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Two new species of *Siconema* (Drilonematoidea: Ungellidae) parasitic in earthworms in Vietnam, and systematic relationships as inferred from ribosomal sequence data

E. S. IVANOVA[1,3], PHAM VAN LUC[2] & S. E. SPIRIDONOV[1]

[1]*Centre of Parasitology of the A.N. Severtsov Institute of Ecology and Evolution RAS, Leninskii prospect 33, 119071, Moscow, Russia*
[2]*Vietnam National Museum of Nature, Vietnam Academy of Science and Technology., 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam*
[3]*Corresponding author. E-mail: elena_s_ivanova@rambler.ru*

Abstract

Two new species of *Siconema* (Drilonematoidea: Ungellidae) from Pu Mat Nature Reserve, Vietnam are described and illustrated. *Siconema ovicallosum* **sp. n.** is characterised by a wide female body, female caudal organs displaced to posterior extremity followed by short tail tip; males short, slim with caudal organs near cloacal opening and long, conical posterior portion of tail; cephalic hooks in both sexes with sturdy base and thin, diverging blade tips; tubular stoma incorporated in hooks base; eggs with very thick shells and two suberose polar caps. It is most similar to *S. laticaudatum* Ivanova & Pham Van Luc, 1997 but is distinguished by much smaller males, their caudal organs, and the shape and ornamentation of eggshells. *Siconema diducuncinum* **sp. n.** is characterised by a stoma that is confined in a tube that protrudes between thin hooks with widely distributed blades, caudal organs situated at long distance from the tail tip, eggs in the shape of a double-poled elongated lemon and presence of a copulatory disc in males. *Siconema diducuncinum* **sp. n.** is most closely related to *S. sinense* Timm, 1966 but differs from it by having rounded *vs* elongate pharyngeal bulb, thinner and smoother eggshells with more pronounced polar caps, twisted *vs* straight ovary and a copulatory disc in males. By an elongated tail spike and bristling hook blades, *S. diducuncinum* **sp. n.** shares similarities with *S. neozelandicum* Yeates & Spiridonov, 1996 but differs in having a non-amalgamated hook base, presence of projecting stomatal tube, differently ornamented eggshells and a copulatory disk in males. A key to species of *Siconema* is given. Phylogenetic relationships of the new species and other drilonematids are discussed.

Key words: new species, drilonematids, ungellids, earthworms, Vietnam

Introduction

The genus *Siconema* Timm, 1966 parasitizes coelomic cavities of terrestrial oligochaetes of the superfamily Megascolicoidea (Timm 1966, Spiridonov & Ivanova 2005, Spiridonov, Ivanova & Pham Van Luc 2007). It predominates in pheretimoid oligochaetes native to South–East Asia and Australasia. Several pheretimoid species (*Amynthas leucocircus* (Chen), *A. lignophilus* (Thai), *A. mucrorimus* (Chen)) are known to be particularly susceptible to invasion of drilonematids, playing host to several genera and families of parasites (Spiridonov & Ivanova 2005).

A specimen of the earthworm host can be found infected by 1–5 species of drilonematids (Ivanova & Spiridonov 1987, Spiridonov & Ivanova 1998). Typically, each nematode genus except *Siconema* and, rarely, *Homungella*, is represented by a single species. Two new species of *Siconema* described below were found in the same host, *Amynthas tuberculatus* (Gates), in Vietnam.

The genus *Siconema* presently contains 25 valid species: *S. aculeatum* Spiridonov, 1992, *S. aequicrassum* Spiridonov, 1993, *S. annamense* Spiridonov & Danilova, 1986, *S. bahli* Spiridonov & Danilova, 1986, *S. baviense* Spiridonov & Ivanova, 1998, *S. duplicoeca* Spiridonov & Danilova, 1986, *S. hatayense* Ivanova & Pham Van Luc, 1997, *S. inaequicrassum* Spiridonov, 1993, *S. laotense* Spiridonov, 1993, *S. laticaudatum* Ivanova & Pham Van

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Luc, 1997, *S. lignophilae* Spiridonov & Danilova, 1986, *S. limonovatum* Timm, 1966, *S. micrurum* Timm, 1966, *S. mucrorimae* Ivanova & Spiridonov, 1987, *S. neozelandicum* Yeates & Spiridonov, 1996, *S. ovicoronatum* Timm, 1966, *S. ovicostatum* Timm, 1966, *S ovimamillatum* Timm, 1966, *S. ovispicatum* Spiridonov, Ivanova & Pham Van Luc, 2007, *S. saccaturum* Timm, 1966, *S. siamense* Timm, 1966, *S. sinense* Timm, 1966, *S. tonkinense* Spiridonov & Ivanova, 1998 and *S. turgidum* Timm, 1966. Within Drilonematoidea, *Siconema* Timm, 1966 is already the most species-rich genus, and owing to the fact that only the smallest part of numerous pheretimoids was examined for the presence of drilonematids is expected to acquire dozens of new species. A relatively rich morphology of *Siconema* (including, for instance, a variety of body and caudal organs' shapes) together with the species richness can make the genus a useful analytical tool for the identification of units of DNA that correspond to morphological diversity within the genus. However, so far molecular data for the new species presented below are only the second report for this genus.

Material and methods

Parasitological procedures. Two infected earthworms (*Amynthas tuberculatus*) were collected during field trips in Pu Mat Nature Reserve in Central North Coastal Vietnam, prov. Nghe An, in November 2008, by S. Spiridonov. The earthworms were found in the upper soil layer on the verge of the vegetable patch in the Nature Reserve. Both earthworms were found heavily infected with 5 species of Drilonematoidea (*Iponema* sp., *Homungella* sp., *Perodira* sp., and 2 species of *Siconema* which are described below). In a single earthworm specimen, intestinal *Mesidionema* sp. was also found. Several specimens were fixed and preserved in 96% ethanol for DNA study and the rest in hot 4–5% formaldehyde for light microscopy and SEM. Formaldehyde-fixed nematodes were then processed into anhydrous glycerol for mounting according to Seinhorst (1959). Light microscopy studies and drawings were performed using a Zeiss Jenaval microscope equipped with a drawing apparatus. Abbreviations used: a, b, c—de Manian indexes, V%—percentage of anterior—vulva distance to body length. Illustrations were finished using a WACOM Intuos A4 USB drawing tablet and Adobe Illustrator CS3 according to Coleman (2003). Three specimens were used for SEM studies as follows. Material for SEM was re-hydrated after formaldehyde, dehydrated in a graded ethanol series, critical-point dried using a HCP-2 HITACHI dryer, mounted on aluminium stubs and coated with gold in a BIO-RAD SC502 sputter coater. Specimens were studied in a JCM-6380 LA SEM.

DNA analysis. DNA extraction and LSU rDNA sequencing was done as described for *Dicelis* nematodes (Spiridonov *et al*. 2005). Primer pair 'D2A' (5'_-ACAAGTACCGTGAGGGAAAGT-3') and 'D3B' (5'-TGCGAAGGAACCAGCTACTA-3') proposed by Nunn (1992) was used (citation after Ye *et al*. 2007).

The following D2D3 LSU rDNA sequences were obtained: *Siconema ovicallosum* **sp. n.**, 674 bp long (deposited in NCBI GenBank with accession number JF274483); *Siconema laticaudatum* Ivanova & Pham Van Luc, 1996, 674 bp long, JF323054; *Siconema diducuncinum* **sp. n.**, 670 bp long, JF 323055; *Homungella tonkinense* Spiridonov & Ivanova, 1997, 660 bp long, JF323056. For comparative purposes and phylogeny reconstruction, the following 28S (LSU) rDNA sequences deposited in GenBank were used: *Plectus aquatilis* Andrássy, 1985—EF417147; *Richtersia* sp.—DQ077762; *Panagrolobus vanmegenae* Holovachov & Boström, 2006—U8HM060686; *Chiloplacus demani* (Thorne, 1925) Thorne, 1937—GU062819; *Deficephalobus desenderi* Holovachov, Esquivel & Bongers, 2005—GU06282; *Pseudacrobeles bostromi* Holovachov & de Ley, 2001—HM439772; *Eucephalobus mucronatus* Kozlowska & Roguska-Wasilevska, 1963—HM439767; *Eucephalobus* sp.—HM439770; *Heterocephalobellus* sp.—DQ145638; *Cephalobus persegnis* Bastian, 1865—DQ903077; *Acrobeloides* sp.—DQ903096; *Placodira lobata* Thorne, 1937—*HM060685*; *Dicelis caledoniensis* Spiridonov *et al*., 2005—AY967867; *Dicelis lovatiana* Ivanova, 1993—AY967868; *Dicelis ussuriensis* Spiridonov *et al*., 2005—AY967869; *Dicelis rubidi* Ivanova, 1994—AY967866; *Scottnema lindsayae* Timm, 1971—HM439774; *Penjatinema novaezelandiae* Holovachov *et al*., 2009—FJ744540.

Sequence alignments (about 710 bp long) were generated using Clustal X (Thompson *et al*. 1997) under default values for gap opening and gap extension penalties. All alignments were analyzed using PAUP* 4.0b10 (Swofford 1998) for maximum parsimony (MP), distance method (NJ) and maximum likelihood (ML). The model for ML-analysis was selected with ModelTest 5.0 (Posada & Crandall 1998). The programme MtGui (Nuin 2005) was used as an interface to prepare Modeltest results for ML analysis in PAUP* 4.0b10.

Taxonomy

***Siconema ovicallosum* sp. n.**
(Fig. 1 & 2)

Measurements: Table 1.

Adult: Larger ungellids. Whole body covered by loose cuticular membrane prominent on curves and bends of body. Cuticle thin, smooth. Lateral fields not discernible. Head inclined dorsally bearing pair of dorsally directed head hooks. Hook base thick; embedded in head tissue; not divided into left and right parts. Hook blades proximally close; distal tips diverging. Blades conical in shape, proximally curved then directed parallel to hook base. Mouth aperture situated beneath distal blades. Stoma tubular, incorporated in hooks base; apical portion slightly projecting from base. Amphids situated closely to hooks on lateral sides of head; pocket-like with small pouches and half-moon-like apertures. Cephalic sensilla absent. Pharynx comprising long, curved, uniformly broad, very finely muscled corpus and twice as shorter and twice as wider glandular bulb. Bulb displaced to dorsal side of body. Fine nerve ring crossing corpus anterior to bulb. Excretory pore located opposite anterior part of bulb. Excretory duct cuticularised. Excretory gland extending to mid-body; constituting from two parts, anterior of which with darker than posterior one, content. Enormous nucleus present in each part of gland. Excretory channels wide, weakly cuticularised. Cardia and intestine developed.

Female: Body tapering towards head end, wide, tail end only slightly expanded due to vast caudal organs. Short conical tail tip present. Body maximum width at level of uterus. Overall height of hooks measured from base to tip blades 11–12 µm. Hook base 6–7µm high and 15–18 µm wide; blades 11–12 µm long, proximally 4–6 µm wide. Stoma 5–7 µm long and 2–4 µm wide. Pharyngeal corpus 9–15 µm wide; bulb 50–58 µm long and 30–38 µm wide. Excretory duct 74–90 µm long and 3–5 µm wide. Monodelphic, prodelphic. Ovary tip located in caudal region. Ovary running anteriad by dorsal side of caudal organs, twisting 2–3 times posterior to vulva level then leading anteriad by ventral body side. Developing oocytes initially spherical, then elongated. Large elongated spermatheca (80–150 µm long and 40–90 µm wide) located at flexure in 243–428 µm from anterior. Globular sperm in spermatheca 3–4 µm in diameter. Oviduct thick-walled, containing a few immature eggs. *Ca*. 20 fully formed, not embrionated eggs in thin-walled uterus. Eggs truncate-ovoid with suberose caps *ca*. 12 µm in diam. covered by *ca*. 40 tiny papilliform tubercles *ca*. 1.5–2 µm high; eggshell walls 4–5 µm thick, composed of 3 layers. Surface between caps composed from low knob-like protuberances densely covered by tinier than on poles papilliform tubercles. Vagina straight, 50–70 µm long, with thick muscular walls. Vulva lips slightly inflated. No post-uterine sack present. Vulva posterior. Anal aperture not detected. Caudal organ region occupies hind portion of postvulval region. Caudal organs externally broadly elliptical, surrounded by thin rim. Surface of caudal organs lacking cuticle. Internally, body of each organ consisting of spongy tissue penetrated by sinuous channel. Anterior to elliptical portion of organ, irregularly shaped diverticulum bulging laterad present. Posterior to diverticulum, on ventral and dorsal body sides glands (or two lobes of the same gland) of unknown function visible. Intestine thin, transparent, probably ending blindly (anal aperture not detected).

Male: Smaller in size than female. Head, pharynx, excretory system and caudal organs structure as in females. Hooks' total height 9 µm, base height 6 µm, blades 6 µm long and proximally 3 µm wide. Stoma *ca*. 2 x 2 µm. Amphid aperture 2 µm in diam. Pharynx 1.5 times shorter than in female with smaller bulb (20 x 10 µm) and thinner corpus (8 µm). Excretory duct 55 µm long and 1 µm wide. Monorchic. Testis 530 µm long, reflexes in 376 µm from anterior end, flexure 200 µm long. Spermatocytes spherical, in 2, then 3 rows. *Vas deferens* as wide as testis. Immature sperm in long ejaculatory duct *ca*. 5 µm in diam. Prominent pericloacal elevation. No genital papillae present. Tail expanding to 50 µm shortly behind cloaca due to the presence of caudal organs. Posterior to caudal organs, tail conical, narrowing from 37 µm to 3 µm. Conical portion of tail 110 µm long. Caudal organs nearly circular externally, *ca*. 31 µm in diam.

Type material. Holotype female (on the slide accession No. 1125) and paratype male (on the slide accession No. 1126) deposited in the Museum of the Helminthological Collections of the Centre of Parasitology at the Severtsov Institute of Ecology and Evolution, Moscow.

Type-host and locality. *Amynthas tuberculatus* collected in Pu Mat Nature Reserve, prov. Nghe An, Vietnam, November 2008, by S. Spiridonov.

Etymology. The species name is derived from Latin words '*ovum*'—egg and '*callosum*'—thick-skinned and refers to the structure of eggshells.

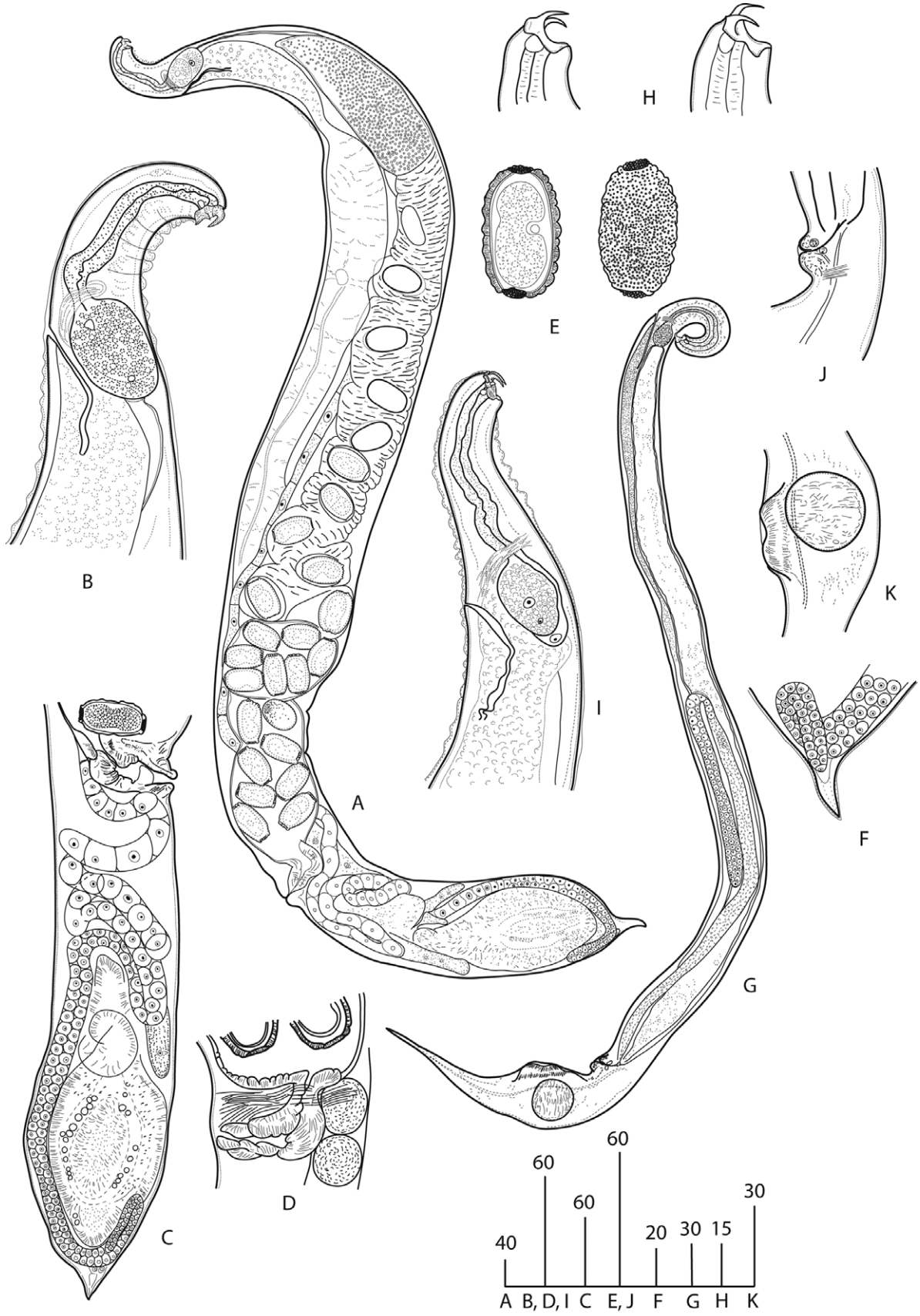

FIGURE 1. *Siconema ovicallosum* **sp. n.** A–G, female; H–K, male. A: entire worm; B: pharynx region; C: posterior region; D: vulval region; E: eggs; F: tail tip; G: hooks; H: entire worm; I: pharynx region; J: cloaca; K: caudal organ. All in lateral position. Scale bars in µm.

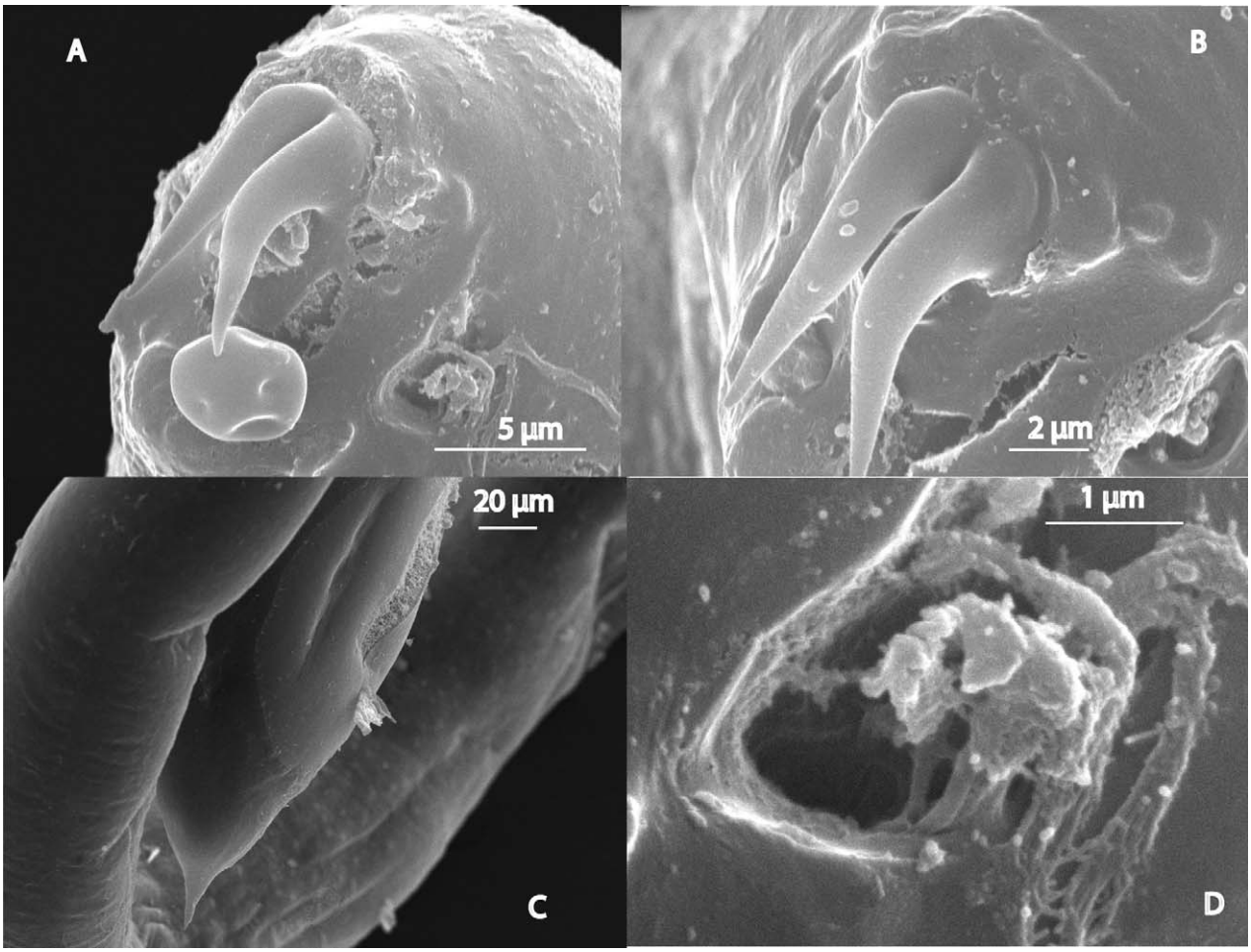

FIGURE 2. *Siconema ovicallosum* **sp. n.** SEM images. Female. A, B: head hooks, latero-dorsal view; C: caudal organ, ventral view; D: amphid, lateral view. Scale bars in µm.

TABLE 1. Morphometrics of *Siconema ovicallosum* **sp. n.** Measurements in µm.

	Female		Male
	Holotype	Paratypes	Paratype
n		2	1
L	1987	2089, 2227	895
a	11.7	12.0, 12.3	22.4
b	14.1	13.6, 13.8	9.2
c			4.69
V	75.2	76.5, 78.0	
Mid-body diam.	120	110, 117	40
Length of pharynx	141	154, 161	97
Distance from head to excretory pore	128	129, 129	81
Distance from head to nerve ring	93	106, 122	75
Length of tail			191
Egg length	56	58, 61	
Egg diam.	34	31, 34	

Diagnosis and relationships. *Siconema ovicallosum* **sp. n.** is characterised by females with a wide body, an insignificantly expanded post-vulval region, caudal organs displaced to posterior extremity, a short tail tip; short

and slim males with caudal organs located closely to a cloacal opening and a long, conical posterior portion of tail; cephalic hooks in both sexes with sturdy base and thin, diverging blade tips; the tubular stoma incorporated in hooks base; eggs with very thick shells and two suberose polar caps.

The species most closely resembles another Vietnamese species, *S. laticaudatum* Ivanova & Pham Van Luc, 1997 by having similar hooks, eggs with two polar caps and the similar position of caudal organs in both sexes. It can be reliably differentiated by males being twice shorter than females *vs* insignificantly shorter with much smaller, circular (*ca*. 31 µm in diameter) caudal organs *vs* elliptical, 55–70 x 47–95 µm in size. Cloacal flaps are less prominent in the present species and no projection protruding from anal opening is present. Females of the new species differs from *S. laticaudatum* by the shape (truncate *vs* ovoid) and ornamentation of eggshells which are much thicker (4–5 *vs* 2 µm). In *S. laticaudatum*, the surface of an eggshell is evenly ovoid and heavily mammilate, caps are convex and bearing each 14–17 tubercles *ca*. 2–3 µm high, whether in the new species eggshells uneven, knobbly (*ca*. 5 x 7 µm) and covered by tubercles less than 1 µm high. Eggshell caps in the new species are flat and bear more numerous (*ca*. 40 *vs* 14–17) tubercles. Additionally, *S. ovicallosum* **sp. n.** differs from *S. laticaudatum* by having the longer pharynx in females (141–161 *vs* 85–110 µm) with the larger, elongated bulb (50 x 30 *vs* 30 x 30 µm) and lacking a post-uterine sack. The female tail is less expanded compared to *S. laticaudatum* (129–155 µm *vs* 185–260 µm) and caudal organs more elongated (150–193 x 114–125 µm *vs* 112–140 x 92–115 µm). No transversal groove in a caudal organ of either sex is detected as well as two cuticularised channels running through the organ. Head sensilla are absent *vs* present. From *S. micrurum* Timm, 1966 females, to which *S. ovicallosum* **sp. n.** females are similar by the general appearance, they can be differentiated by the twice larger body size and wider eggs with thicker eggshells.

***Siconema diducuncinum* sp. n.**
(Fig. 3 & 4)

Measurements: Table 2

Adult: Body moderately slim, expanding at level of caudal organs and tapering to both ends, covered by folded cuticular membrane apparent in anterior region. Cuticle thin, smooth. Lateral fields *ca*. 23–27 µm wide at mid-body, displaced dorsally in posterior region; smooth, margins marked by 2 rows of ladder-like 'stitches' of modified cuticle 3–4 µm wide. Head inclined dorsally bearing pair of dorsally directed head hooks. Hook base thick; embedded in head tissue; divided into left and right parts. Hook blades widely spaced, proximally curved but distally directed almost parallel to hook base; thin, conical, with rounded tips. Mouth confined in heavily cuticularised tube *ca*. 3–5 µm high and 2–3 µm in diam. situated between hooks. Amphids situated closely to hook base; pocket-like with small pouches and half-moon-like apertures surrounded by thickened rim *ca*. 1 µm thick. Pharynx reaching bottom of mouth tube; comprising long, curved, uniformly broad, very finely muscled corpus and twice shorter and wider glandular, pear-shaped, displaced dorsally bulb. Isthmus not pronounced. Thick nerve ring crossing corpus anterior to bulb. Excretory pore situated on eminence opposite anterior part of bulb. Excretory duct heavily cuticularised, *ca*. 70 µm long and 2 µm wide. Excretory gland extending from proximity of excretory pore at least to mid-body. Excretory channels weakly cuticularised, *ca*. 5 µm wide. Intestine transparent. Cardia small. Caudal organs paired, causing swelling of middle part of tail; longitudinally elliptical, flat or hollow-like, symmetrically disposed. Each organ externally surrounded by thin rim. Posterior to caudal organs, tail narrowing to conical tip.

Female: Total hook height 17.3 ± 2.3 (16–20) µm. Blades 12.3 ± 1.5 (11–14) µm long. Stomatal tube 3.5 ± 0.7 (3–4) µm high and 3 ± 1.4 (2–3) µm wide. Amphids *ca*. 5–8 x 2–4 µm in size. Anus and rectum not discernible. Prodelphic, monodelphic. Ovary tip located near tail proximity. Ovary rounding caudal organs on dorsal side and making several twists between caudal organs and vulva, then running anteriad and turning back in *ca*. 100 µm posterior to pharynx. Spermatheca 173.3 ± 45.1 (130–220) µm long and 20 ± 2 (38–42) µm wide, not offset but morphologically distinct from oviduct. Oviduct long, thick-walled; uterus thin-walled. Small appendage *ca*. 25–30 µm long (nonfunctioning post-uterine sac?) present posterior to vulva. Vagina *ca*. 27 µm long, straight or slightly inclined posteriad. Vulva lips very slightly inflated. Eggs prolate-oval, with two slightly protruding knobs *ca*. 7 µm in diam. Eggshells 1.7 ± 0.6 (1–2) µm wide, covered in small dots more densely distributed on poles. Caudal organs elliptical, 108.3 ± 7.6 (100–115) µm long and 51.7 ± 10.4 (40–60) µm wide.

FIGURE 3. *Siconema diducuncinum* **sp. n.** A & B, female & male *in copula*; C–H, female; I, J, male. C: pharynx region; C: posterior region; D: hooks; and G, I: hooks into D, G, I: hooks; J: posterior region. Except I, all in lateral position. Scale bars in µm.

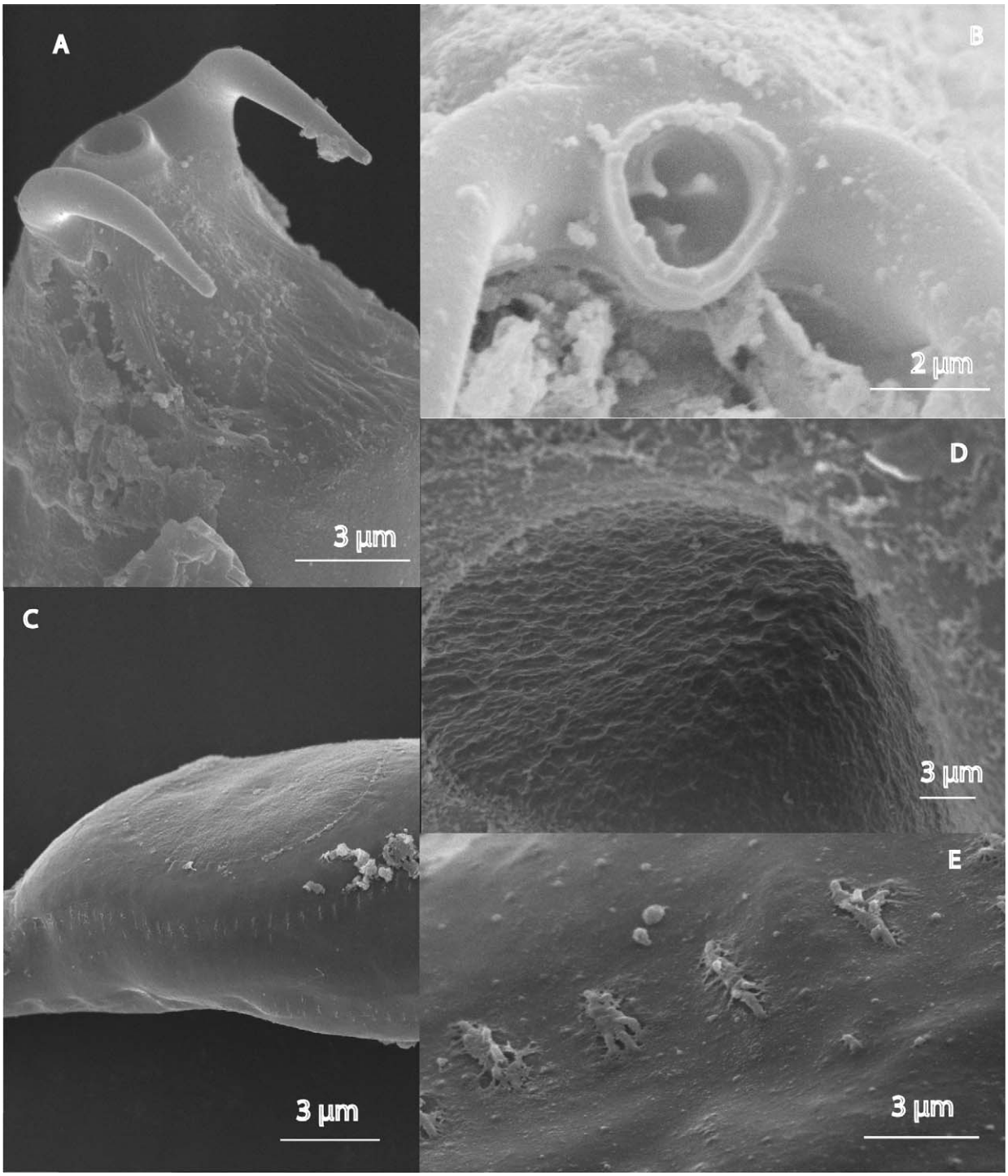

FIGURE 4. *Siconema diducuncinum* **sp. n.** SEM images. Female. A: hooks, dorsal view; B: stomatal tube, *en face* view; C: caudal organ, dorsal view; D: caudal organ, lateral view; E: margin of sub-lateral field. Scale bars in µm.

Male: About twice shorter than females. Anterior end similar in structure to female. Stomatal tube 5 ± 1.4 (4–6) µm high and 3.5 ± 0.7 (3–4) µm wide. Total hook height 16.5 ± 1.7 (15–19) µm. Blades 13.3 ± 0.3 (13–14) µm long. Intestine transparent, thin. Testis thin, reflexing in 528 ± 38 (478–564) µm from anterior, flexure 176 ± 22 (143–190) µm long. Spermatocytes in 2, then 3 rows, spherical. Spermatids *ca*. 6–8 x 7–10 µm in size. Immature sperm spherical; *ca*. 5 µm in diam. *Vas deferens* set off by constriction. Cloaca encircled by thickened copulatory disc *ca*. 30 µm in diameter. Tail elongated and slightly swollen anterior to its middle because of caudal organs. These are longitudinally oval to circular spots lacked cuticle with a centrally positioned pore not opened on surface. One pair of short ventral papillae on level of anterior third of caudal organ. Portion of tail posterior to caudal organs long, narrowly conical.

TABLE 2. Morphometrics of *Siconema diducuncinum* **sp. n.** Measurements are ranges in µm.

	Female		Male
	Holotype	Paratypes	Paratype
n		3	4
L	2080	2246±169	1228±162
a	13.9	24.2±15	24.3±3.3
b	13.9	4.4±1	8.4±0.7
c			4.5±0.3
V	57.7	60.9±1.3	
Mid-body diam.	150	94±12	47±2
Length of pharynx	150	150±6	150±9
Distance from head to excretory pore	113	124±10	131±5
Distance from head to nerve ring	90	103±5	88±5
Length of tail			273±53
Egg length	58	58±3	
Egg diam.	25	26±1	

Type-material. Holotype female (on the slide accession No. 1127) and paratype male and female *in copula* (on the slide accession No. 1128) deposited in the Museum of the Helminthological Collections of the Centre of Parasitology at the Severtsov Institute of Ecology and Evolution, Moscow. Three paratype males and 3 paratype females, each on a separate slide, are in the senior author's collection.

Type-host and locality. *Amynthas tuberculatus* collected in Pu Mat Nature Reserve, prov. Nghe An, Vietnam, November 2008, by S. Spiridonov.

Etymology. The species name is derived from Latin words '*diduco'*—spread apart and '*uncinus'*—small hook and reflects the particular appearance of cephalic hooks.

Diagnosis and relationships. *Siconema diducuncinum* **sp. n.** is characterised by a stoma confined in the tube protruding between long, thin cephalic hooks with widely distributed blades, caudal organs situated at a long distance from the tail extremity, eggs shaped similarly to a double-poled elongated lemon and a copulatory disc in males.

By having similar body size and shape, *S. diducuncinum* **sp. n.** resembles *S. sinense* Timm, 1966, *S. ovicoronatum* Timm, 1966, *S. bahli* Spiridonov & Danilova, 1986, and *S. neozelandicum* Yeates & Spiridonov, 1996.

It is most similar to *S. sinense* due to its similar body and cephalic hooks size, the caudal organs and vulva position and long, similarly shaped cephalic hooks. It differs from *S. sinense* by having a rounded *vs* elongate pharyngeal bulb, thinner (1 *vs* 5 µm) and smoother eggshells with more pronounced polar caps, a twisted *vs* straight ovary and a copulatory disc in males. The original description of *S. sinense* had presented no indication to whether cephalic hooks were diverged or closely placed but from an illustration it can be assumed that the latter is more probable.

From *S. ovicoronatum* the present species can be distinguished by a twisted *vs* straight ovary, smaller (av. 57.7 x 26.3 µm *vs* av. 70 x 40 µm) eggs with differentiated poles *vs* not differentiated and much thinner eggshells (*ca*. 1.7 *vs* 6–7 µm thick) with different pattern of ornamentation (thinly mamillate *vs* mamillate, marked with thin irregular filamentous rods and the shorter, tapering tail spike (av. 215.7 µm *vs* av. 320 µm long).

By similar body size and conical tails *S. diducuncinum* **sp. n.** shares similarities with *S. bahli* but differs by having longer, diverging hook blades with a thinner base, more posteriorly placed caudal organs and the different ornamentation of eggshells (punctated with two polar caps *vs* papillate with a single polar cap).

By having an elongated tail spike and bristling hook blades, *S. diducuncinum* **sp. n.** shares similarities with *S. neozelandicum*. However, in the latter the base of hooks is solid *vs* consisting from two not joined parts and mouth is not confined into a projecting tube but reduced; eggshells tuberculate *vs* punctate and without prominent polar differentiation *vs* with polar differentiation. Males of *S. diducuncinum* **sp. n.** can be distinguished from *S.*

neozelandicum by the presence *vs* absence of the copulatory disk and the absence *vs* presence of an aperture in the caudal organ.

Remarks. Males of *Siconema* are characterised as being of smaller size than females (from only slightly smaller to nearly twice as that) and different from female shape of caudal region and caudal organs while retaining the same structure of anterior end. Owing to the fact that males were described for not all members of the genus, we present a key to *Siconema* based of female characters only. Main diagnostic characters of *Siconema* females include the size and shape of head hooks, the size, shape and ornamentation of eggshells, the shape of a posterior body portion determined by the presence of caudal organs, the location and appearance of caudal organs and the arrangement of the ovary. Such important features as head hooks were less used in the key because many of original descriptions illustrate head hook structure in lateral position only. The natural dorsal inclination of a nematode's anterior end and dorsally directed head hooks rarely permit mounting nematodes in a way showing *en face* or dorsal view of hooks unless provided by occasional contortion of body.

Key to females of the species of *Siconema*

1 Swelling at posterior followed by long, thin, conical part of tail (caudal organs distanced from tail extremity) . . . 2
- Swelling at posterior followed by broadly conical part or short spike (caudal organs situated closely to tail extremity) . . . 13
2 Head hook blades widely spaced . . . 3
- Head hook blades situated closely to each other . . . 4
3 Eggs prolate-ovoid, not protruding on poles, av. 70 x 32 µm in size, stoma reduced . . . *S. neozelandicum* Yeates & Spiridonov, 1996
- Eggs with knobs on both poles, 58 x 26 µm in size, stoma a protruding tube 2 µm high . . . *S. diducuncinum* **sp. n.**
4 Ovary convoluted . . . 5
- Ovary straight . . . 6
5 Body 1–2.8 mm long, slim except of swelling, eggshells thick with double corona and no caps . . . *S. siamense* Timm, 1966
- Body 370–750 µm long, bloated, eggshells with single cup. . . . *S. baviense* Spiridonov & Ivanova, 1998
6 Eggs 52–53 x 31–33 µm in size, eggshells longitudinally wrinkled, margin of only pole marked by groove, caudal organs covered by hairs 30 µm long. . . . *S. lignophilae* Spiridonov & Danilova, 1986
- Caudal organs not covered by hairs, eggs of different size and coat pattern . . . 7
7 Body dramatically narrowing just posterior to caudal organs. . . . 8
- Body gradually tapers posterior to caudal organs . . . 11
8 Body 1.3 mm long or shorter . . . 9
- Body 1.8 mm or longer . . . 10
9 Body 580–790 mµ long, caudal organs *ca*. 45 x 30 µm in size, hooks 7–8 x 5–6 µm, pharynx 92–14 µm long, eggs moderately tuberculate with two tuberculate caps . . . *S. tonkinense* Spiridonov & Ivanova, 1998
- Body 690–1230 µm long, caudal organs 42–72 x 28–42 µm in size, hooks 10–11 x 6–8 µm, pharynx 135–150 µm long, eggshells moderately mamillate with two tuberculate caps . . . *S. hatayense* Ivanova & Pham Van Luc, 1997
10 Body 1800–2400 µm long, eggshells av. 70 x 40 µm in size and 6–7 µm thick with rod-like ornamentation . . . *S. ovicoronatum* Timm, 1966
- Body 2.3–3.2 mm long, eggshells 54–73 x 29–32 µm and 5 µm thick, with two polar caps . . . *S. sinense* Timm, 1966
11 Eggshells lemon-shaped, smooth, 50 x 28 µm in size . . . *S. limonovatum* Timm, 1966
- Eggshells ovoid, ornamented . . . 12
12 Eggshells 52–55 x 27–30 µm in size, surface papillate, single cap bearing ring-shaped structures, caudal organs 150 x 100 µm . . . *S bahli* Spiridonov & Danilova, 1986
- Eggshells 50–56 x 27–32 µm in size, surface cristate (covered by ridges), single cap tuberculate, caudal organs 100 x 80 µm . . . *S. annamense* Spiridonov & Danilova, 1986
13 Caudal organs broadly elliptical or circular . . . 14
- Caudal organ narrowly elliptical . . . 24
14 Ovary tip at mid-body or at vulva . . . 15
- Ovary tip in post-vulval region or near tail extremity. . . . 16
15 Body 760–1340 µm long, eggshells 57–62 x 31–35 µm in size, strongly echinate, no caps . . . . *S. aculeatum* Spiridonov, 1993
- Body 1700–2380 µm long, eggshells 62–68 x 23–25 µm in size, finely mamillate with two polar knobs . . . *S. aequicrassum* Spiridonov, 1993
16 Ovary tip in post-vulval region . . . 17
- Ovary tip near tail extremity . . . 23
17 Anus and rectum well developed, hooks displaced ventrally, eggshells 45–52 x 20–25 µm in size, thinly mamillate, with two knob-like caps, occasionally one of the caudal organs reduced lacking spongy tissue . . . . *S. inaequicrassum* Spiridonov, 1993
- Anus and rectum poorly developed or absent, hooks displaced dorsally or apical, eggs of different size and coat pattern . . . 18
18 Hook blades fused on nearly whole length, eggshells 61–62 x 28–30 µm in size with widely set acute pyramid-shaped spikes and two tuberculate caps . . . *S. ovispicatum* Spiridonov, Ivanova & Pham Van Luc, 2007

- Hook blades separated. 19
19 Eggshells with polar differentiation 20
- Eggshells without polar differentiation 21
20 Eggshells 46–49 x 24–26 µm in size, echinate, with a single, poorly differentiated cap . . . . . *S. duplicoeca* Spiridonov & Danilova, 1986
- Eggshells heavily mammilate, 56–59 x 31–36 µm in size, with 2 convex, tuberculate caps . . . . . *S. laticaudatum* Ivanova & Pham Van Luc, 1997
21 Eggshells 5 µm thick, with irregular hexagonal pattern and no polar differentiation, caudal organs 105 x 74 µm in size . . . . . *S. ovicostatum* Timm, 1966
- Eggshells thinner, with different ornamentation and polar differentiation, caudal organs of different size 22
22 Eggshells narrowly elliptical (52–64 x 19–23 µm in size), moderately mamillate, caudal organs 80 x 38 µm in size. . . . . *S. saccaturum* Timm, 1966
- Eggshells av. 54 x 20 µm in size, narrowly rounded at ends, finely punctated, caudal organs 64 x 44 µm in size . . . . . *S. micrurum* Timm, 1966
23 Ovary convoluted, eggshells truncate-ovoid, 56–61 x 31–34 µm in size, surface uneven, knobby, weakly tuberculate, 2 flat, tuberculate caps, caudal organs 150–193 x 114–125 µm in size . . . . . *S. ovicallosum* **sp. n.**
- Ovary slightly winding but making no loops, eggshells ovoid, 55–60 x 27–29 µm in size, surface with rod-like pattern with delicate coating, 2 caps from flattened knobs, caudal organs 55–90 µm in diameter. . . . . .*S. laotense* Spiridonov, 1993
24 Eggshells without polar differentiation 25
– Eggshells with two polar caps, finely mamillate. . . . . *S. mucrorimae* Ivanova & Spiridonov, 1987
25 Ovary tip near tail extremity, eggshells av. 51 x 27 µm in size and 4 µm thick, heavily mamillate. . . . . *S ovimamillatum* Timm, 1966
- Ovary tip near anterior end of caudal organs, eggs of different size and coat pattern 26
26 Body *ca.* 2 µm long, pharynx 160–208 µm, hooks 12 x 11 µm in size, eggshells av. 58 x 34 µm in size, bearing irregular hexagonal pattern . . . . . *S. turgidum* Timm, 1966
- Body 1.1–1.5 mm long, pharynx 80–100 µm long, hooks 9 x 8 µm in size, eggshells narrowly rounded at ends, av. 52 x 20 µm in size, finely mamillate . . . . . *S. micronchium* Timm, 1966

Phylogeny

Three algorithms (maximum parsimony, neighbor joining and maximum likelihood) were used to reconstruct relationships within the superfamily Drilonematoidea on the basis of LSU rDNA sequences. The GTR+G+I model was selected (-lnL = 6293.36186) for maximum likelihood analysis. The topology of MP and NJ trees was very similar with all main clades present in trees obtained with both methods. Slight differences were related to the support level of basal nodes in the trees. In the clades representing the Drilonematoidea nematodes, the support levels for both basal and inner nodes were nearly identical in both MP and NJ trees (Fig. 5, A, B). Remarkably, *Pseudacrobeles bostromi* was in the basal position in the well-supported clade containing also *Homungella* and *Siconema*, both in MP and NJ trees. In the ML tree, the majority of inner nodes of the tree collapsed but two moderately supported clades containing Drilonematoidea nematodes were still present (Fig. 5, B). As in MP and NJ trees, the clade containing *Siconema* and *Homungella* also has *Pseudacrobeles bostromi* as a basal taxon in the ML tree.

Discussion

The current morphology-based systematics places the coelomic nematode parasites of earthworms into the subfamily Drilonematoidea (Rhabditida). The Drilonematoidea includes families Drilonematidae, Homungellidae, Ungellidae and Scolecophilidae which placement into Rhabditida was never argued, and the contradictory Creagrocercidae which many morphological characters do not support its placement with Drilonematoidea. It was discovered earlier (Spiridonov *et al*. 2005) based on analysis of 18S SSU and 28S LSU rDNA sequences that the genus *Dicelis* (Drilonematidae, Drilonematoidea) constitutes a well supported clade with several cephalobid genera. When representatives of other Drilonematoidea families, Ungellidae (*Siconema ovispicatum*) and Homungellidae (*Perodira minuta*), were collected and SSU rDNA sequences obtained, the further analysis was carried out and the more extensive trees were constructed. In this analysis, *Siconema* and *Perodira* clustered together forming a clade positioned at the base of the phylogenetic tree including nearly all studied cephalobid genera (Spiridonov *et al*. 2007). The position of *Dicelis*, which was not a member of the *Siconema* and *Perodira* clade, was consistent with the previous analysis (Spiridonov *et al*. 2005).

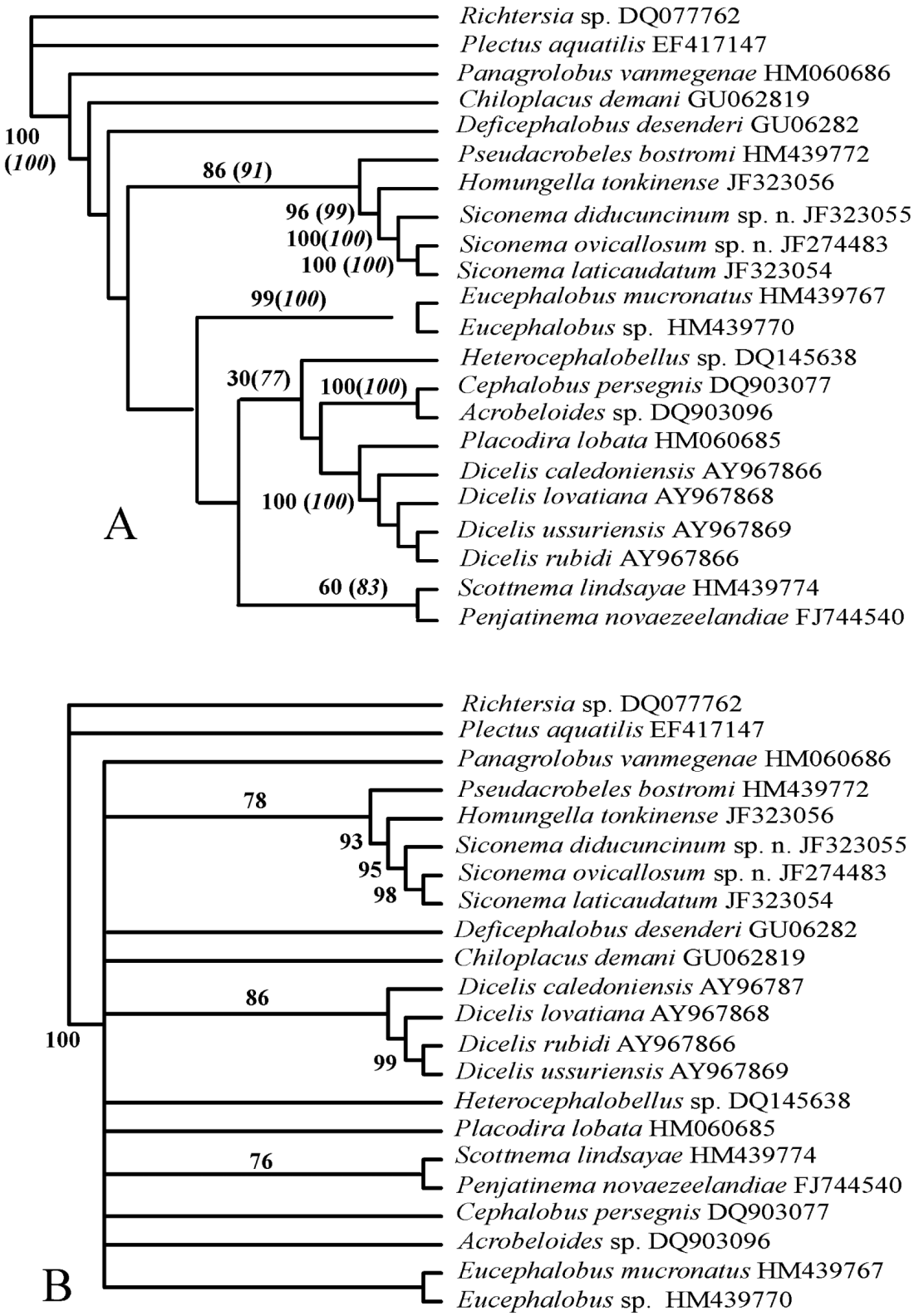

FIGURE 5. Phylogram of the relationships between nematodes of the genera *Siconema* and *Homungella tonkinense* from Pu Mat National Park and other nematode genera based on the partial sequence of D2D3 expansion segment of LSU rDNA. A—Tree obtained with maximum parsimony and distance (NJ) optimality criteria. Number of bootstrap replicates = 10000; of 718 total characters: 276 characters are constant, 127 variable characters are parsimony-uninformative, number of parsimony-informative characters = 315. Bootstrap values of MP and NJ analysis (in parentheses) are indicated near appropriate nodes. B—Maximum likelihood analysis, model selected - Model selected: GTR+G+I; – lnL = 6293.3618, Number of bootstrap replicates = 100, Bootstrap values are indicated near appropriate nodes.

In the current analysis based on LSU (28S) rDNA sequences for three newly sequenced representatives of Ungellidae and one sequence for a species of *Homungella* (type genus of the family Homungellidae), similar patterns demonstrating the paraphyletic nature of Drilonematoidea were shown. Similarly to SSU rDNA analysis (Spiridonov *et al*. 2007), two well-supported and distantly related clades (one of *Dicelis* and another including Ungellidae and Homungellidae) were defined.

The long-suspected non-relatedness of *Creagrocercus* (Creagrocercidae) to the rest of Drilonematoidea was recently confirmed by LSU and SSU rDNA analyses which demonstrated its close affinity with *Domorganus* (Ohridiidae, Plectida) (Ivanova & Spiridonov 2011). Still, more sampling of other drilonematid taxa is needed to resolve relationships within several groups of a vague systematic position, particularly in the Drilonematidae family, and to place Scolecophilidae.

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