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DESCRIPTION AND MOLECULAR DIFFERENTIATION OF A NEW *FALCAUSTRA* (NEMATODE: KATHLANIIDAE) FROM THE INDOCHINESE WATER DRAGON, *PHYSIGNATHUS COCINCINUS* (SQUAMATA: AGAMIDAE) IN NORTH-CENTRAL VIETNAM

Nguyen Van Ha[1], Ong Vinh An[2], Hiroshi Sato[3], Stephen E. Greiman[4], Le Thi Mai Linh[5], and Tran Thi Binh[1]

[1] Department of Parasitology, Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam.

[2] Department of Biology, Vinh University, 182 Le Duan Street, Vinh City, Nghe An Province, Vietnam.

[3] Laboratory of Parasitology, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan.

[4] Department of Biology, Georgia Southern University, 4324 Old Register Road, Statesboro, Georgia 30460.

[5] Department of Molecular Systematics and Conservation Genetics, Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam.

Correspondence should be sent to Tran Thi Binh at: *tranthibinhs@yahoo.com*

KEY WORDS

Falcaustra vietnamensis n. sp.
Nematoda
Physignathus cocincinus
Squamata
Agamidae
Vietnam

ABSTRACT

Falcaustra vietnamensis n. sp. is described from the small intestine of *Physignathus cocincinus* from north-central Vietnam. The new species is characterized by the large male worms (20.2–28.8 mm in length and 557–724 μm in width) relative to known members of the genus, 2 sharply pointed alate spicules of equal length (1,128–1,256 μm in length), gubernaculum including 2 separate pieces, 1 ventral with a pointed distal end and 1 dorsal with a blunt distal end (164–192 μm and 155–172 μm in length, respectively), and 12 pairs of caudal papillae. Female worms are larger than male worms (24.2–34.1 mm in length and 532–735 μm in width), with the vulva situated in the posterior half of body, and elliptical eggs, 60–70 μm long by 42–47 μm wide. *Falcaustra vietnamensis* n. sp. represents the 38th species assigned to the genus and the third species recorded from a lizard host in the Oriental biogeographical region. Partial sequences of the *18S* ribosomal RNA gene (rDNA), internal transcribed spacer regions (*ITS*), and cytochrome c oxidase subunit 1 (*COI*) are provided for the new species. The molecular phylogenetic position of the genus *Falcaustra* is briefly discussed.

The Indochinese water dragon, *Physignathus cocincinus* Cuvier, 1829, is widely distributed in tropical forests of south Asia, including Vietnam, southern China (Yunnan and Guangdong), Laos, Myanmar, southeastern Thailand, and Cambodia. This lizard lives mostly in trees but may be found in other foliage, vines, and shrubs at low altitude (mostly around 300 m above sea level) (Nguyen et al., 2009, 2017). To our knowledge, the parasite fauna of this lizard species has not yet been studied until now.

A total of 104 species within the genus *Falcaustra* Lane, 1915, have been recorded from fishes, amphibians, and reptiles around the world. Two species, *Falcaustra desilvai* Bursey, Goldberg and Bauer, 2009, and *Falcaustra malaysiaia* Bursey, Goldberg and Grismer, 2014, have been recorded from lizards in the Oriental biogeographical region (Bursey et al., 2020). *Falcaustra vietnamensis* n. sp. is the second species recorded in Vietnam after *F. stewarti* Baylis and Daubney, 1922, from *Coura mouhotii* (Gray, 1862) (syn: *Cyclemys mouhotii*) in southern Vietnam (Berry, 1984; Nguyen et al., 2009) and the 38th species within the Oriental biogeographical region.

Very little molecular data are available for the genus *Falcaustra*. Until now, only 3 species, *Falcaustra araxiana* Massino, 1924, *Falcaustra sinensis* Liu, Zhang and Zhang, 2011, and *Falcaustra catesbeianae* Walton, 1929, have DNA sequences available (Hasegawa et al., 2013; Rajabloo et al., 2016; Li et al., 2018). In this study, we conducted morphological and molecular genetic characterizations of the new species from the Indochinese water dragon. Following comparison with its congeners, it is described as a new species, *Falcaustra vietnamensis* n. sp.

MATERIALS AND METHODS

Animals and parasitological examination

Twelve Indochinese water dragons were collected by hand in September and October 2018 from 2 localities in the central region (Nghe An Province: 3 water dragons; Thua Thien-Hue Province: 9 water dragons) of Vietnam. The nematode *Falcaustra*

Version of Record, first published online with fixed content and layout, in compliance with ICZN Arts. 8.1.3.2, 8.5, and 21.8.2 as amended, 2012. ZooBank publication registration: urn:lsid:zoobank.org:pub: D8A8C164-C310-46B8-A331-019A62907782.

Table I. Recovery of *Falcaustra vietnamensis* n. sp. from Indochinese water dragons in north-central Vietnam.

No.	ID	Locality	Date of collection	No. of nematodes
1	VN-NA-A.2018.06	Tuongduong, Nghe An Province 16°15′11″N, 104°30′25″E	25 Sept 2018	—
2	VN-NA-A.2018.07	Tuongduong, Nghe An Province 16°15′11″N, 104°30′25″E	25 Sept 2018	—
3	VN-NA-A.2018.08	Tuongduong, Nghe An Province 16°15′11″N, 104°30′25″E	25 Sept 2018	—
4	VN-HUE.2018-01	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	11 Oct 2018	Small intestine: 1
5	VN-HUE.2018-02	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	11 Oct 2018	—
6	VN-HUE.2018-03	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	13 Oct 2018	Small intestine: 3
7	VN-HUE.2018-04	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	13 Oct 2018	Small intestine: 3
8	VN-HUE.2018-05	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	14 Oct 2018	Small intestine: 11
9	VN-HUE.2018-06	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	14 Oct 2018	Small intestine: 12
10	VN-HUE.2018-07	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107° 52′47″E	14 Oct 2018	Small intestine: 7
11	VN-HUE.2018-08	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	14 Oct 2018	Small intestine: 17
12	VN-HUE.2018-09	Bachma National Park, Thua Thien-Hue Province 16°14′56″N, 107°52′47″E	14 Oct 2018	Small intestine: 50
Total				**104**

sp. was found in the small intestine of 8 (66.7%) water dragons. The lizards were euthanatized with ether and immediately dissected. Details of the collection sites are shown in Table I. Body cavities of the large water dragons were opened by longitudinal incision, and the gastrointestinal tracts were removed by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, and large intestine of each lizard were examined separately for endoparasites. Collected nematodes were killed in hot water at 70 C, fixed, and preserved in 70% alcohol for later use. For morphological observation, nematodes were placed in a clearing solution with glycerin and examined under a light microscope (Olympus BX53) (Olympus Corporation, Shinjuku City, Tokyo, Japan). Drawings were made with the aid of a camera lucida. Measurements are in micrometers (μm) unless otherwise stated, with mean ± 1 SD and range in parentheses. Nematodes were deposited in the Vietnam National Museum of Nature, VAST, Hanoi, Vietnam, under specimen numbers VNMN-IZ000.000.160–000.000.162.

Scanning electron microscopy (SEM)

Samples preserved in 70% alcohol were washed 3 times in 0.2 M Na_2HPO_4–NaH_2PO_4-buffered solution (PBS), pH 7.8, and immersed in 2.5% glutaraldehyde in PBS overnight. Subsequent processing was similar to that described previously (Tran et al., 2015).

DNA extraction, polymerase chain reaction (PCR), and sequencing

Individual nematodes stored in 70% alcohol were cut into 2 parts. The anterior parts of 2 males and a female were individually washed with distilled water and used for DNA extraction using DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland) according to the manufacturer's recommended protocol. DNA extracts were kept at –30 C until used. DNA of each specimen was PCR amplified using the following sets of primers: 18SF: CGCGA-ATRGCTCATTACAACAGC and 18SR: GGCGGTATCT-GATCGCC) for *18S rDNA* (Floyd et al., 2005); AB28: 3′-ATATGCTTAAGTTCAGCGGGT-5′ and TW81: 3′-GTTTCCGTAGGAACCTGC-5′ for *ITS rDNA* (Joyce et al., 1994); and Jb3: 3′-TTTTTTGGGCATCCTGAGGTTTAT5′ and Jb4: 3′TAAAGAAAGAACATAATGAAAATG-5′ for *COI* mtDNA (Bowles et al., 1992). The PCR reaction mixture contained 25 μl Hotstart PCR Mastermix (Promega, Madison, Wisconsin), 1 μM forward and reverse primers, 5 μl crude DNA-extract, and sterile water up to a volume of 50 μl. The PCR program settings were as follows: 95 C for 5 min; 35 cycles with 96 C for 35 sec, 52 C for 30 sec, and 72 C for 45 sec; and a final extension at 72 C for 3 min. Successful PCR products were purified with ExoSAP-IT PCR Product Cleanup Reagent kit (ThermoFisher, Waltham, Massachusetts) and sequenced (using the PCR primers) at Apical Scientific Sdn Bhd, Selangor, Malaysia. DNA sequences were submitted to GenBank under accession numbers MN729570–MN729572 and MN727387–MN727392.

Phylogenetic analysis

Newly obtained sequences of *F. vietnamensis* n. sp. and sequences of related taxa were used in the phylogenetic analyses. DNA sequences of related nematodes were obtained from GenBank and aligned using the geneious alignment tool in the bioinformatics program Geneious (Kearse et al., 2012). Three distinct alignments, *18S*, *ITS*, and *COI*, were prepared, and 3

analyses were run. Due to the interspecific hypervariability of the *ITS* gene, we used the Gblocks program to remove poorly aligned positions and divergent regions within the alignment (Castresana, 2000). The *18S* alignment was 836 bp long, and the *ITS* alignment (after removal of hypervariable regions using Gblocks) was only 277 bp long. The *COI* nucleotide sequences were translated using the invertebrate mitochondrial genetic code, and the resulting amino-acid sequences were aligned using the geneious alignment tool (122 amino acids long).

Phylogenetic analyses were carried out using Bayesian inference implemented in MrBayes version 3.2.7 (Huelsenbeck and Ronquist, 2001) through the CIPRES science gateway (Miller et al., 2010). A preliminary analysis (*18S*) was run using a larger dataset (39 taxa), with special attention to including Ascaridida taxa. For the *18S* alignment, *Protostrongylus rufescens* was used as the outgroup, and the following nucleotide substitution parameters were used: lset nst=6, rates=invgamma, ngammacat=4, prset shapepr=fixed (0.3070), pinvarpr=fixed (0.5150), which corresponds to the TrNef+I+G substitution model (Tamura and Nei, 1993). For the *ITS* alignment, related in-group taxa and outgroup, *Ascaris lumbricoides*, were selected based on our larger *18S* phylogeny and the phylogeny published by Chen et al. (2018). The following nucleotide substitution parameters were used: lset nst=2, rates=invgamma, ngammacat=4, prset shapepr=fixed (1.420), pinvarpr=fixed (0.3220), which corresponds to the K80+I+G substitution model (Kimura, 1980). For the *COI* alignment, related in-group taxa and outgroup (*Steinernema carpocapsae*) were chosen based on the published phylogeny by Kim et al. (2006). The following amino-acid substitution parameters were used: lset rates=propinv, ngammacat=4, prset aamodelpr=mixed, statefreqpr=fixed (empirical, pinvarpr=fixed (0.477), which corresponds to the MtArt+I substitution model (Abascal et al., 2007). All analyses were run for 3,000,000 generations, and log-likelihood scores were examined to ensure convergence. The final 75% of trees were used to produce the consensus tree. The substitution models for *18S* and *ITS* were selected based on results obtained from jModelTest version 0.1.1 (Posada, 2008), and the substitution model for *COI* was selected based on results obtained from ProtTest version 3.4.2 (Darriba et al., 2011).

DESCRIPTION

Falcaustra vietnamensis n. sp.

(Figs. 1–3)

General: Stout, large-sized among recorded *Falcaustra* spp. Sexual dimorphism not significantly evident, male worms smaller than female worms. Anterior end blunt and posterior end pointed. Cuticle with fine, regular transverse striations. Mouth with 3 lips of equal size; dorsal lip with 2 papillae, each subventral lip with 1 papilla and 1 amphid. Esophagus with spherical isthmus, valved bulb. Tail conical in both sexes.

Male (holotype and 8 paratypes): Body length 25.21 ± 2.52 mm (20.2–28.8 mm), width at level of esophageal-intestinal junction 642 ± 60 (557–724). Pharynx 195 ± 18 (170–210) long; corpus 3,182 ± 249 (2,672–3,445) long; isthmus 244 ± 20 (200-266) long, 148 ± 11 (134–168) wide; valved bulb 267 ± 19 (230–285) long, 250 ± 22 (218–279) wide. Nerve ring 624 ± 34 (580–667) and excretory pore 2,299 ± 132 (2,114–2,490) from anterior end, respectively. One pseudosucker, middle of pseudosucker to posterior end 6,175 ± 790 (4,920–7,505), 28–36 pairs of muscles terminating on rim of pseudosucker. Caudal end with well-developed radial muscles, 60–67 oblique muscle bands in a single field beginning at anterior of cloaca and extending to pseudosucker. Twelve pairs of caudal papillae: 4 pairs precloacal, ventrolateral in position; 1 pair adcloacal; 7 pairs postcloacal (4 pairs ventrolateral and 3 pairs lateral in position), single median papilla immediately anterior to cloaca. Two alate spicules, equal in length, lightly sclerotized, curved ventrally, and sharply pointed at the distant end, 1,193 ± 47 (1,128–1,256) long; alate reaching nearly to pointed tip. Gubernaculum including 2 separate pieces, 1 ventral with pointed distal end 179 ± 10 (164–192) in length, and 1 dorsal with blunt distal end 165 ± 6 (155–172). Tail 699 ± 73 (563–782) in length, conical, terminating in sharp point.

Female (allotype and 8 paratypes): Body length 27.77 ± 4 mm (24.2–34.1 mm), width at level of esophageal-intestinal junction 662 ± 64 (532–735). Pharynx 185 ± 14 (176–197) long; corpus 3,169 ± 227 (2,674–3,447) long; isthmus 239 ± 31 (177–247) long, 155 ± 14 (140–170) wide; valved bulb 270 ± 28 (205–295) long, 257 ± 24 (222–290) wide. Nerve ring 626 ± 44 (570–673) and excretory pore 2,439 ± 68 (2,348–2,496) from anterior end, respectively. Vulva located at the posterior end of the body, 16.44 ± 2 mm (12.8–18.6 mm) from the anterior end. Vagina running anteriorly from vulva and dividing into 2 divergent uteri. Eggs oval with thick shell, unembryonated, 65.80 ± 3 (60–70) long by 45.80 ± 1 (42–47) wide. Tail conical but sharply pointed, 1,233 ± 118 (1,000–1,408) long.

Taxonomic summary

Type specimens: Holotype, male (VNMN_IZ000.000.160); allotype, female (VNMN_IZ000.000.161); and paratypes (VNMN_IZ000.000.162) in Vietnam National Museum of Nature, VAST, Hanoi, Vietnam.

Other materials: Voucher IEBR (86 specimens were fixed in 70% alcohol).

Type host: Physignathus cocincinus Cuvier, 1829.

Type locality: Bachma National Park (16°14′56″N, 107°52′47″E), Thua Thien-Hue Province, Vietnam.

Site of infection: Small intestine.

Prevalence: 8 (66.7%) of 12 water dragons examined.

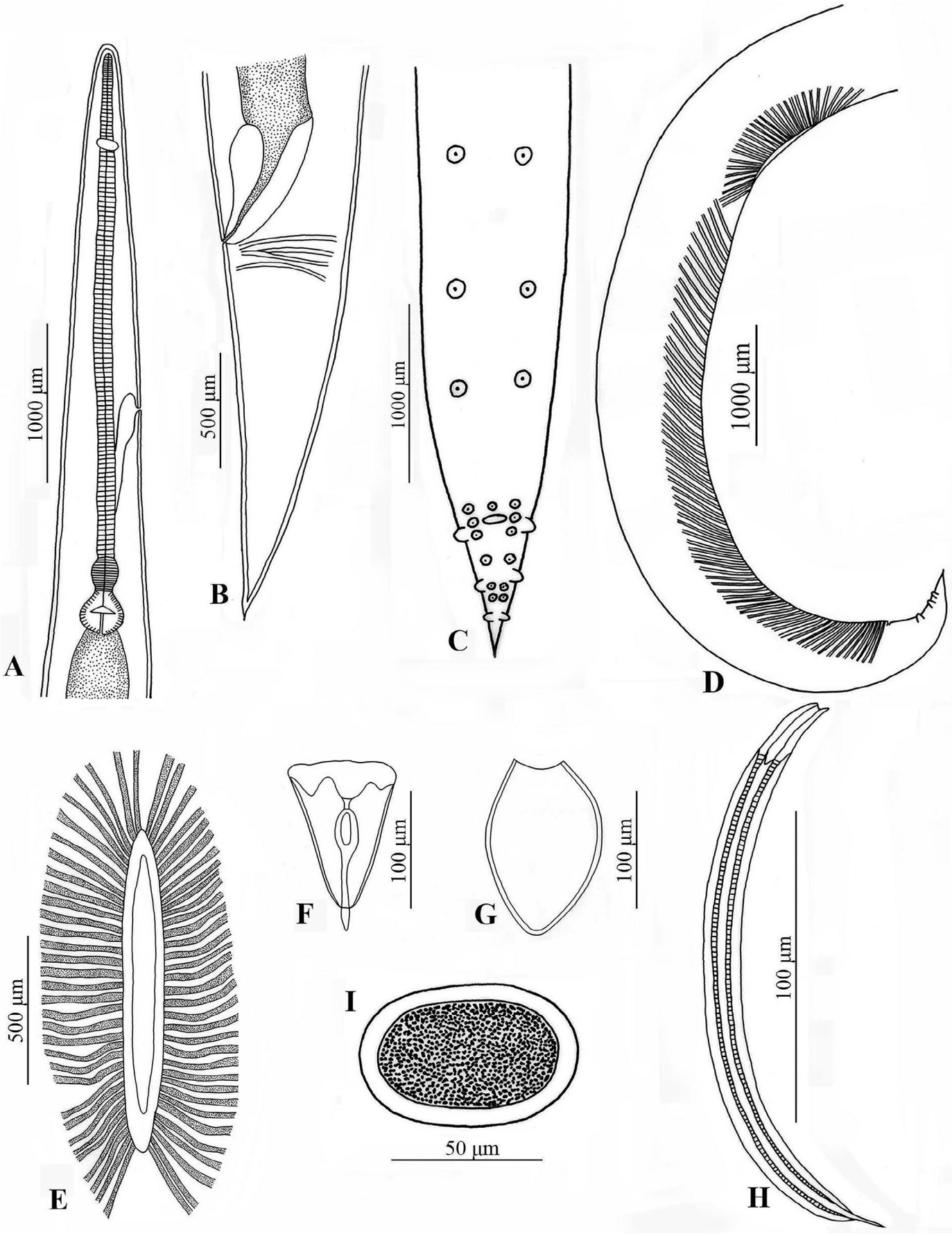
Mean intensity of infection: 13 ± 15.9 (range: 1–50).

ZooBank registration: urn:lsid:zoobank.org:act:8483C81C-883F-455C-B5A6-0937104DAB58.

Etymology: This species is named referring to the country where infected lizards were collected.

Remarks

A total of 37 *Falcaustra* species was previously reported from the Oriental biogeographical region; among them, 35 species were recorded from fish, amphibian, and turtle hosts (Bursey et al., 2020). *Falcaustra vietnamensis* n. sp. is distinguished from all 35 species by the pattern and number (12 pairs) of caudal papillae. Two more species have been described from lizard hosts in the Oriental biogeographical region, *F. desilvai* Bursey, Goldberg and Bauer, 2009, and *F. malaysiaia* Bursey, Goldberg and Grismer, 2014, of which *F. desilvai* are shorter in overall length (males), possess a different pattern of caudal papillae, and lack a pseudosucker, and *F. malaysiaia* are shorter in overall length (males) and possess a different number and pattern of caudal



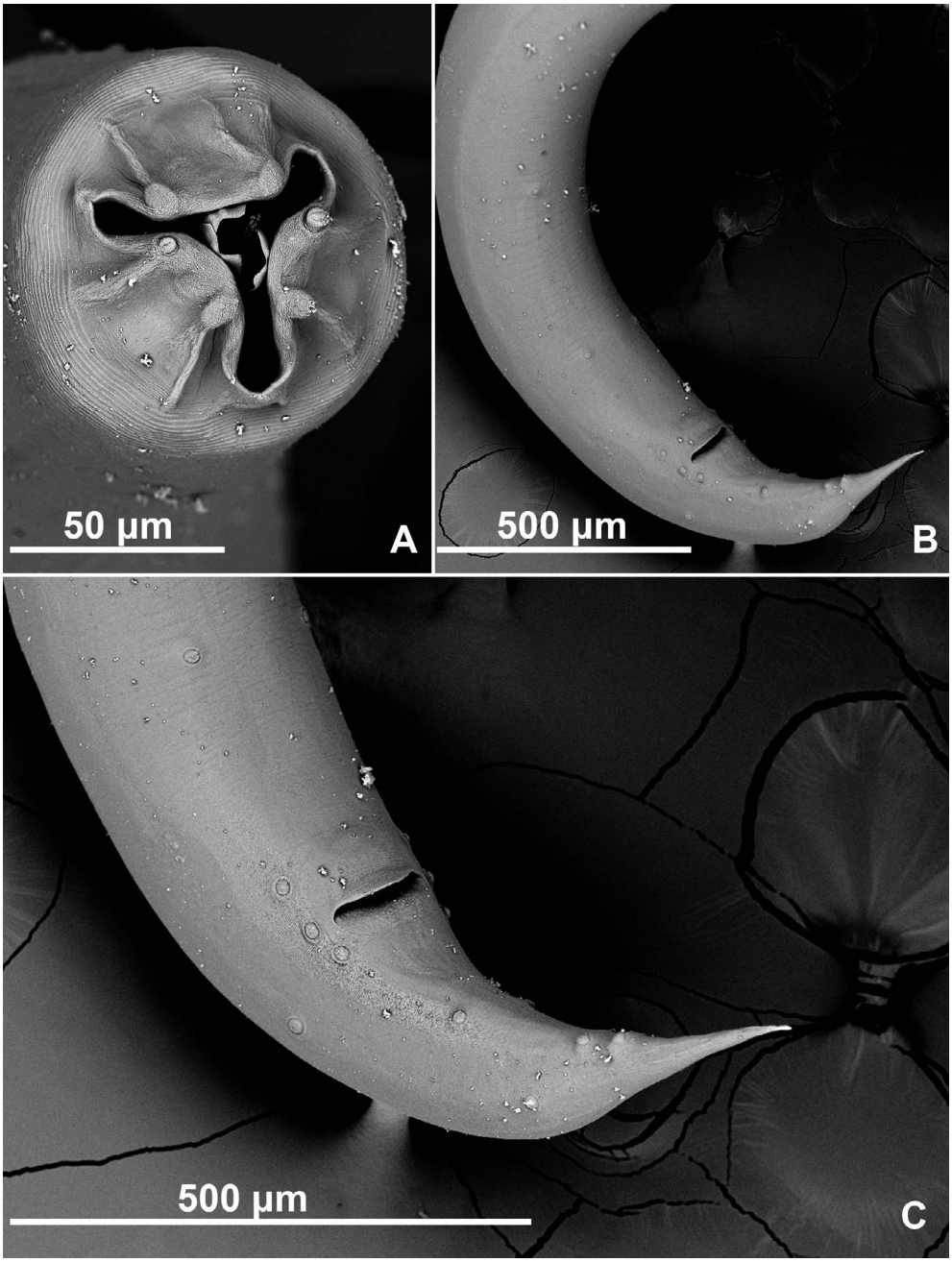


Figure 2. SEM view of male worm, *Falcaustra vietnamensis* n. sp. (**A**) En face view of the anterior end. (**B**) Lateral view of the posterior end, lower magnification. (**C**) Lateral view of posterior end, higher magnification.

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Figure 1. *Falcaustra vietnamensis* n. sp. (**A**) Female, anterior end, lateral view. (**B**) Female, posterior end, lateral view. (**C**) Male, posterior end, ventral view. (**D**) Male, posterior end, lateral view. (**E**) Pseudosucker, ventral view. (**F**) Ventral piece of gubernaculum with pointed distal end, ventral view. (**G**) Dorsal piece of gubernaculum with blunt distal end, ventral view. (**H**) Spicule, lateral view. (**I**) Egg.

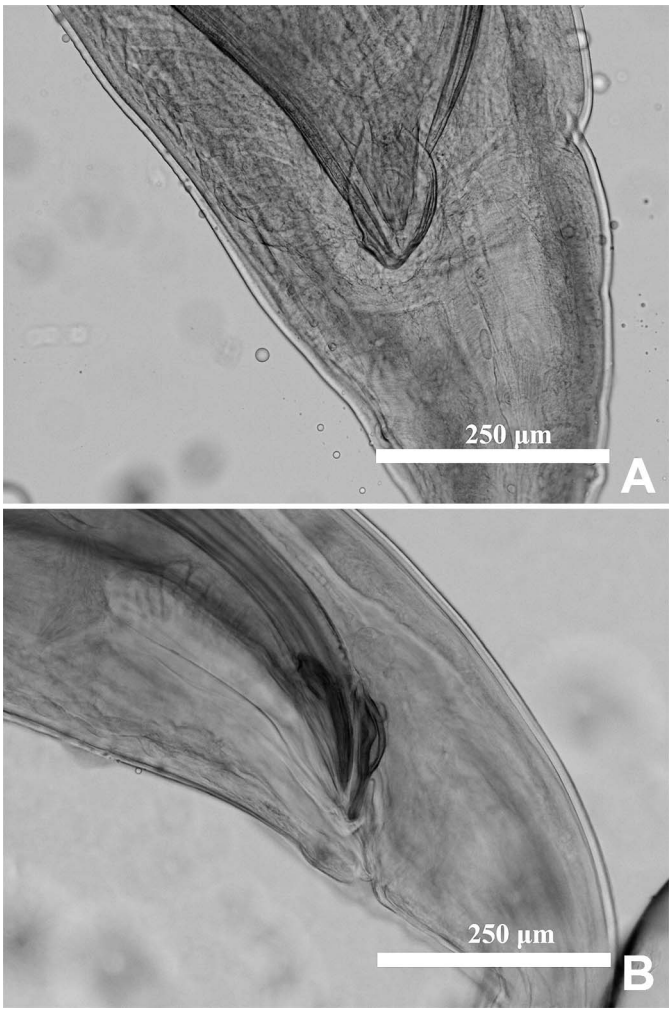

Figure 3. Microscopic images of *Falcaustra vietnamensis* n. sp. (**A**) Ventral view of gubernaculum. (**B**) Lateral view of gubernaculum.

papillae (see Table II). Moreover, 5 species have been described from lizards in the other biogeographical regions: *Falcaustra papuensis* Bursey, Goldberg and Kraus, 2007, *Falcaustra tannaensis* Bursey, Goldberg, Hamilton and Austin, 2010 (Australian biogeographical region), *Falcaustra belemensis* Baker and Bain, 1981, *Falcaustra costaricae* Bursey, Goldberg and Miller, 2004 (Neotropical biogeographical region), and *Falcaustra stellionis* (Chatin 1875) Chabaud and Golvan, 1957 (Ethiopian biogeographical region) (Bursey et al., 2014). Among them, *F. stellionis* (*species inquirenda*) was found from a lizard (*Agama stellio L.*) in North Africa without the description of a male (Skrjabin et al., 1964). The new species is easily distinguished from the above species by the overall length of the body in males, length of spicules, and number and pattern of caudal papillae (Bursey et al., 2014). Furthermore, *F. vietnamensis* n. sp. is differentiated from *F. stewarti* by the lack of a pseudosucker, number of caudal papillae, and size of spicules (see Table II).

Molecular genetic analysis

Molecular characterization: Three *18S* sequences of *F. vietnamensis* were obtained at 945 bp in length, displaying no intraspecific nucleotide variability. Comparative *18S* sequence analysis of *F. vietnamensis* with other related taxa in the GenBank database demonstrated the highest homology with 99% sequence similarity with *Falcaustra catesbeianae* AB818380, *Falcaustra araxiana* KM200715, and *Paraquimperia africana* JF803925.

Three *ITS1-5.8S-ITS2* sequences of *F. vietnamensis* were obtained at 771–813 bp in length, displaying only 1–2 intraspecific nucleotide variability. Comparative *ITS1-5.8S-ITS2* sequence analysis of *F. vietnamensis* with other related taxa in the GenBank database demonstrated the highest homology with *Megalobatrachonema hainanensis* MH545567 and MH545568 at 96% and *Falcaustra sinensis* MF061681 at 96% within only its *ITS2* sequence. Only 1 species, *F. sinensis*, had an *ITS* sequence available in GenBank.

The length of 3 *COI* sequences obtained was 560 bp, displaying no intraspecific nucleotide variability. Limited sequences of closely related taxa are available in GenBank; based on only a 366 bp fragment of the *COI* sequence the closest related taxon in GenBank is *F. sinensis* (87%).

Phylogenetic analyses: The *18S rRNA* gene is highly conserved, and thus the resulting phylogeny provides limited phylogenetic resolution among a majority of the included taxa; however, *F. vietnamensis* fell into a clade (albeit poorly supported, and therefore a polytomy) with the other 2 *Falcaustra* species (*F. catesbeianae* and *F. araxiana*) (Fig. 4). The phylogenetic tree resulting from the *ITS1-5.8S-ITS2* alignment produced a more strongly supported tree (Fig. 5); however, due to its high level of sequence variability significant portions of the alignment were removed for phylogenetic analysis. Additionally, we could not concatenate the *18S* and *ITS* sequences for a larger phylogeny, given the current limited overlap in sequences available in GenBank. All ingroup taxa are ascaridid nematodes. *Falcaustra vietnamensis* formed a slightly weaker supported (91% posterior probability) clade with other Kathlaniidae nematodes in the ITS tree (Fig. 5), although *Falcaustra* is paraphyletic with sampled species of *Megalobatrachonema* more closely related to *F. vietnamensis*. The COI amino-acid–based phylogeny includes limited taxon sampling and, for the most part, was not strongly supported. However, *Falcaustra* is monophyletic in the COI tree with 100% posterior probability (Fig. 6).

DISCUSSION

Falcaustra is characterized by a spherical isthmus and well-developed valved bulb (Chabaud, 1978). The genus currently is distributed in all regions of the world and found infecting a wide range of hosts (fishes, amphibians, and reptiles). The most diversity occurs within the Oriental biogeographical region (37 species) including 20 species from turtles, 10 species from fishes, 3 species from frogs, 2 species from lizards, and 2 species from toads (Bursey et al., 2020). None of the described species within the genus have males that possess a gubernaculum with 2 separate pieces, which is a unique character of the new species. *Falcaustra vietnamensis* n. sp. is the 38th species assigned to the genus and the third species from a lizard host in the Oriental biogeographical region. Additionally, the new species is the second recorded from Vietnam after *F. stewarti* Baylis and Daubney, 1922, which was also reported from various turtles (*Coura mouhotii*, *Heosemys grandis*, and *Kachuga dhongoka*) (Berry, 1984).

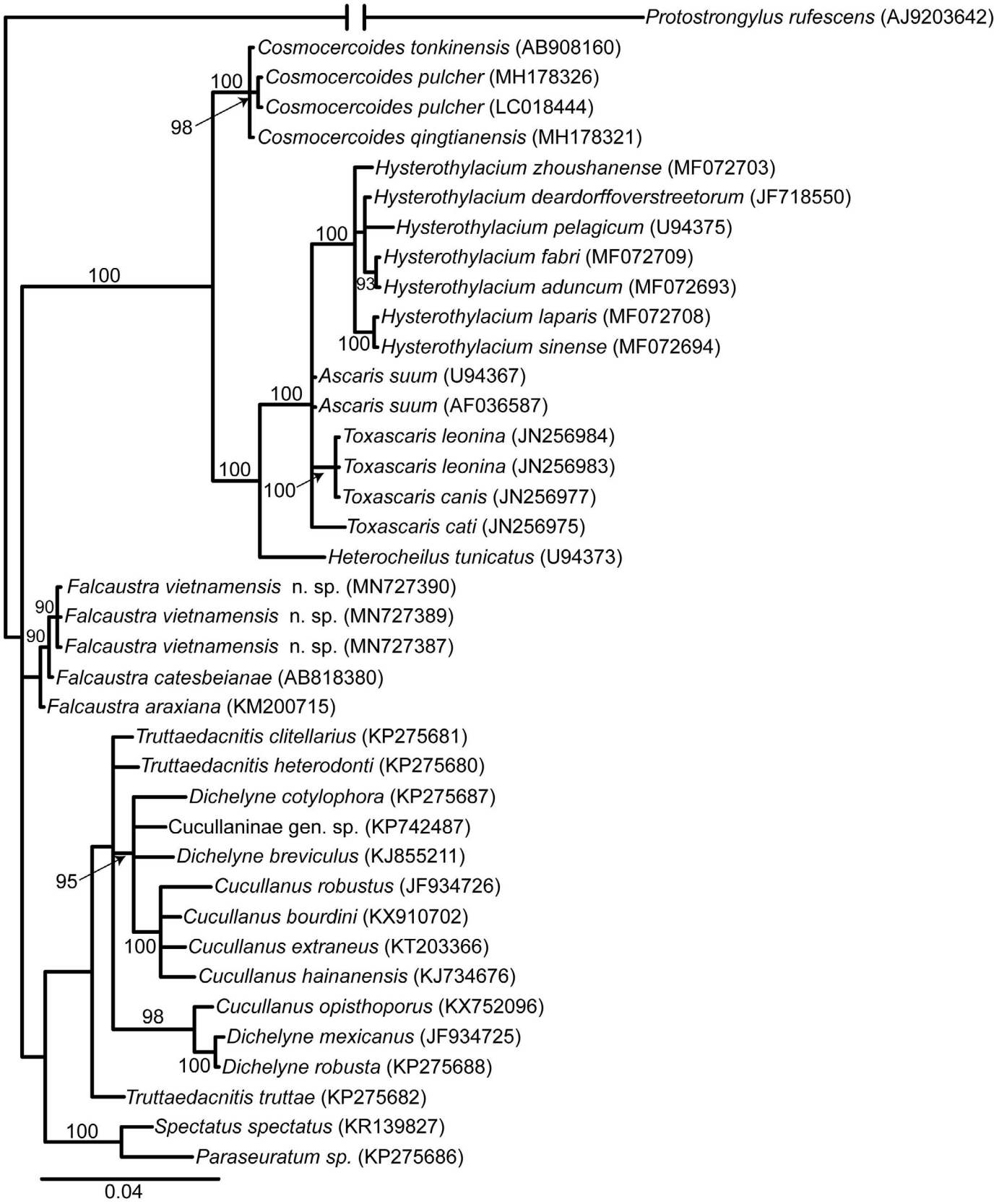

Figure 4. Phylogenetic relationships among 39 nematode specimens resulting from Bayesian analysis (3,000,000 generations) of partial sequences of the *18S* ribosomal small subunit DNA locus. Posterior probabilities greater than 90% are shown above internodes.

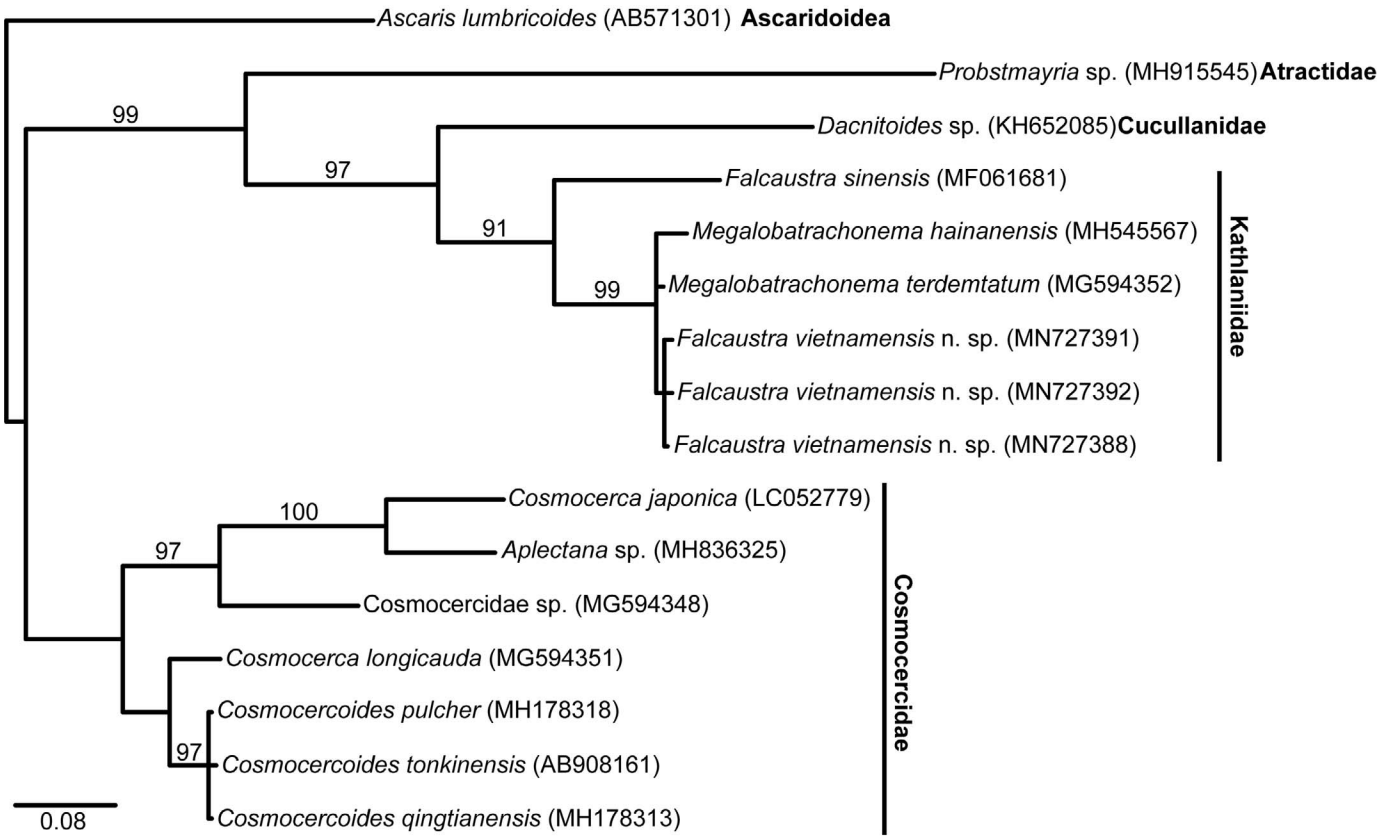

Figure 5. Phylogenetic relationships among 16 ascaridid nematode specimens resulting from Bayesian analysis (3,000,000 generations) of partial sequences of the ribosomal *ITS1-5.8S-ITS2* DNA locus. Posterior probabilities greater than 90% are shown above internodes. Nematode family indicated in bold beyond taxon names.

Table II. Morphometric comparison of *Falcaustra vietnamensis* n. sp. with morphologically related species from the Oriental biogeographical region.

Species	*F. vietnamensis* n. sp.	*Falcaustra desilvai*	*Falcaustra malaysiaia*	*Falcaustra stewarti*
Host	Lizard	Lizard	Lizard	Turtle
	Physignathus cocincinus	*Cnemaspis* aff. *tropidogaster*	*Cnemaspis mcguirei*	*Kachuga smithii*, *Hardella thurgi*
Locality	Vietnam	Sri Lanca	Malaysia	India
Reference	This study	Bursey et al., 2009	Bursey et al., 2014	Baylis and Daubney, 1922
Male	n = 9	n = 6	—	—
Body length (mm)	20.2–28.8	6.27–8.00	8.3–8.9	17.0–19.8
Body width*	557–724	230–332	281–383	600–700
Excretory pore to AE†	2,114–2,490	816–944	1,377–1,428	1,600–1,650
Preanal pseudosucker	1	Absent	One	Absent
Caudal papillae pattern‡	8-2-14+1	12-2-10+1	6-2-12+1	6-2-24 (28)+1
No. caudal papillae†	12 pairs	12 pairs	10 pairs	16–18 pairs
Spicule length	1,128–1,256	956–1,046	1,310–1,367	500
Gubernaculum				
Ventral piece length	164–192	85–116	73	Present
Dorsal piece length	155–172	Absent	Absent	Absent
Tail length	563–782	214–293	287–306	1,400–1,700
Female	n = 9	n = 6	—	—
Body length	24.2–34.1	9.09–14.08	10.9	19.0–22.6
Body width*	532–735	319–408	332	650–750
Vulva to AE†	9.5–14.0	2.82–4.67	4.34	7.75–10.3
Position of vulva	Posterior	Posterior	Posterior	Posterior
Tail length	1,000–1,408	383–561	281	2,250–2,600
Egg length	60–70	61–73	58	—
Egg width	42–47	40–49	46	—
	Unembryonated	Unembryonated	Unembryonated	Embryos

* At esophago-intestine junction.
† AE = anterior end.
‡ Precloacal-adcloacal-postcloacal+median.

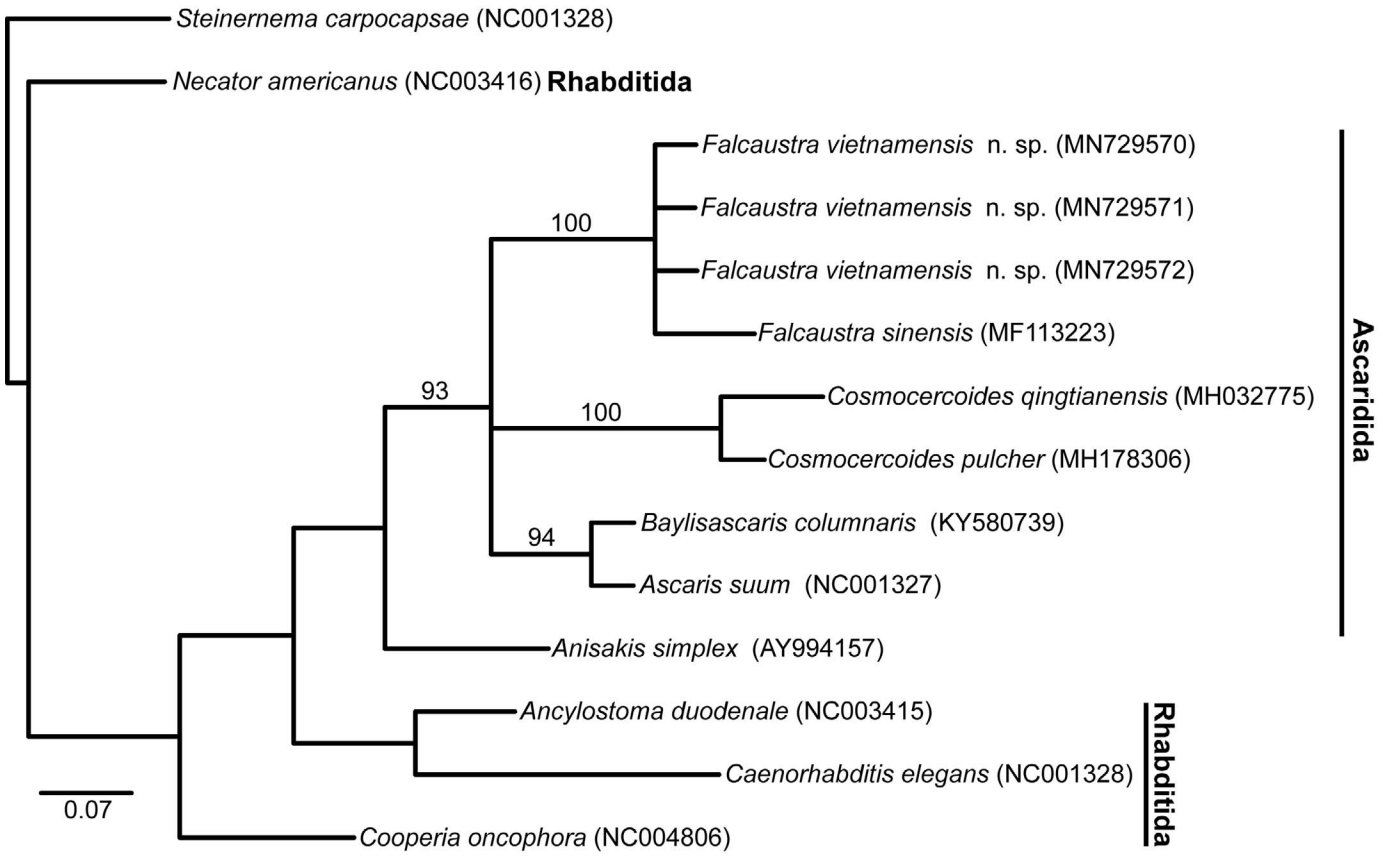

Figure 6. Phylogenetic relationships among 9 ascaridid and 4 rhabditid nematode specimens resulting from Bayesian analysis (3,000,000 generations) of partial amino acid sequences of the mitochondrial *COI* locus. Posterior probabilities greater than 90% are shown above internodes. Nematode order is indicated on vertical lines.

Sequence comparison and phylogenetic analyses support *Falcaustra vietnamensis* n. sp. as a new species. Previous studies have focused exclusively on the morphology of the genus; however, recent studies have sequenced several genes from members of the genus (Hasegawa et al., 2013; Rajabloo et al., 2016; Chen et al., 2018). In this study, genetic comparisons among *F. vietnamensis*, *F. catesbeianae*, and *F. araxiana* showed between 2 and 4 bp (including the 3 ambiguous base pairs in the *F. araxiana* sequence) differences within the 897 bp fragment of *18S rRNA* gene. Comparative analysis of the *18S* sequence also showed a close relationship between *F. araxiana* and *P. africana* (0.11% divergence within a 912 bp fragment); however, given the highly conserved nature of the *18S rRNA* gene, this further iterates the difficulty of using this gene to examine the evolutionary relationships among ascaridid nematodes. Future work including more sequences of species within the genus *Falcaustra* and more genera within the family Kathlaniidae is necessary to better elucidate their evolutionary interrelatedness.

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