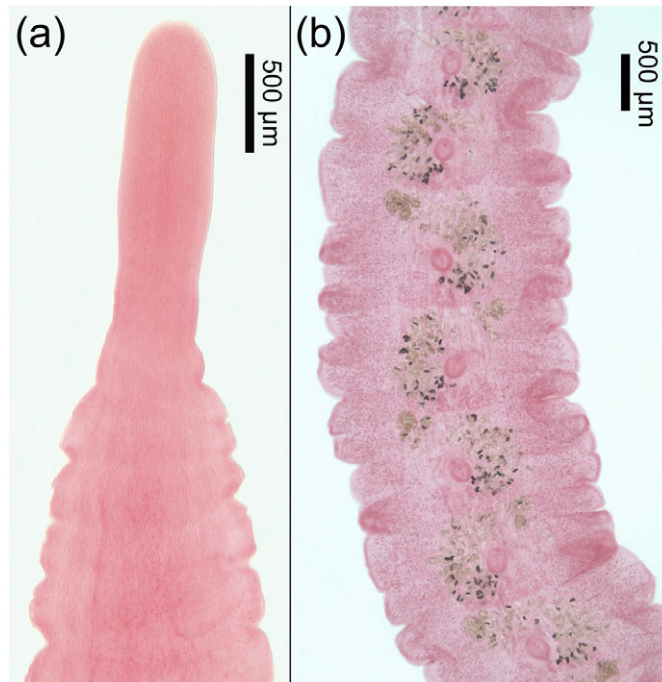
Figure 1. *Anantrum gallopintoi* sp. nov. from *Synodus scituliceps.* Microphotographs of a ventrally mounted paratype showing the scolex (a) and a section of the spirally twisted strobila with mature proglottids (b).
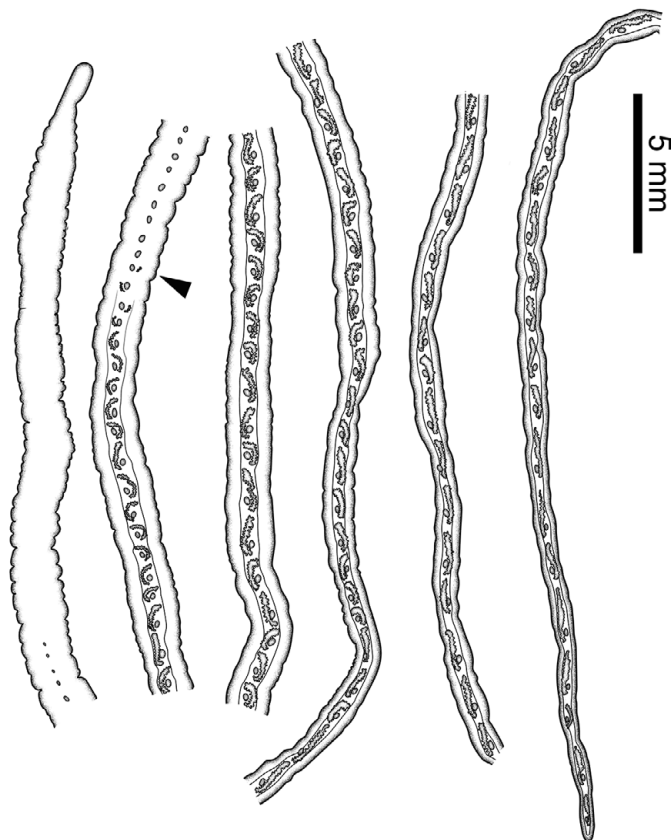

Figure 2. *Anantrum gallopintoi* sp. nov. from *Synodus scituliceps.* Line drawings of the strobila sections (paratype, ventral view). The arrowhead indicates where the mature proglottids start.
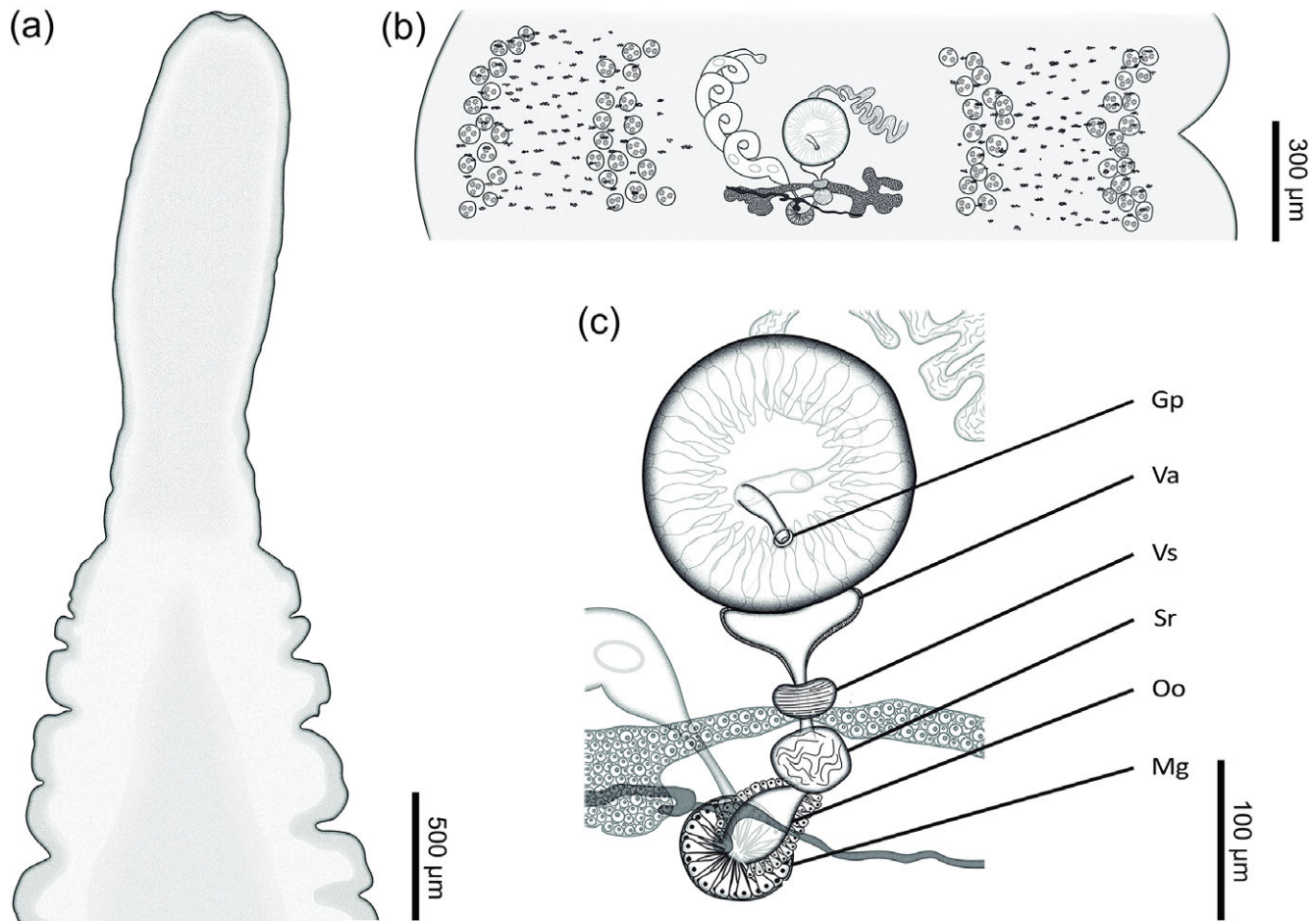
Figure 3. *Anantrum gallopintoi* sp. nov. from *Synodus scituliceps.* Line drawings of the holotype in ventral view. Scolex (a), mature proglottid (b), and genitalia (c). Abbreviations: Gp, genital pore; Mg: Mehlis' gland; Oo, ootype; Sr, seminal receptacle; Va, vagina; Vs, vaginal sphincter.

(Figures 1a, 3a, 5a). Strobila spirally twisted with rippled margins (Figure 1b), anapolytic, external segmentation absent with indistinguishable number of immature proglottids occupying 10.3%–25.5% (16.7% ± 5.5%; n = 6) of body length (Figure 2). Mature proglottids wider than long (Figures 1b, 3b); gravid proglottids longer than wide (Figure 4a); gravid proglottids (counting reproductive system sets with eggs), 83–154 (104 ± 28.6; n = 6) in number occupying 72.9%–87.8% (82.2% ± 5.5%; n = 6) of body length. Pygidium (posterior end of strobila) longer than wide (Figure 4b) (length: width ratio 1:2.4–4.4 [1:3.1 ± 0.7; n = 6], 1312–2901 [1848 ± 578.2; n = 6] long, 466–649 [584 ± 68.4; n = 6] wide [measured from its posterior extremity to anterior margin of uterus]). Tegument thick. Internal longitudinal muscles well developed. Nerve cord situated lateral on each side of proglottids. Osmoregulatory canals medullary, four pairs on each side of proglottids. Tegumental microtriches not observed.

Testes medullary, spherical 52–87 (67 ± 8.1; n = 40) in diameter; 50–84 (68 ± 8.4; n = 12) in number per proglottid, forming two longitudinal layers on each side of proglottids (Figures 3b, 4a), 23–50 in number on each side of longitudinal layers. Vas deferens strongly coiled, situated anteromedially. Genital pore dorsal; genital atrium (Figures 5b, 5d, 5e), sub-median, round with irregular tegumental papilliform processes on its margin. Cirrus-sac oval (Figure 3c), large, thick-walled, median, 213–305 (249 ± 29.1; n = 20) long, 160–202 (185 ± 11.9; n = 20) wide. Cirrus elongate, unarmed, 117–201 (159 ± 30.2; n = 9) long, 13–22 (19 ± 3.2; n = 9) wide.

Ovary bilobed (Figures 3b, 4a), median, transversely elongated near posterior margin of proglottid, 481–847 (651 ± 116.4; n = 20) long, 99–435 (251 ± 84.5; n = 20) wide; isthmus conspicuous. Vagina posterior to cirrus sac in midline of proglottid between ovary lobes, vaginal sphincter 29.4–44.5 (36.5 ± 3.6; n = 20) long, 58.9–75.9 (67.6 ± 5.4; n = 20) wide (Figure 3c). Seminal receptacle round, 37.7–58.87 (50.5 ± 5.1; n = 20) in diameter. Mehlis' gland round, 37–61 (47 ± 7.1; n = 10) in diameter. Vitelline follicles irregular, densely distributed in two cortical layers around testes (Figures 3b, 4a). Vitelline follicles, 20.9–45.1 (30.1 ± 6.1; n = 20) long, 30.4–52.9 (41.9 ± 5.4; n = 20) wide in mature proglottids, and 43.7–59.7 (50.4 ± 4.4; n = 20) long, 38.5–60.5 (52.5 ± 5.6; n = 20) wide in gravid proglottids. Vitelline reservoir dorsal. Uterus sinuous, coiled, irregularly alternating dextrally or sinistrally to midline, occupying 79%–100% (85% ± 5.6%; n = 20) of proglottid length. Uterine pore ventral (Figures 5c, 5f), anterolateral. Eggs operculate (Figures 4c, 5f), unembryonated, oval 45–61 (53 ± 4.4; n = 25) long, 24–34 (30 ± 2.1; n = 25) wide.

Taxonomic summary

Type host: shorthead lizardfish *Synodus scituliceps* Jordan & Gilbert, 1882 (Aulopiformes: Synodontidae).
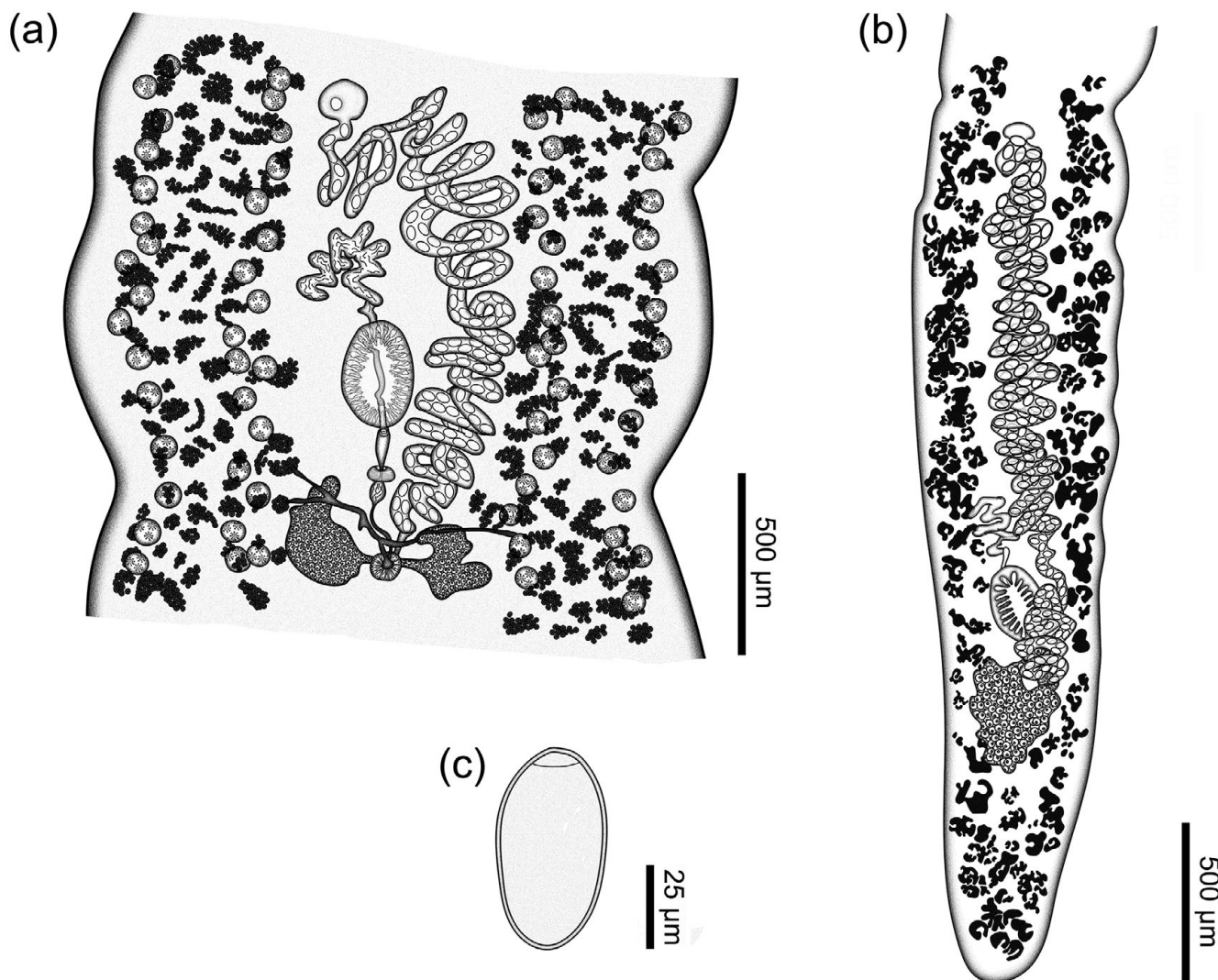
Figure 4. *Anantrum gallopintoi* sp. nov. from *Synodus scituliceps.* Line drawings of the holotype in ventral view. Gravid proglottid (a), pygidium (b), and egg (c).

Type locality: Gulf of Santa Elena off Playa Cuajiniquil (10° 56′04.38″N, 85°42′14.09″W), Guanacaste province, north Pacific coast of Costa Rica (collected on April 7, 2023).

Site of infection: Posterior intestine.

Type material: Holotype (MHNG-PLAT-0159514) and two paratypes (MHNG-PLAT-0159515 and MHNG-PLAT-0159516) in the Parasite Collection of the Natural History Museum of Geneva in Geneve (Switzerland); two paratypes in the Helminthological Collection of Costa Rica (CHCR-215-1 and CHCR-215-2) at the Universidad de Costa Rica, San José (Costa Rica).

Prevalence and intensity: two (males: 15 and 18 cm total length) of seven individuals infected with two and nine worms, respectively.

Etymology: The new species is named after the typical Costa Rican breakfast food.

Remarks

There are only three species of *Anantrum*, including the new one. These are: *A. tortum* (Linton, 1905) described from *Synodus foetens* at Beaufort, North Carolina (North Atlantic) (Linton, 1905), and later redescribed from *S. intermedius* at St George's West, Bermuda (Western Atlantic) (Rees, 1969), and *S. foetens* from South Florida (Everglades National Park, Atlantic) (Overstreet 1968), and *A. histocephalum* Jensen & Heckmann, 1977 described from *S. lucioceps* from coastal waters of Los Angeles County, California (North Pacific) (Jensen & Heckmann, 1977).

Anantrum gallopintoi sp. nov. can be distinguished from both its conspecifics by having a vaginal sphincter, different number of testes per proglottid (50–84 *vs* 12–16 in *A. tortum*, and 31–101 in *A. histocephalum*), and different type host. *Anantrum gallopintoi* sp. nov. is most closely related to *A. tortum*; however, it is larger (119–153 *vs* 36–70 mm), has larger number of sets of reproductive organs along the strobila (mean: 104 *vs* 45), and has larger cirrus sac (249 × 185 *vs* 50 × 76 in diameter), and different geographical distribution (Pacific *vs* Atlantic). *Anantrum gallopintoi* sp. nov. can be also distinguished from *A. histocephalum* by body length (119–153 *vs* 81–552 mm), different scolex shape (elongate *vs* mushroom shape), presence of tissue-associated scolex in *A. histocephalum*, and absence *vs* presence of neck, and different type host. Table 2 lists the main ecological, morphological, and morphometrical differences among the known species of *Anantrum*.
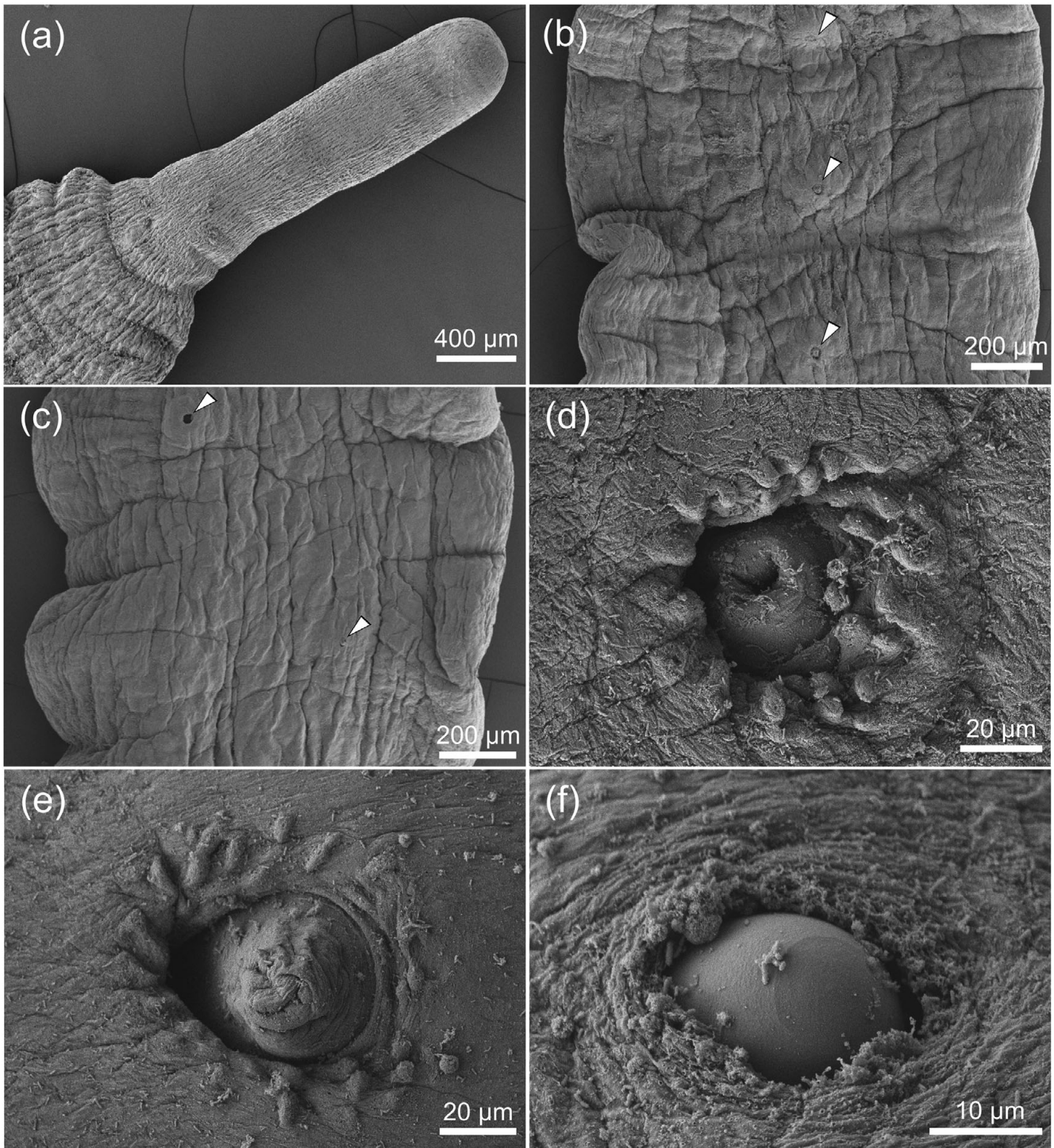
Figure 5. Scanning electron microscopy of *Anantrum gallopintoi* sp. nov. Scolex (a); dorsal view of mature proglottid showing the genital pore (b, arrowheads); ventral view of mature proglottids showing the irregularly alternating uterine pore (c, arrowheads); genital pore partially obliterated showing contracted tegumental papilliform processes (d); genital pore showing around the tegumental papilliform processed (e); operculate egg coming out of the uterine pore (f).

Molecular and phylogenetic analyses

A sequence of 1438 bp of the 28S rDNA gene was obtained from a specimen of *A. gallopintoi* sp. nov. which was deposited in GenBank under the accession number PP756387. Results of the query of the BLASTn tool showed that sequences possessing the highest degree of similarity were *Anantrum* sp. PBI_609 (KR780919), *A. tortum* isolate PBI_014 (KR780883), and *A. tortum* (AF286941), with 100% of coverage and about 92% of similarity.

BI and maximum likelihood results were identical, showing the same topology and strong clade and subclade supports, hence only BI tree is shown (Figure 6). The BI analysis from the alignment, of final length 1774 bp solved the tree, clearly separating the genera in the family Bothriocephalidae, and distinguishing between the two clades of freshwater and marine taxa (Figure 6). No sequences were available in GenBank for the remaining genera reported in Caira *et al.* (2024) which included *Andycestus* Kuchta, Scholz & Bray,

Table 2. Main comparative data available in literature for the valid species of *Anantrum* (measurements are expressed as the mean with the range in parentheses).

Species	*A. tortum*	*A. tortum*	*A. histocephalum*	*A. gallopintoi*
Reference	Overstreet, 1968	Rees, 1969	Jensen & Heckmann, 1977	This study
Host	*S. foetens*	*S. intermedius*	*S. lucioceps*	*S. scituliceps*
Locality	South Florida (Everglades National Park, Atlantic)	Bermuda (Western Atlantic)	Los Angeles County, California (North Pacific)	Gulf of Santa Elena, Costa Rica (North Pacific)
Body length by body width	(10–161.5) mm	41.4 (3–70) × (1.8–2.1) mm	321 (81–552) by 4.2 (1.6–6) mm	135.8 (119–153) × 1.9 (1.6–2.5) mm
Scolex shape	Elongate	Elongate	Mushroom-shaped	Elongate
Scolex length	(0.6–1.5) mm	(0.6–0.7) mm	1.61 (1.14–2.21) mm	2 (1.4–2.6) mm
Number of mature proglottids	321	45		104 (83–154)
Testes number		(12–16)	(31–101)	68 (50–84)
Testes diameter	(25–57) μm	(35–39) μm		67 (52–87) μm
Cirrus sac	(50–76) μm in diameter	(80–110) × 57 μm		249 (213–305) × 185 (160–202) μm
Ovary			592 (289–935) × 225 (75–374) μm	651 (481–847) × 251 (99–435) μm
Mehlis' gland		(38–67) by (35–50) μm	(53–75) × (53–57) μm	47 (37–61) μm in diameter
Vaginal sphincter				36.5 (29.4–44.5) μm
Vitelline follicles	(18–43) μm in diameter	(9–14) × (35–39) μm	42 (20–66) × 34 (13–57) μm	(20.9–59.7) × (30.4–60.5) μm
Egg	(48–58) × (27–34) μm	50 × 28 μm	60 (48–70) × 43 (31–53) μm	53 (45–61) × 30 (24–34) μm

2008, *Plicatobothrium* Cable & Michaelis, 1967, *Plicocestus* Kuchta, Scholz & Bray, 2008, and *Taphrobothrium* Lühe, 1899.

Our new species clustered with other sequences of the genus *Anantrum*, forming the most basal lineage of the genus. The most closely related taxa are members of the genus *Clestobothrium* and bothriocephalids from other synodontid fishes, such as *Penetrocephalus ganapattii* and *Oncodiscus sauridae*. The tree resolved well the phylogeny of Bothriocephalidae (Figure 6), in agreement with Brabec *et al.* (2015), who reconstructed the molecular phylogeny of Bothriocephalidea. Freshwater Bothriocephalidae consisted of two groups, the first comprising Nearctic species of *Bothriocephalus* sensu lato with Holarctic distribution (Clade J in Brabec *et al.*, 2015). The second included *Bothriocephalus travassosi* Tubangui, 1938 on a separate branch, and subsequently *Senga* spp. Dollfus, 1934, *Schyzocotyle* spp. Akhmerov, 1960, and a group of bothriocephalids designated as Clade K in Brabec *et al.* (2015). As expected, this group also included *Regobothrium microhamulinum* Scholz, Takemoto & Kuchta, 2017 which grouped with African taxa (Scholz *et al.*, 2017). Marine taxa comprised almost all parasites of coastal demersal marine teleosts with the exception of *Bothriocephalus manubriformis* (Linton, 1889) Ariola, 1900. The most basal taxon was represented by *Ptychobothrium belones* (Dujardin, 1845) Lönnberg, 1889, whereas on the subsequent branch separated Clade G (Brabec *et al.*, 2015), then *Anantrum* species, and finally *Bothriocephalus* spp. (Clade H according to Brabec *et al.*, 2015), which was sister to the three *Clestobothrium* Lühe, 1899 species available (Fig. 6).

The close relationship between *Anantrum* species and Clade H + *Clestobothrium* species was also confirmed by the results of pairwise distances shown in Table 3. The present analysis clearly showed that our sequence was phylogenetically different from other species of *Anantrum*. In addition to be located on a well-supported separate branch of *Anantrum* genus, demonstrating its distinctiveness, the described species presented genetic distances between congeners ranging from 0.060 to 0.069 (Table 3), well above commonly used

thresholds for species discrimination. In *Clestobothrium* or *Bothriocephalus*, smaller differences could be observed among congeners, similar to those between other *Anantrum* species; thus, the *A. gallopintoi* sp. nov. higher degree of dissimilarity confirmed its status as new species.

Discussion

The family Bothriocephalidae was established by Bray *et al.* (1994) and later slightly modified in the identification key by Kuchta *et al.* (2008b) to accommodate the cestode genera of aquatic environment by having a median genital pore. The present specimens well agree with the diagnostic morphological characters of the genus *Anantrum*, as previously described in Kuchta *et al.* (2008b). In particular, the main characters used for their identification as belonging to *Anantrum* were: the spirally twisted strobila with rippled margins, the absence of strobila segmentation, and the elongated scolex without bothria (Bray *et al.*, 1994; Kuchta *et al.*, 2008b).

Species of the genus *Anantrum* are parasites with strong host species-specificity for marine teleost of *Synodus* spp. Members of this genus have been previously identified in *S. foetens*, *S. intermedius*, and *S. lucioceps* along its range of distribution (Linton, 1905; Overstreet, 1968; Rees, 1969; Jensen & Heckmann, 1977). An additional undescribed putative new species found in *Trachinocephalus myops* (previously assigned to the genus *Synodus*) from the Gulf of Mexico and referred to in GenBank as *Anantrum* sp. n. PBI_609 (KR780919) has been deposited by Brabec *et al.* (2015). The latter is confirmed by our phylogenetic analysis to be a distinct entity from known species of *Anantrum* waiting to be described as new species.

Morphological, molecular, and phylogenetic analyses of the newly described species supported its status as a new taxon. *Anantrum gallopintoi* sp. nov. parasitizes *S. scituliceps*, an endemic
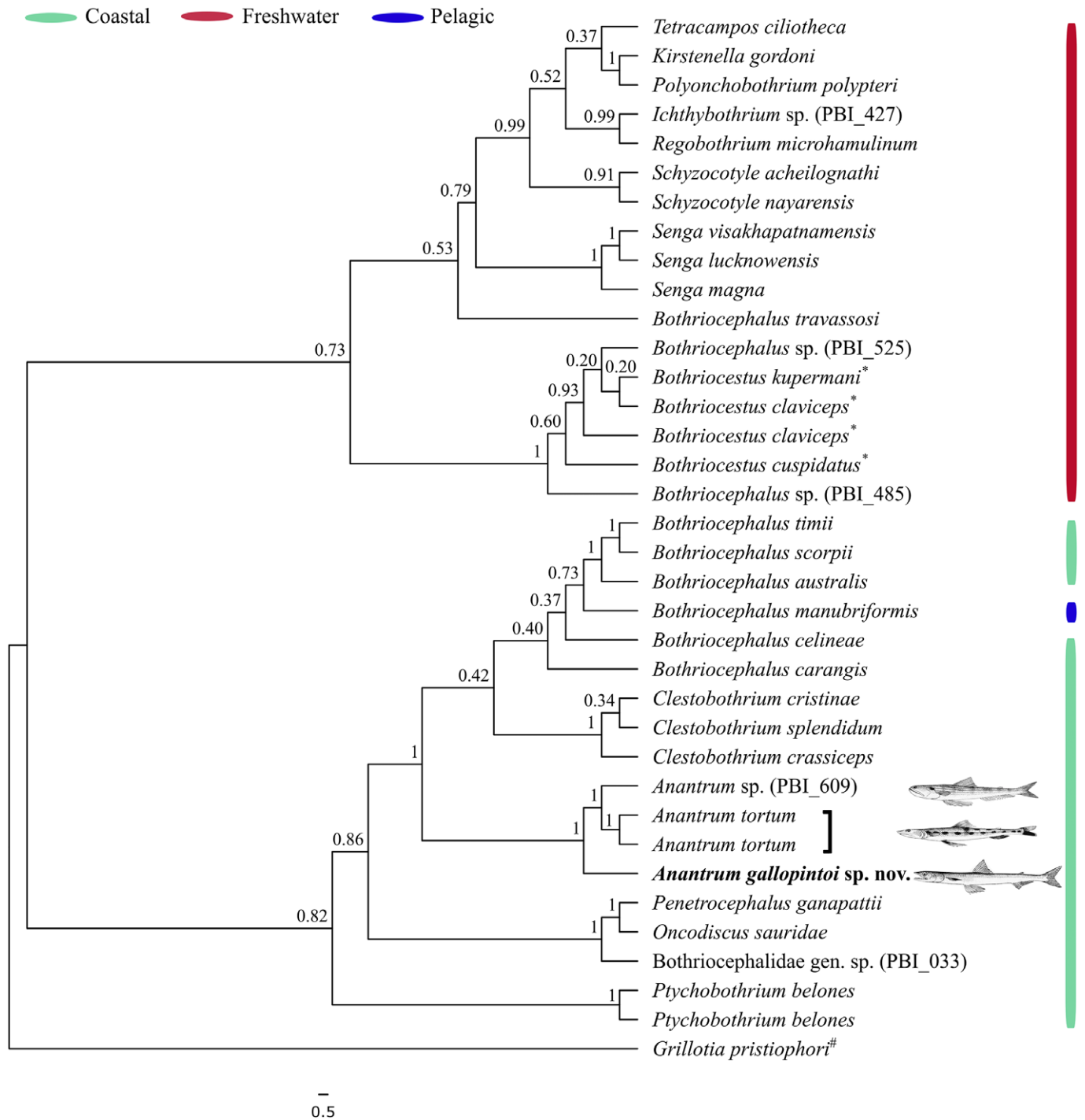
Figure 6. Bayesian inference (BI) tree for the analyses based on the 28S rDNA sequences (1774 bp). Nodal support is given as posterior probabilities. The scale bar indicates the expected number of substitutions per site. The sequence generated in this study is shown in bold. Coloured bars represent fish host habitats (green: coastal; red: freshwater; blue: pelagic). Fish outlines represent, from top to bottom, *Trachinocephalus myops*, *Synodus foetens*, and *S. scituliceps* (the *Anantrum* spp. hosts, see text). *Reassigned to this genus (see Caira *et al.*, 2024). #Outgroup.

species of the eastern Pacific with a distributional range limited to the coasts from Mexico to Chile and the Galapagos Islands. The geographical distribution of the present host does not overlap the distributional ranges of other *Synodus* and *Trachinocephalus* spp. in which the other *Anantrum* spp. have been found (Froese & Pauly, 2024). According to the present finding, *S. scituliceps* represents a new host record and Costa Rica a new geographical record for *Anantrum* species, and, in general, for a bothriocephalid.

The other two known species of the genus *Anantrum* had historically been described or redescribed based solely on their traditional morphological characters. Unfortunately, they are still poorly known. For instance, in the redescription of *A. tortum*, Overstreet (1968) mentioned that the genital atrium has approximately 30 papillae which are placed in four circular rows. Rees (1969) redescribing the same species mentioned that the genital atrium is lined by tegument raised into prominent closely packed

Table 3. Differences among representatives of the genera *Anantrum*, *Bothriocephalus*, and *Clestobothrium* for 28S rDNA sequences, p-distances (below the diagonal), and pairwise nucleotide differences (above the diagonal) (alignment 1774 bp); the sequence generated in this study is shown in bold.

	Species (GenBank accession number)	1	2	3	4	5	6	7	8	9	10	11	12
1	***Anantrum gallopintoi* sp. nov. (PP756387)**	-	**85**	**87**	**86**	**95**	**86**	**104**	**89**	**93**	**86**	**85**	**85**
2	*Anantrum* sp. PBI_609 (KR780919)	**0.060**	-	35	27	119	103	115	109	110	104	103	103
3	*Anantrum tortum* (AF286941)	**0.069**	0.027	-	10	117	107	124	108	110	101	100	98
4	*Anantrum tortum* (KR780883)	**0.061**	0.018	0.008	-	116	106	122	107	108	100	99	99
5	*Bothriocephalus australis* (KR780886)	**0.069**	0.080	0.093	0.078	-	42	65	26	26	47	46	46
6	*Bothriocephalus carangis* PBI_020 (KR780888)	**0.062**	0.069	0.085	0.071	0.028	-	58	35	38	38	37	36
7	*Bothriocephalus manubriformis* (KR780887)	**0.075**	0.077	0.099	0.082	0.044	0.039	-	57	60	67	66	66
8	*Bothriocephalus scorpii* (KY909259)	**0.064**	0.074	0.086	0.073	0.018	0.024	0.039	-	10	38	37	37
9	*Bothriocephalus timii* (KR780885)	**0.067**	0.074	0.088	0.073	0.017	0.025	0.040	0.007	-	41	40	40
10	*Clestobothrium crassiceps* (KR780884)	**0.062**	0.070	0.080	0.067	0.031	0.025	0.045	0.026	0.027	-	1	1
11	*Clestobothrium cristinae* (KR780901)	**0.061**	0.069	0.080	0.067	0.031	0.025	0.044	0.025	0.027	0.001	-	0
12	*Clestobothrium splendidum* (KR780920)	**0.061**	0.070	0.078	0.067	0.031	0.024	0.044	0.025	0.027	0.001	0.000	-

papilliform processes. The present study of the tegumental surface of the genital atrium of *A. gallopintoi* sp. nov., as revealed by SEM, allowed to detect the presence of some tegumental papilliform processes on its external margin, which could obliterate the pore when contracted, as suggested previously by Rees (1969). Moreover, Linton (1905) did not mention whether the egg of *A. tortum* is operculate or not, whereas Overstreet (1968) and Rees (1969) redescribed the same cestode species with operculate and unoperculate eggs, respectively. All these incongruences suggest that morphological characters of this cestode family should be also investigated using SEM because they may be difficult to assess using traditional microscopy alone, as confirmed in the present study.

Members of the most closely related genera to *Anantrum* (i.e. *Bothriocephalus* species in Clade H + *Clestobothrium* spp.) present remarkable morphological differences and host associations, including hosts with dissimilar biological and ecological features, as well as a wide geographic distribution (Bray *et al.*, 1994; Kuchta *et al.*, 2008b). In addition, *Anantrum* species are associated with members of the Synodontinae sub-family presenting significant differences in their distribution and ecology. However, this is not uncommon for Bothriocephalidea, and for Bothriocephalidae in particular (Brabec *et al.*, 2015). For example, similar morphological features appear in not closely related lineages across the phylogenetic tree of the Bothriocephalidea, being likely the result of convergent evolution (Brabec *et al.*, 2015). Furthermore, the same species might occur in various orders of teleosts, differing in their biology, ecology, habitat, and other characters potentially affecting parasite transmission, whereas, at the same time, be phylogenetically most related to species recovered from very distant geographic locations (e.g. *R. microhamulinum*) (Scholz *et al.*, 2017). Most Bothriocaphalidae lack morphological synapomorphies and can only be defined unequivocally by a combination of biological and ecological characteristics (Brabec *et al.*, 2015; Kuchta *et al.*, 2008b). Therefore, results of the present analysis, in agreement with the latest taxonomic classification of the Bothriocephalidae family, highlights how the integration of molecular and morphological characterisation is essential to clarify taxonomic relationships of these cestodes. Future efforts should be focused on finding novel morphological, biological, and ecological traits, in addition to increasing efforts to expand resolution of molecular markers, to improve taxonomic reliability.

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