



## DESCRIPTION AND MOLECULAR DIFFERENTIATION OF A NEW *SKRJABILOPTERA* (NEMATODE: PHYSALOPTERIDAE) FROM *EUTROPIS MACULARIA* (SAURIA: SCINCIDAE) IN NORTH-CENTRAL VIETNAM

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### KEY WORDS ABSTRACT

*Skrjabinoptera vietnamensis* n. sp.  
*Eutropis macularia*  
Sauria  
Scincidae  
Vietnam

*Skrjabinoptera vietnamensis* n. sp. is described from specimens recovered from the stomach of *Eutropis macularia* in north-central Vietnam. The new species is characterized by the medium-sized male worms (6.7–8.7 mm in length and 154–182 µm in width) relative to known members of the genus, 2 pointed spicules of unequal length (87–112 µm and 56–72 µm in length), and 10 pairs of caudal papillae. Female worms are larger than male worms (10.7–18.4 mm in length and 264–411 µm in width), with the vulva situated in the anterior part, and embryonated, elliptical eggs, 35–46 µm long by 20–24 µm wide. *Skrjabinoptera vietnamensis* n. sp. represents the ninth species assigned to the genus and the first species recorded from the Oriental region. Partial sequences of the 18S ribosomal RNA gene (rDNA), and cytochrome c oxidase subunit 1 (*COI*) are provided for the new species. The molecular phylogenetic position of the genus *Skrjabinoptera* is briefly discussed.

Lizards within the genus *Eutropis* Fitzinger, 1843 (syn. *Mabuya* Fitzinger, 1826), are widely distributed throughout Asia with more than 15 species (Miralles et al., 2005), five of which are found throughout Vietnam (Nguyen et al., 2009). Herein, we examined the species *Eutropis macularia* (Blyth, 1853) (Bronze mabuya) for endoparasites for the first time in Vietnam. Additionally, this is the second parasitological investigation of *E. macularia*; the other one is from an imported lizard to Slovenia from Pakistan through the pet trade, which was found to harbor no parasites (Rataj et al., 2011).

*Skrjabinoptera* was erected by Schulz (1927) to include the type species, *Skrjabinoptera colubri* (Rudolphi, 1819), collected from a snake (*Coronella austriaca* Laurenti, 1768) in Austria and divided the genus into 2 subgenera. Later the genus was revised, and the 2 subgenera were recombined based on possessing a mouth surrounded by 2 single-lobed lateral pseudolabia, each with a single internolateral tooth (Chabaud, 1978). Currently, 8 valid species within the genus have been described from reptiles around

the world. *Skrjabinoptera vietnamensis* n. sp. is the first species recorded from a skink from Vietnam and the Oriental biogeographical region. Herein, we describe this new species and differentiate it from morphologically similar species parasitic in lizards. We also provide the first DNA sequences of any *Skrjabinoptera* and provide a molecular analysis revealing phylogenetic relationships of the new species.

### MATERIALS AND METHODS

#### Sample collection and morphological analysis

Fifteen Bronze mabuya were collected by hand using a fishing rod from July 2018 to May 2020 in the central region (Bachma National Park, Thua Thien-Hue Province) of Vietnam, with body length 50–66 mm (57.6 ± 4.9). The nematode *Skrjabinoptera* sp. was found in the stomach of 9 (60.0%) skinks. The skinks were euthanized with ether and immediately dissected. Body cavities of the skinks 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

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:52B1909F-3FDE-448C-BB1E-ED217BC1DE9F](https://zoobank.org/pub:52B1909F-3FDE-448C-BB1E-ED217BC1DE9F).

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 ( $\mu\text{m}$ ) unless otherwise stated, with mean  $\pm$  1 SD and range in parentheses. Nematodes were deposited in the Vietnam National Museum of Nature, VAST, Hanoi, Vietnam, under specimen numbers VNMN\_IZ000.000.190–000.000.192.

### Scanning electron microscopy (SEM)

Samples preserved in 70% alcohol were washed 3 times in 0.2 M  $\text{Na}_2\text{HPO}_4\text{--NaH}_2\text{PO}_4$ -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 part of 2 males and a female were washed with distilled water and used for DNA extraction using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland) according to the manufacturer's recommended protocol. DNA extracts were kept at  $-30^\circ\text{C}$  until used. DNA of each specimen was PCR amplified using the following sets of primers: 18SF: CGCGAATRGCTCATTACAACAGC and 18SR: GGCGGTATCTGATCGCC for 18S rDNA (Floyd et al., 2005); and Jb3: 3'-TTTTTTGGGCATCCTGAGGTTTAT5' and Jb4: 3'TAAAGAAAGAACATAATGAAAATG-5' for COI mtDNA (Bowles et al., 1992). The PCR reaction mixture contained 25  $\mu\text{l}$  Hotstart PCR Mastermix (Promega, Madison, Wisconsin), 1  $\mu\text{M}$  forward and reverse primers, 5  $\mu\text{l}$  crude DNA extract, and sterile water up to a volume of 50  $\mu\text{l}$ . The PCR program settings were as follows: 95  $^\circ\text{C}$  for 5 min; 35 cycles with 96  $^\circ\text{C}$  for 35 sec, 52  $^\circ\text{C}$  for 30 sec, and 72  $^\circ\text{C}$  for 45 sec; and a final extension at 72  $^\circ\text{C}$  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 MW016950–MW016952 and MW015827–MW015829.

### Phylogenetic analysis

Newly obtained 18S rDNA sequences of *S. vietnamensis* n. sp. and sequences of related taxa were used in the phylogenetic analyses. GenBank numbers for taxa used in the phylogeny are provided within the tree figure and were selected based on a phylogeny published by Maldonado et al. (2020) and were obtained from GenBank and aligned using the Geneious alignment tool in the bioinformatics program Geneious (Kearse et al., 2012). The Gblocks program was used to remove poorly aligned or ambiguous positions within the alignment using the default settings outlined by Castresana (2000). The resulting 18S alignment was 679 bp long. A phylogenetic analysis was 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). *Gnathostoma turgidum* was used as an outgroup based on the phylogeny by Maldonado et al. (2020). The nucleotide substitution parameters used correspond to the TPM3+G substitution model (Kimura, 1981) as determined by jModelTest version 0.1.1 (Posada, 2008). The analysis was 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.

## DESCRIPTION

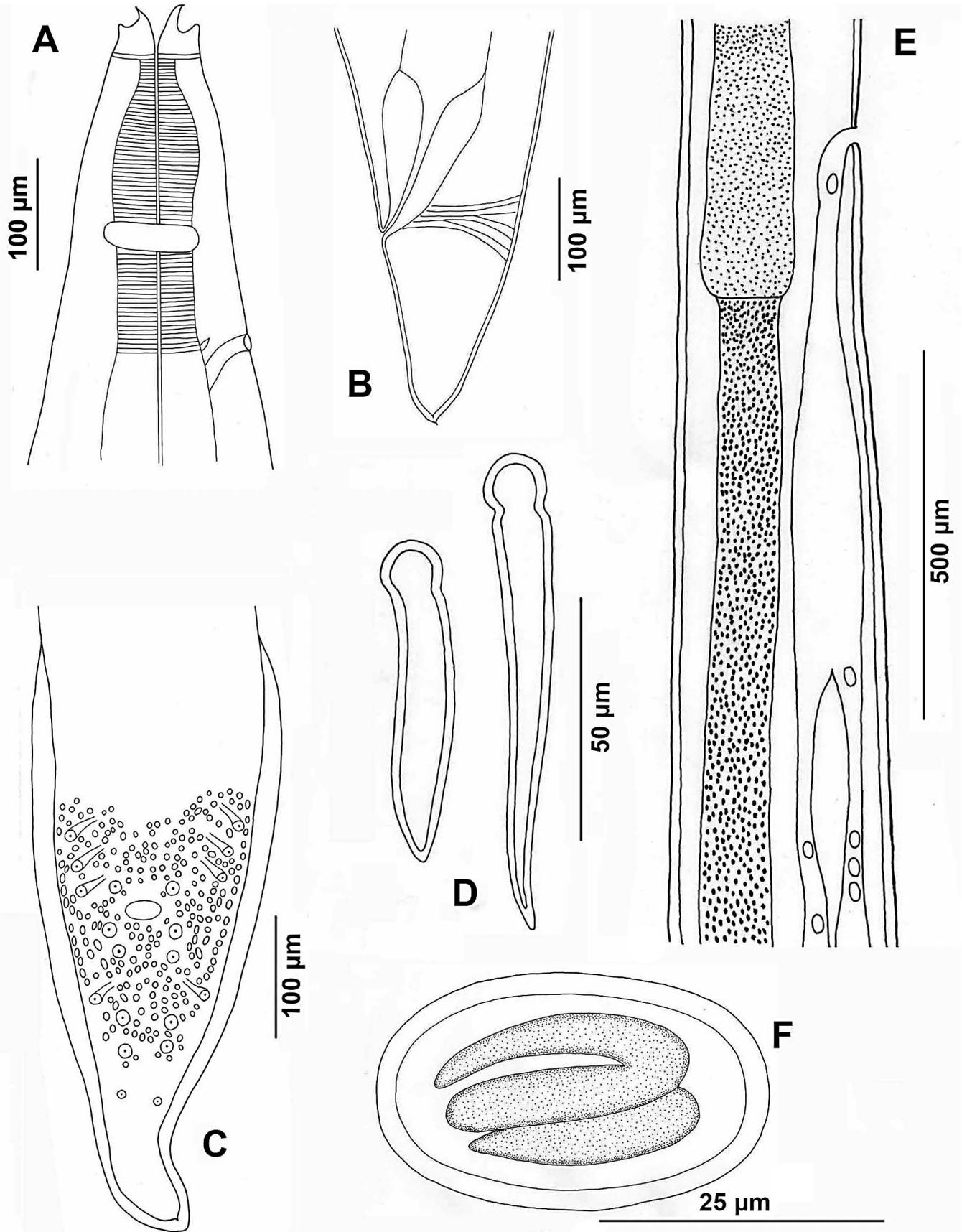
### *Skrjabinoptera vietnamensis* n. sp.

(Figs. 1 and 2)

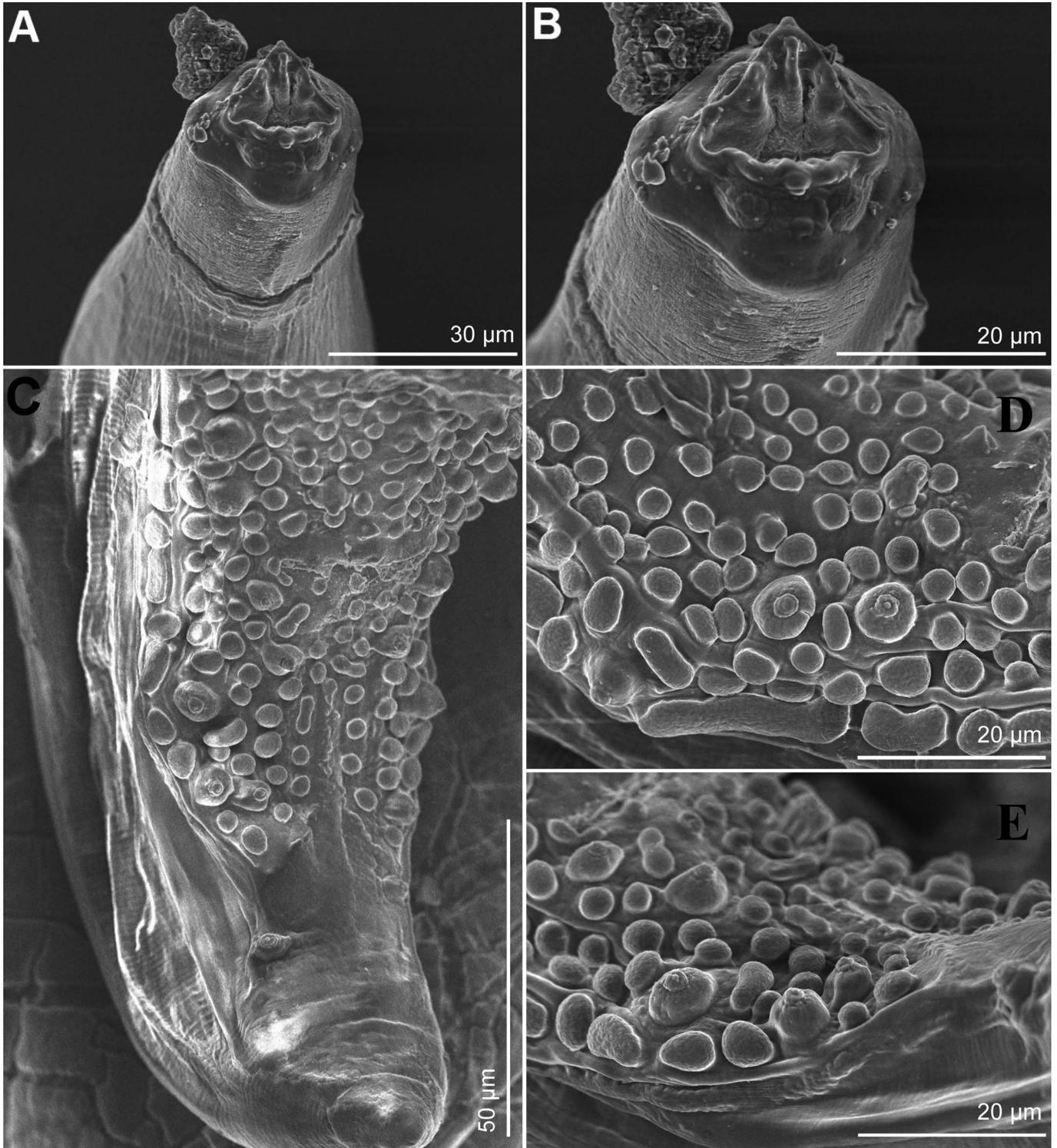
*General:* Physalopteridae Leiper, 1903, Physalopterinae Raillet, 1893, *Skrjabinoptera* Schulz, 1927. Stout, cylindrical nematodes. Sexual dimorphism not hugely evident, male worms smaller than female worms. Head with cuticular collar. Esophagus divided into anterior muscular and posterior glandular portions. Mouth elongated dorso-ventrally, surrounded by 2 single-lobed lateral pseudolabia, each with an internolateral tooth, sclerotized. Deirid located at level of anterior muscular and posterior glandular junction. Narrow caudal alae.

*Male (holotype and 8 paratypes):* Body length  $7.7 \pm 0.8$  mm (6.7–8.7 mm), width at level of buccal cavity-esophageal junction  $51 \pm 7$  (44–62), width at level of esophageo-intestinal junction  $171 \pm 13$  (154–182). Cervical collar length  $5 \pm 0.9$  (4–6). Buccal cavity  $28 \pm 2$  (25–32) deep. Esophagus divided into anterior muscular portion  $199 \pm 24$  (169–212) long and posterior glandular portion  $1,476 \pm 123$  (1,283–1,625) long. Nerve ring  $174 \pm 14$  (154–196) and excretory pore  $229 \pm 7$  (218–240) from anterior end, respectively. Deirid at level of anterior muscular and posterior glandular junction. Posterior end of body flexed ventrally. Tail  $222 \pm 18$  (192–250) with pointed end. Two unequal spicules, left, slender  $101 \pm 9$  (87–112) long; right, broader  $65 \pm 6$  (56–72) long. Narrow caudal alae extending from anterior of pedunculate papillae to end of tail. Ten pairs of caudal papillae: 4 pairs precloacal, 3 pairs ventrolateral (pedunculate papillae), and 1 pair ventral (sessile papillae) in position; 6 pairs postcloacal, 1 pair ventrolateral (pedunculate papilla), and 5 pairs ventral (sessile papillae) in position. Small cuticular verrucae filling area defined by pedunculate papillae anterior and laterally and reaching base of fifth pair of postcloacal sessile papillae posterior.

*Female (allotype and 8 paratypes):* Body length  $13.4 \pm 2.4$  mm (10.7–18.4 mm), width at buccal cavity-esophageal junction  $64 \pm 10$  (51–86), width at level of esophageo-intestinal junction  $318 \pm 49$  (264–411). Cervical collar length  $6 \pm 1$  (4–7). Buccal cavity  $35 \pm 2$  (33–40) deep. Esophagus divided into anterior muscular portion  $244 \pm 25$  (238–274) long and posterior glandular portion  $2,296 \pm 358$  (1,877–2,972) long. Nerve ring  $207 \pm 18$  (185–234) and excretory pore  $254 \pm 25$  (212–282) from anterior end, respectively. Deirid at level of anterior muscular and posterior glandular junction. Vulva located at the anterior part of the body, at level of esophageo-intestinal junction  $2,549 \pm 478$  (1,879–3,344) from the anterior end, vagina directed posteriorly and then dividing into two uteri. Eggs, oval with thick shell, embryonated  $37 \pm 3$  (35–46) long by  $20 \pm 2$  (20–24) wide. Tail pointed end, anus  $188 \pm 18$  (174–220) from posterior end of body.



**Figure 1.** *Skrjabinoptera vietnamensis* n. sp.: (A) Female, anterior end, lateral view. (B) Female, posterior end, lateral view. (C) Male, posterior end, ventral view. (D) Spicules, ventral view (E) Female, Vulva region, lateral view. (F) Egg.



**Figure 2.** SEM view of male worm, *Skrjabinoptera vietnamensis* n. sp.: (A) En face view of the anterior end, lower magnification. (B) En face view of the anterior end, higher magnification. (C) Ventral view of the posterior end, lower magnification. (D) Lateral view of precloacal papillae, higher magnification. (E) Lateral view of postcloacal papillae, higher magnification.

**Table I.** Selected characteristics of *Skrjabinoptera* spp.

Species	Host	Locality	Reference	Body length (mm)	Body width*	Spicule left	Spicule right	Papillae pattern†	No. of uteri
<i>Skrjabinoptera vietnamensis</i> n. sp.	<i>Eutropis macularia</i>	Vietnam	This study	6.7–8.7	154–182	87–112	56–72	8-0-12	2 uteri
<i>Skrjabinoptera colubri</i> (Rudolphi, 1819)	<i>Coronella austriaca</i>	Austria	Ortlepp, 1922; Drasch, 1882	5	—‡	—	—	6-0-12+1	4 uteri
<i>Skrjabinoptera phrynosoma</i> (Ortlepp, 1922)	<i>Phrynosoma cornutum</i> , <i>Phrynosoma regale</i>	Brazil	Ortlepp, 1922	11	470	530	180	6-0-14+1	2 uteri
<i>Skrjabinoptera leiocephalorum</i> Greve and Powell 1989	<i>Leiocephalus schreibe</i>	Dominican Republic	Greve and Powell, 1989	5.1–8.5	100–160	134–200	120–195	6-0-14+1	2 uteri
<i>Skrjabinoptera chamaeleontis</i> (Gedoelst, 1916)	<i>Chamaeleon gracilis</i>	Congo	Gedoelst, 1916	13.4	—	2,100	370	6-0-14+1	2 uteri
<i>Skrjabinoptera wetzeli</i> Horchner and Weissenburg 1965	<i>Agama hispida aculeata</i>	Congo	Horchner and Weissenburg, 1965	9.8–10.0	—	1,910	221	6-0-14+1	4 uteri
<i>Skrjabinoptera scelopori</i> Caballero Rodríguez, 1971	<i>Sceloporus torquatus torquatus</i>	Mexico	Caballero Rodríguez, 1971	12.5	163	274	66	6-0-14+1	2 uteri
<i>Skrjabinoptera goldmanae</i> Mawson 1970	<i>Amphibolurus barbatus</i>	Australia	Mawson, 1970	9.1–14.1	—	410–550	170–200	8-2-10+1	4 uteri
<i>Skrjabinoptera simplicidens</i> Ortlepp 1922	Sleeping lizard	Australia	Ortlepp, 1922	14–21	—	510	410	6-0-14+1	4 uteri

\* At esophageo-intestine.

† Precloacal-adcloacal-postcloacal+median.

‡ Data not available.

### Taxonomic summary

*Type specimens:* Holotype, male (VNMN\_IZ000.000.190); allotype, female (VNMN\_IZ000.000.191); and paratypes (VNMN\_IZ000.000.192) in Vietnam National Museum of Nature, VAST, Hanoi, Vietnam.

*Other materials:* Voucher IEBR (24 specimens were fixed in 70% alcohol).

*Type host:* *Eutropis macularia* (Blyth, 1853).

*Type locality:* Bachma National Park (16°14'52"N, 107°52'04"E), Thua Thien-Hue Province, Vietnam.

*Site of infection:* Stomach.

*Prevalence:* 9 (60.0%) of 15 skinks examined.

*Mean intensity of infection:* 5.7 ± 3.4 (range: 2–12).

*ZooBank registration:* urn:lsid:zoobank.org:act:846306AF-C8AE-418F-9AF4-020189A764D0.

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

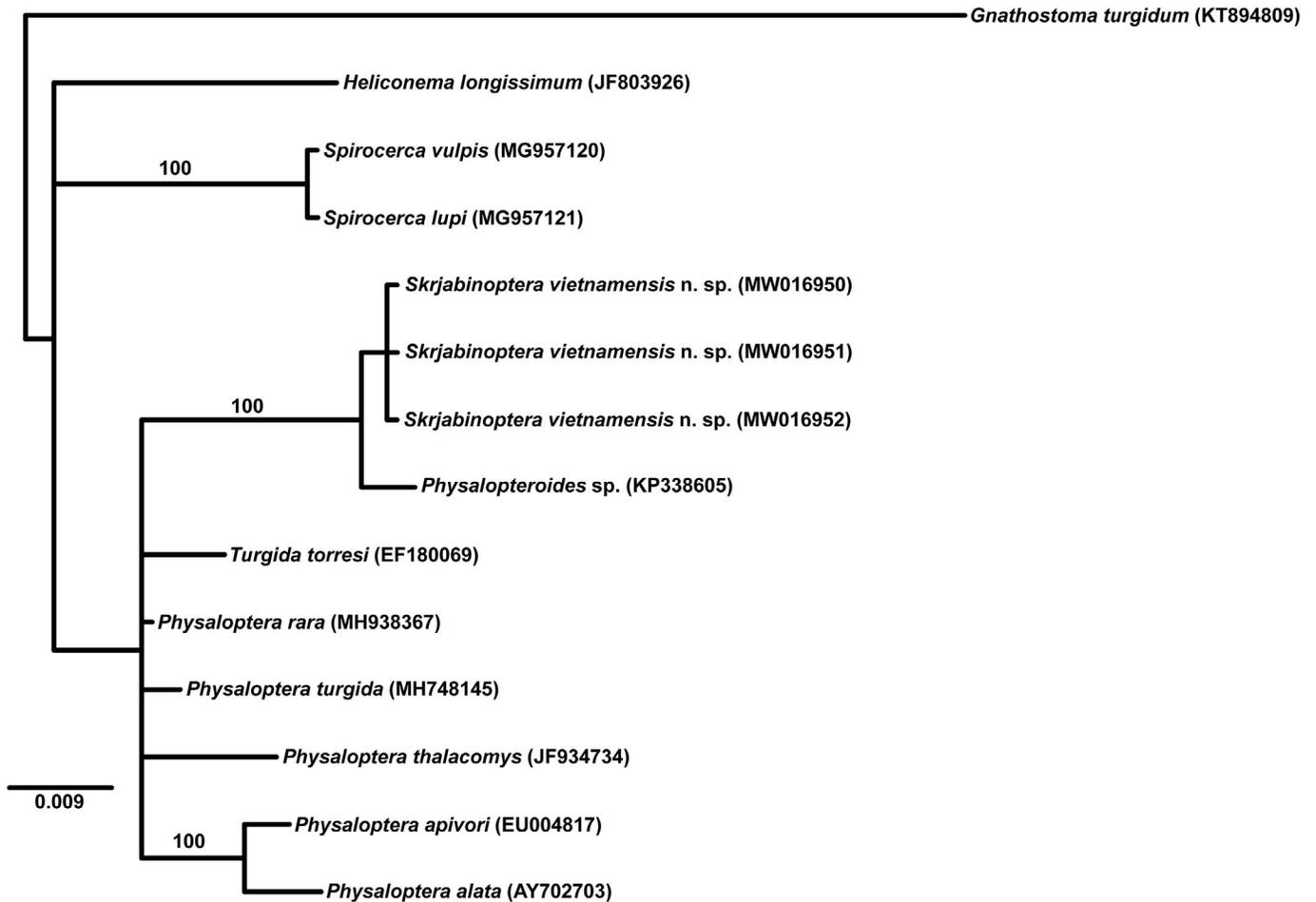
*DNA sequence data:* Nuclear 18S rDNA and mitochondrial COI sequences were generated from 3 individual worms. Sequence comparison showed 0 bp differences across 825 bp of the 18S rDNA gene and only 1 bp difference across 370 bp of the COI gene. On the phylogenetic analysis, *S. vietnamensis* n. sp. formed a strongly supported (100% posterior probability) clade with the only other lizard nematode included in the phylogeny, *Physalopteroides* sp. Relationships within Physalopteridae, however, were poorly resolved.

### Remarks

The new species, *S. vietnamensis*, is assigned to *Skrjabinoptera* based on the structure of each pseudolabium having a single internolateral tooth without an externolateral tooth. Species within *Skrjabinoptera* are differentiated by basic characteristics of the uteri number in females, length of spicules, number, and the caudal papillae pattern in males. A total of 8 *Skrjabinoptera* species were previously recognized around the world; among them, *S. vietnamensis* is distinguished from the 4 species *Skrjabinoptera colubri* (Rudolphi, 1819); *Skrjabinoptera wetzeli* Horchner & Weissenburg, 1965; *Skrjabinoptera goldmanae* Mawson, 1970; and *Skrjabinoptera simplicidens* Ortlepp, 1922, by having 2 uteri instead of 4 uteri in females (see Table I). Of the 4 species with 2 uteri (*Skrjabinoptera phrynosoma* (Ortlepp, 1922); *Skrjabinoptera leiocephalorum* Greve and Powell, 1989; *Skrjabinoptera chamaeleontis* (Gedoelst, 1916); and *Skrjabinoptera scelopori* Caballero Rodríguez, 1971), *S. vietnamensis* has the shortest spicules, without single median papilla immediately anterior to the cloaca, and differentiated by the pattern of caudal papillae in males (see Table I).

### DISCUSSION

The history of research on lizard parasites in Vietnam has tended for more than 50 yr from the first described species of *Abbreviata deschiensi* Le and Nguyen, 1966, in *Calotes versicolor* from southern Vietnam (Le and Nguyen, 1966). Up to now, only 14 lizard species have been studied for endoparasites with more



**Figure 3.** Phylogenetic relationships among 14 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. GenBank numbers given in parentheses.

than 50 parasite species, including 6 new species to science, being found (Tran et al., 2016, 2020). Isolation of the new species, *S. vietnamensis*, is of great interest for the helminth fauna of Vietnam and the Oriental biogeographical region. Identification of the new species is interesting, given the wide distribution of the *Skrjabinoptera* genus worldwide. With the inclusion of our new species, the genus includes 9 valid species mostly parasitizing lizards, except *S. colubri* (Rudolphi, 1819) from a snake, distributed in 7 biogeographical regions: Panamanian (1 species), Neotropical (1 species), Ethiopian (2 species), Nearctic (1 species), Palearctic (1 species), Australian (2 species), and Oriental (1 species).

### Molecular characterization

Our newly obtained sequences are the first representatives of *Skrjabinoptera* in GenBank and therefore the first to be included in a phylogenetic analysis of the Physalopteridae. Sequence comparison of 3 individuals of the new species showed 0 bp differences across 825 bp of the 18S rDNA gene and only 1 bp difference across 370 bp of the *COI* gene.

The topology of the tree resulting from the 18S rDNA phylogeny is very similar to that of Maldonado et al. (2020),

except for the inclusion of *Skrjabinoptera* (Fig. 3). Relationships within Physalopteridae were poorly resolved, with most species forming polytomies. However, the 3 individuals of *S. vietnamensis* formed a strongly (100%) supported clade with the only other lizard nematode included in the phylogeny, *Physalopteroides* sp. Although GenBank does not indicate the species designation of *Physalopteroides*, the authors of the sequence published it as *P. dactyluris* collected from *Hemidactylus brooki* (Brooke's house gecko) in India (Goswami et al. 2016). Future work including additional sequences of representative taxa within *Skrjabinoptera* and other Physalopteridae genera will help elucidate the phylogenetic relationships of these nematodes.

### ACKNOWLEDGMENT

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.05-2017.17.

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