Phasmarhabditis meridionalis sp. n. (Nematoda: Rhabditidae) from a land snail *Quantula striata* (Gastropoda: Dyakiidae) from southern Vietnam

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Summary. A new nematode species, *Phasmarhabditis meridionalis* sp. n., was isolated from the land snail *Quantula striata* in Cat Tien Natural Park in southern Vietnam. The nematode is characterised by a wide stoma in adult nematodes, a cupola-shaped female tail with a filamentous spike, long, thin, slightly projecting phasmids in females and males with the longest spicules (76 (71-83) µm) within the genus featuring hook-like distal tips. Dauer larvae of *P. meridionalis* sp. n. are 839 (770-912) µm long; a lateral field in adults is a simple narrow band with marginal, slightly elevated ridges and, in dauer larvae, expressed as a central band flanked by 4 ridges (3 incisions) at each side. The molecular analysis based on partial sequences of LSU, SSU and ITS rDNA regions has been performed. Both morphologically and genetically, the new species is close to another Asian species, *P. huizhouensis* Huang, Ye, Ren & Zhao (2015).

Key words: description, ITS rDNA sequences, LSU sequence, molecular, Mollusca, morphology, morphometrics, new species, *Pellioditis*, phylogeny, SSU, taxonomy.

A new species of the genus *Phasmarhabditis* Andrássy, 1976 was found in the pallial cavity of a terrestrial bioluminescent gastropod, *Quantula striata* (Gray, 1834) (Gastropoda: Stylommatophora: Dyakiidae), in Cat Tien Natural Park in southern Vietnam in January 2017. Recently, a number of new species of the genus was described all but one in close association with slugs (Tandigan *et al.*, 2014, 2016; Nermuť *et al.*, 2016a, b, 2017; Huang *et al.*, 2015). Except for *P. huizhouensis* Huang, Ye, Ren & Zhao, 2015 found in rotten leaves, all *Phasmarhabditis* species have similar host associations. Sudhaus (2011) has considered the relationships between these nematodes and their gastropod and earthworm hosts as necromenic. According to Huang *et al.* (2015), members of *Phasmarhabditis* either are slug-parasites or associated with slugs, snails or earthworms.

The situation with the status of *Phasmarhabditis* was discussed in Huang *et al.* (2015) who showed that the revised *Pellioditis sensu* Sudhaus "is a taxon basically equivalent to *Phasmarhabditis sensu* Andrássy containing stem species of the '*Papillosa*' group, *i.e. P. papillosa*, *P. hermaphrodita* and *P. neopapillosa*". Despite the priority of *Pellioditis* over *Phasmarhabditis*, all recently discovered novel species were treated under the name of *Phasmarhabditis*. To avoid further taxonomic confusion in the present paper, we will use this name as well. Moreover, Nermuť *et al.* (2016a) suggested that synonymy of *Pellioditis* Dougherty, 1953 and *Phasmarhabditis* Andrássy, 1976 'can be rejected' based on molecular studies. The opinion on the monophyly of this group of nematodes in the recent studies based on the molecular approach (Tandigan *et al.*, 2014, 2016; Huang *et al.*, 2015; Nermuť *et al.*, 2016a, b, 2017) was controversial.

MATERIAL AND METHODS

Two specimens of medium-sized snail, *Q. striata* were collected in Cat Tien Natural Park in southern Vietnam in January 2017. Snails were dissected and nematode juveniles found in the mantle cavity, three in each snail. One juvenile from each snail was frozen individually for DNA extraction and the rest was used for propagation. Each pair of juveniles

was placed in a Petri dish containing small parts of the snail body on a wet filter paper and kept at room temperature. The progeny was collected and preserved in hot 4-5% formalin for morphological examination and several specimens were also frozen for DNA extraction. After depletion of the food resources, nematodes died away (after 3 weeks of growth) before the mass production of infective juveniles occurred. Live nematodes from the culture were photographed using a Leica microscope equipped with a digital camera. Nematodes preserved in formalin were processed to anhydrous glycerin for light microscopy as described by Seinhorst (1959).

Light microscopic studies and drawings were done using Nicon Eclipse 200 microscope equipped with a drawing attachment. Scale bars are given in micrometres (µm). Abbreviations: V% – distance from anterior extremity to vulva to body length in %; a, b, c – de Manian indices. Illustrations were prepared using WACOM Intuos A4 USB drawing tablet and Adobe Illustrator CS5. For the SEM studies, formalin-preserved material was dehydrated, critical point dried and coated with gold. Images were taken on a CamScan (Cambridge).

Molecular characterisation and phylogenetic analysis. For DNA extraction, juveniles recovered from the snail and mature females and males from cultures were frozen individually in 0.7 ml Eppendorf tubes. DNA was extracted according to the method described by Holterman *et al.* (2006). The worm-lysis solution (950 µl of a mixture of 2 ml of 1M NaCl, 2 ml of 1M Tris-HCl, pH 8 plus 5.5 ml of deionised water plus 10 µl of mercaptoethanol and 40 µl of proteinase K, 20 mg ml^{-1}) was prepared directly before DNA extraction. Aliquots of 25 µl of sterile water and 25 µl of worm-lysis solution were added to each tube with a nematode and incubated at 65°C for 90 min. The tubes containing the homogenate were then incubated at 99°C for 5 min to deactivate proteinase K. About 1.0 µl of homogenate was used as PCR template.

PCR reactions were performed using Encyclo Plus PCR kit (Evrogen®, Moscow, Russia) according to the manufacturer's protocol. Primer pairs Nem18S_F (5'-CGC GAA TRG CTC ATT A CA ACA GC-3') and Nem18S_R (5'-GGG CGG TAT CTG ATC GCC-3') were used to obtain partial (about 900 bp long) sequence of 5' half of the mitochondrial 18S rDNA (Floyd *et al.*, 2005). PCR cycling parameters included primary denaturation at 94°C for 5 min followed by 34 cycles 94°C for 45 s, 54°C for 60 s and 72°C for 1 min, followed by post-amplification extension at 72°C for 3 min.

A pair of primers LSU391 (5'-AGC GGA GGA AAA GAA ACT AA-3') and LSU501 (5'-TCG GAA GGA ACC AGC TAC TA-3') was used to amplify a 1100 bp long sequence of D2D3 expansion segment of 28S rDNA (Nadler *et al.*, 2000). PCR cycling parameters included denaturation at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 54°C for 35 s, and 72°C for 70 s.

Table 1. Morphometrics of *Phasmarhabditis meridionalis* sp. n. Measurements are in µm and in the form mean (range).

Character	Female		Male	Dauer larva
	Holotype	Paratypes	Paratypes	
n		8	9	9
L	1728	1612±294 (1057-1931)	1317±140 (1159-1526)	839±45 (770-912)
a	19.9	17.8±3.1 (14.3-24.6)	22.1±2.0 (19.2-25.8)	24.5±1.2 (23-25.8)
b	7.9	7.6±1 (5.6-8.5)	7.1±0.5 (6.5-7.6)	5.4±0.2 (5.3-5.7)
c	22.2	24.2±2.3 (19.0-32.9)	41.5±3.9 (34.2-46.8)	7.4±0.5 (7.0-8.0)
V%	56.1	52.5±1.8 (50.3-56.1)	–	–
Mid-body diam.	87	94±27 (43-134)	60±7 (45-67)	34±2 (32-38)
Pharynx length	220	212±16 (185-230)	185±11 (174-208)	156±5 (149-164)
Head to excretory pore	192	172±23 (142-210)	176±13 (164-190)	125±4 (120-130)
Head to nerve ring	168	154±16 (133-180)	141±7 (135-154)	114±6 (103-122)
Tail length	78	68±17 (44-97)	32±5 (25-38)	116±13 (100-135)
Spicule length (chord)	–	–	76±4 (71-83)	–
Gubernaculum length	–	–	43±2 (40-46)	–
Egg length	45	53±5 (45-58)	–	–
Egg width	35	35±3 (32-40)	–	–

A pair of primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') was used to amplify approx. 800 bp long sequence of ITS region of ribosomal DNA (Curran & Driver, 1994). PCR cycling parameters included primary denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 45 s, 56°C for 60 s and 72°C for 70 s.

PCR products were visualised in agarose gel and bands were excised for DNA extraction with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA). Samples were directly sequenced using the same primers as used for primary PCR. The sequences were combined and aligned using the Clustal_X program after the addition of sequences from the GenBank (Thompson *et al.*, 1997). Subsequently, the sequences were edited using the Genedoc 2.7 program (Nicholas *et al.*, 1997), to prepare a file for the analysis in MEGA5 (Tamura *et al.*, 2011). Phylogenetic trees were obtained with different methods (MP – maximum parsimony, NJ – neighbour joining and ML – maximum likelihood) and pairwise nucleotide differences were calculated. Obtained sequences were deposited in GenBank (MG543981 for the18S rDNA sequence; MG543921 for the 28S rDNA and MG543920 for the partial 28S rDNA.

Etymology. The specific epithet reflects the southern location of Cat Tien, home to the host of the new nematode.

DESCRIPTION

Phasmarhabditis meridionalis sp. n. (Figs 1-3)

Adults. Body robust, 1.1-2.2 mm long, straight when relaxed, slightly tapering to anterior. Cuticle about 1 µm thick, bearing rows of transversal and longitudinal striations. Longitudinal striations appearing at some distance from anterior, usually posterior to 20th annulus. Lateral fields narrow (*ca* 2 µm wide), simple, narrow band with 2 marginal ridges appearing posterior to 15th annulus. Deirid located at level of cardia. Head truncate, lip region short, not or very slightly offset. Six lips arranged in three groups, each lip bearing a small, short labial papilla in apical position. Each sublateral lip bearing small cephalic papilla located slightly posterior to labial one. Pore-like amphids situated close to labial papillae on lateral lips. Mouth aperture triangular. Stoma tubular, wide and short (about as long as lip region diameter). Cheilostom not cuticularised. Gymnostom walls parallel, thickened. Stegostom with glottoid apparatus, isomorphic, isotropic, metarhabdions thickened, with three minute warts each. Pharyngeal collar narrow, covering two thirds of stoma length. Cheilostom: gymnostom: stegostom ratio 1:2.5:1. Pharynx muscular, differentiated into corpus expanded posteriorly (median bulb), stout, well defined isthmus and small, pear-shaped terminal bulb as wide as metacorpal expansion or slightly narrower. Terminal bulb with valve and haustrulum. Nerve ring surrounding middle to posterior region of isthmus. Excretory pore position variable, situated from just posterior to nerve ring to level to pharynx base, often obscure. Excretory duct short, weak. Cardia prominent, projecting into intestine. Intestine well developed, forming proventriculus at anterior. Rectal glands present.

Female. Body straight after fixation. Lateral fields running to the end of rounded part of tail or to the base of tail terminus. Stoma 7.2 ± 0.4 (7-8) µm wide and 18.8 ± 1 (18-20) µm long. Cheilostom 4 ± 0.5 (3-5) µm long, gymnostom 10.4 ± 1 (9-12) µm long, stegostom 4.3 ± 0.9 (3-6) µm long. Corpus 119 ± 7 (107-127) µm long or corresponding to 54-61 (57%) of pharynx length, *ca* 19 µm wide at anterior, metacorpal expansion 31 ± 6 (20-40) µm; isthmus 54 ± 7 (40-60) µm long and 14 ± 2 (10-17) µm wide; terminal bulb 41 ± 5 (33-50) µm long and 35 ± 5 (26-42) µm wide. Excretory pore situated at 172 ± 23 (142-210) µm from anterior body end. Oviparous. Gonads amphidelphic, ovaries reflexed on dorsal side. Ovary branches equally long. Oocytes rounded, large, arranged in two or three rows. Oviduct short. Spermathecae filled with sperm cells. Uterus spacious, containing 20-30 eggs, 53 ± 5 (50-67) µm long and 36 ± 3 (30-40) µm wide and with thin, smooth shells. Vagina straight, muscular, less than half corresponding body diam. long or 35 ± 13 (18-55) µm. Vulva median, a wide transverse slit with flat lips. Massive copulatory plug over vulva present in fertilised specimens. Rectum inflated, about corresponding body diameter long. Anus an arcuate slit. Tail end cupola-shaped with filamentous terminus 40 ± 8 (27-50) µm long, corresponding to *ca* 67% of total tail length. Phasmids thin, slightly projecting, located at terminus base.

Male. Slightly shorter and much slimmer than females. Lateral field posteriorly reaching bursa level. Stoma 6 ± 1 (5-7) µm wide and 17 ± 1 (15-18) µm long. Corpus 107 ± 9 (90-118) µm long or corresponding to *ca* 58% of pharynx length, 15 ± 2 (12-17) µm wide anteriorly. Metacorpal expansion (bulb) prominent, 24 ± 3 (19-26) µm wide. Isthmus 41 ± 7 (35-50) µm long and 13 ± 1 (11-14) µm wide. Basal bulb 34 ± 7 (27-51) µm long and 27 ± 3 (22-30) µm

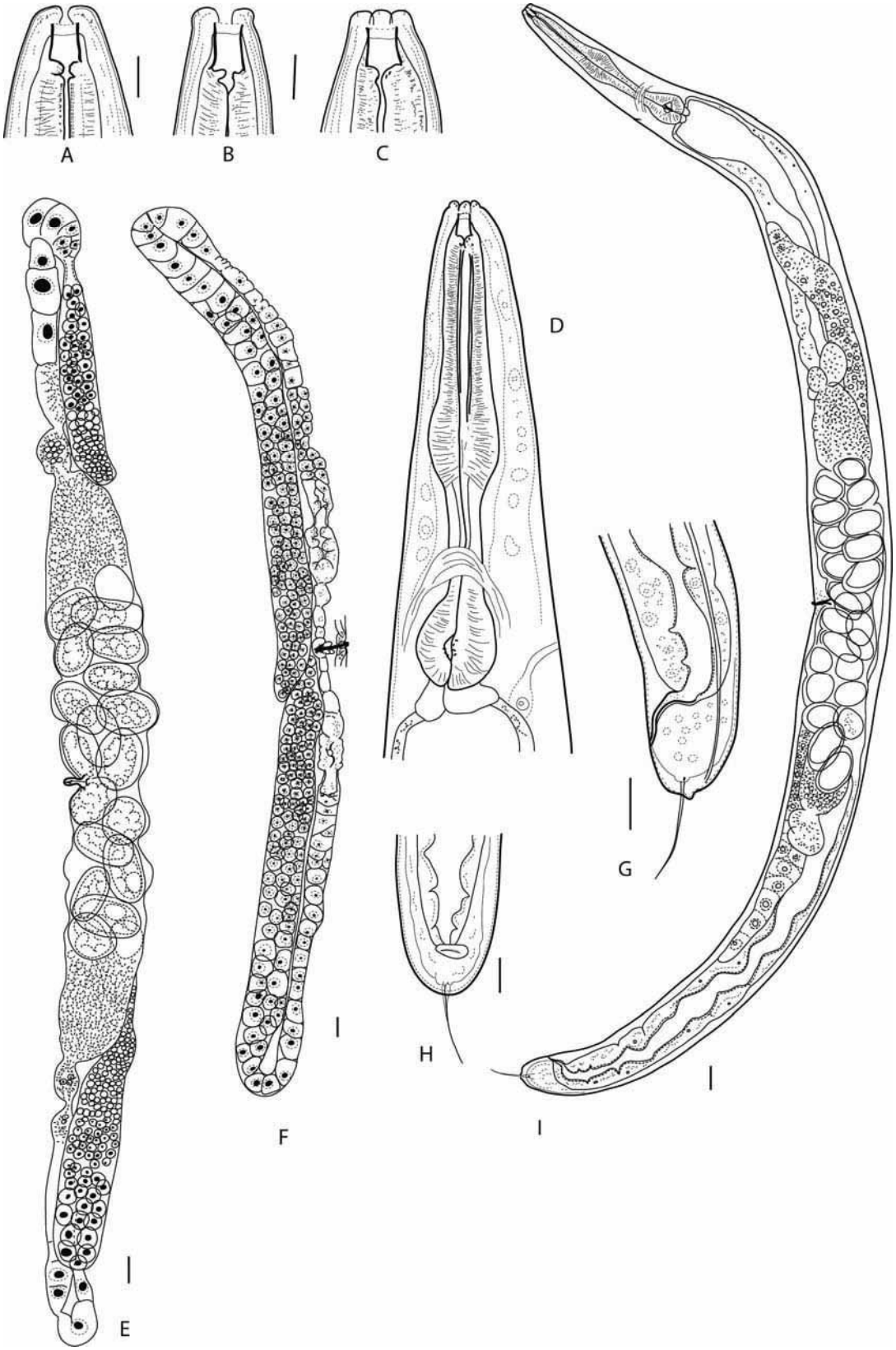

Fig. 1. *Phasmarhabditis meridionalis* sp. n. Female. A, B, C: head, lateral and sublateral; D: anterior end; E: gonad of a mature female; F: gonad of a young female; G: tail lateral; H: tail, ventral; I: entire worm. Scale bars = 10 µm.

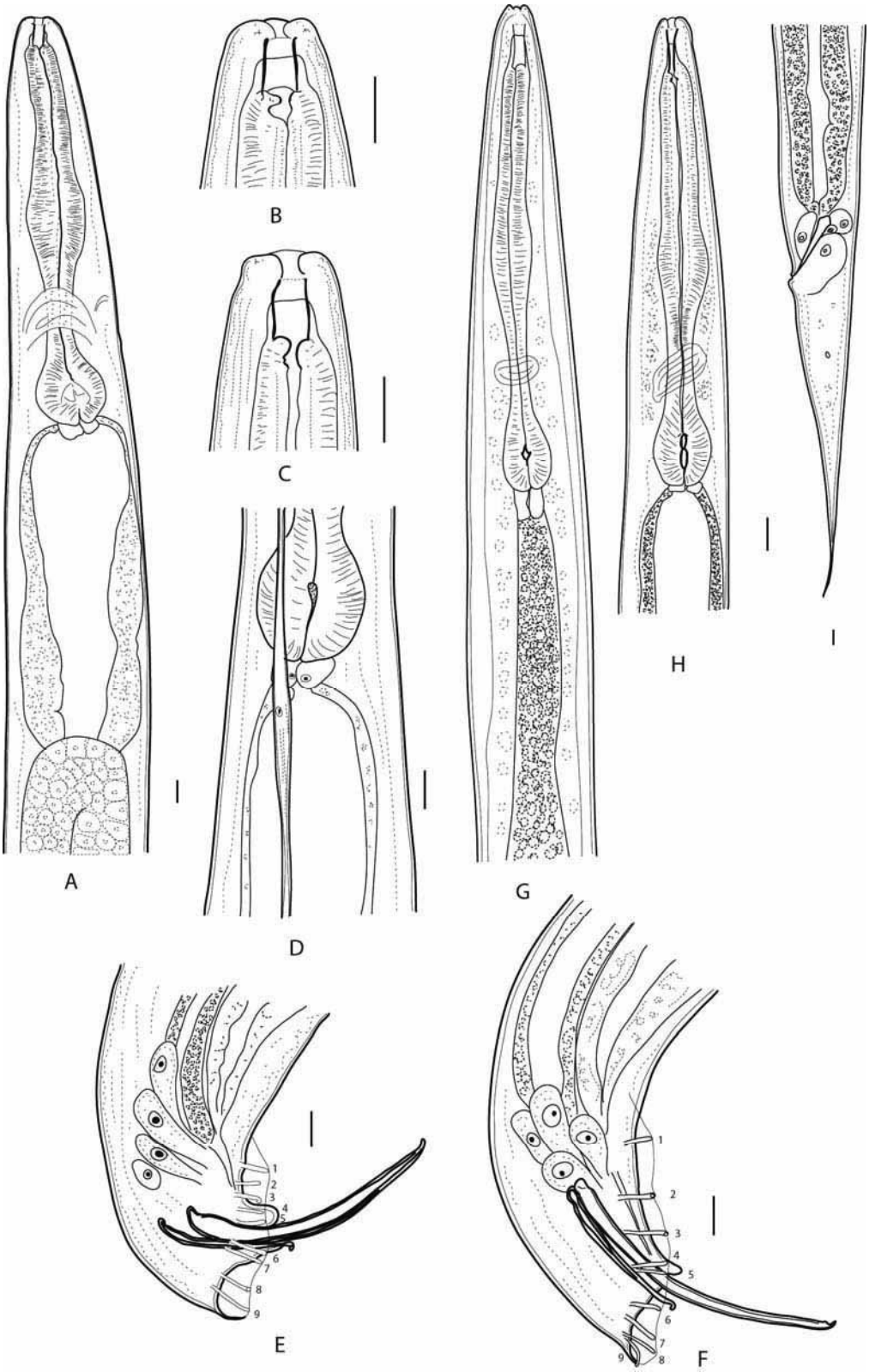

Fig. 2. *Phasmarhabditis meridionalis* sp. n. Male (A-F) and dauer larva (G-I). A: anterior end, lateral; B, C: head, subventral and lateral; D: lateral field showing deirid; E, F: tail, lateral; G: anterior end of ensheathed larva; H: anterior end of exsheated larva; I: tail of ensheathed larva. Scale bars = 10 µm. Caudal papillae are numbered.

wide. Excretory pore poorly conspicuous, situated just posterior to isthmus. Spermatocytes rounded, arranged in two rows. *Vas deferens* wide, filled with large immature sperm cells *ca* 7 × 5 µm in size, ejaculatory duct separating from latter by constriction. Single testis reflexed at 316 ± 38 (260-372) µm from anterior, reflexion 271 ± 33 (233-338) µm long. Sperm cells *ca* 4-6 µm in diam. Bursa open, peloderan. Nine pairs of pedunculate genital papillae (GP) incorporated into bursa (formula 1+1+1+2/1+3+ph). GP 8 and 9 staggered. GP 4 and GP 8 longest and opening dorsally. GP 5-7 and GP 9 not reaching the edge of bursa. Tail tip reaching the edge of bursa. Phasmids posterior to GP 9. Three pericloacal papillae present: precloacal papilla located on the anterior cloacal lip in median or submedian position and two process-like papillae situated at lateral margins of cloacal opening. Spicules nearly straight, uniformly thick at most length, tapering to tips and at least twice longer than corresponding body diameter, with small manubria about 4 µm long and wide. Lamina mean 8 (6-10) µm wide. Velum thin, almost indiscernible. Distal tips hook-like, acute, curved towards ventral body surface. Gubernaculum boat-shaped, about twice shorter than spicules; dorsal process not present; small, distinct, ventral process bent posteriad always present.

Dauer larva (ensheathed). Body slender, tapering gently towards head end. Cuticle with transversal and longitudinal striations; longitudinal striations appearing posterior to the 20-22th annulus. Lateral field with central flat band bearing low, almost indistinct ridge in the middle. Band flanked with four closely situated ridges by each side. Head rounded, lip region flat, not offset from body contour; 6 cephalic papillae; amphidial apertures situated slightly posterior to circle of head papillae. Mouth aperture enclosed. Stoma 19 ± 1 (18-22) µm long and 3.3 ± 0.5 (3-4) µm wide; cheilostom *ca* 4 µm long, not cuticularised, gymnostom *ca* 15-16 µm long, strongly cuticularised, stegostom *ca* 2-3 µm long with cuticularised metarhabdions. Pharynx comprising straight corpus 10 ± 1 (8-11) µm wide and 95 ± 3 (90-98) µm long with metacorpal expansion 15 ± 1 (13-17) µm wide, isthmus 38 ± 3 (35-42) µm long and 8 ± 1 (6-9) µm wide and pear-shaped, valvated basal bulb 23 ± 3 (18-27) µm long and 19 ± 4 (15-25) µm wide. Nerve ring surrounding isthmus in 114 ± 6 (103-122) µm from anterior end. Cardia *ca* 7 µm long, elongated, protruding into intestine. Excretory pore not observed. Deirids inconspicuous. Intestine collapsed, filled with fat globules. Genital primordium at mid-body *ca* 32 µm long. Tail conical, attenuated. Rectum collapsed. Phasmids pore-like, situated in 26-49 µm from anus.

Exsheathed juveniles. Similar to ensheated dauer larvae in general morphology. Stoma open. Cardia smaller, not elongated. Intestine with large proventriculus *ca* 45-50 µm long. Genital primordium 17-30 µm long. Rectum inflated, *ca* 24 µm long.

II stage juvenile or early III stage juvenile. Body 593 µm long and 25 µm wide. Stoma 18 µm long and 3 µm wide (cheilostom 3 µm long, gymnostom 12 µm long, stegostom 3 µm long). Pharynx 125 µm long with similar to other stages structure. Genital primordium 12 µm long.

Type material. Holotype female and paratype male, accession nos 1293 and 1294 are deposited in the Museum of the Helminthological Collections of the Centre of Parasitology at the Severtsov Institute of Ecology and Evolution, Moscow.

Type host. *Quantula striata* (Gray, 1834) (Gastropoda, Dyakiidae).

Type locality. Cat Tien National Park, 11°30’ N, 107°20’ E, collected in January 2017 by I.I. Semenyuk.

Diagnosis and relationships. *Phasmarhabditis meridionalis* sp. n. is recognised by its morphometrics, flat lips, a wide rhabditoid stoma, a high collar, a cupola-shaped female tail with a filamentous spike, long, thin, slightly projecting phasmids, lateral field in adults a simple narrow band with marginal, slightly elevated ridges and in dauer larvae, expressed as a central band flanked by 4 ridges (3 incisions) at each side, with nearly indistinct, low ridge running in the centre of the band; long spicules with hook-like distal tips, dauer larvae possessing a long cylindrical stoma and a filamentous tail and the distinct molecular characteristics of the new species.

The genus *Phasmarhabditis* Andrássy, 1976 currently comprises the following species: *P. hermaphrodita* (Schneider, 1859) Andrássy 1983, *P. neopapillosa* (Mengert in Osche, 1952) Andrássy 1983, *P. papillosa* (Schneider, 1866) Andrássy, 1976, *P. huizhouensis* Huang, Ye, Ren & Zhao, 2015, *P. tawfiki* (Azzam, 2003), *P. californica* Tandingan De Ley, Holovachov, McDonnell, Bert, Paine & De Ley, 2016, *P. bonaquaense* Nermuť, Půža, Mekete & Mráček, 2016, *P. apuliae* Nermuť, Půža & Mráček, 2016 and *P. bohemica* Nermuť, Půža, Mekete & Mráček, 2017.

Morphologically, *Phasmarhabditis meridionalis* sp. n. is most closely related to *P. huizhouensis* having the similar shape of a female tail albeit with the relatively longer filiform terminus (the ratio a wider part of tail/terminus being 2:3 *vs* 1:1). Both

species also have similarly sized spicules. However, spicules differ in the shape of the distal tip, acutely hooked in the new species *vs* obtuse with the shallow indentation in *P. huizhouensis* and female phasmids of the new species project less outside the body contour. The cuticularised cheilostom reported for *P. huizhouensis* was not observed in *P. meridionalis* sp. n. as well as in the rest of the species of *Phasmarhabditis* (Huang *et al.*, 2015).

In having the similar shape of a female tail, spicules of similar length and shape and similar body length of dauer larvae (839 µm *vs* 902 µm), *P. meridionalis* sp. n. is also close to *P. bonaquaense* but can be distinguished by generally smaller body size of adult nematodes, less projecting and thinner phasmids in the female, lateral fields in adult nematodes representing a narrow band with elevated ridges *vs* more elaborated ones with about 14 incisions and much shorter tails of both sexes (32 µm *vs* 51 µm, male and 63 µm *vs* 85 µm, female). The dauer larvae of the new species, contrary to these of *P. bonaquaense*, possess differently structured lateral fields (formed by rows of lower ridges on the sides of a central narrow band bearing even lower central ridge *vs* two prominent ridges with wide band (incisure) between them) (Nermuť *et al.*, 2016).

From another species with a cupola-shaped female tail, *P. papillosa*, *P. meridionalis* sp. n. differs by the significantly shorter female tail (mean 63 µm *vs* 106 µm) with different proportions, *i.e.* having a shorter, perfectly rounded wider part with the much longer and thinner filiform terminus while in *P. papillosa*, the wider part is rather conoid and the tail spike sturdier and is as long as the wider part of the tail. It is also differentiated from *P. papillosa* in having much longer spicules (av. 76 µm *vs* 27.7 µm) and a gubernaculum (av. 43 µm *vs* 23 µm) and flat *vs* inflated vulval lips (Tandingan De Ley *et al.*, 2016).

In having the gonochoristic mode of reproduction and a dome-shaped *vs* a widely conoid female tail, the present species can be differentiated from hermaphroditic species of *P. hermaphrodita* and *P. californica* (Tandingan De Ley *et al.*, 2016).

From the rest of species, *i.e. P. neopapillosa*, *P. tawfiki*, *P. bohemica* and *P. apuliae*, the new species can immediately be distinguished in having a dome-shaped with a filiform terminus female tail *vs* conoid one. By the body length of dauer larvae (mean 839 µm), *P. meridionalis* sp. n. is comparable with *P. neopapillosa* (mean 1010 µm), *P. tawfiki* (mean 965 µm), and *P. apuliae* (mean 812 µm and 986 µm in different strains) (Hooper *et al.*, 1999; Azzam, 2003, Nermuť *et al.*, 2016a), all with a conoid female tail.

Molecular analysis and phylogenetic position of *P. meridionalis* sp. n. The sequences of expected length were obtained for all three studied loci. The BLAST search for the obtained sequences supported the primary morphological identification as all the tested sequences demonstrated the closest relationships with those of *Phasmarhabditis* nematodes and, in some cases, nematodes of the related genus *Angiostoma*. All the sequences of nematodes of these two genera together with those of some other Rhabditida were downloaded as FASTA-files from NCBI site and used for alignment construction. A matrix of nucleotide data with the length of 853 bp was obtained for 18S rDNA sequences (flanking parts of unequal length were discarded). With 588 characters of this alignment being constant and 52 variable characters being parsimony-uninformative, the total number of parsimony-informative characters was 213. A matrix of nucleotide data with the length of 813 bp was obtained for D2-D3 28S rDNA. With 713 characters of this alignment being constant and 59 variable characters parsimony-uninformative, the total number of parsimony-informative characters was 41. A matrix of nucleotide data with the length 743 bp was obtained for ITS rDNA including 5.8S. With 257 characters of this alignment being constant and 235 variable characters being parsimony-uninformative, the total number of parsimony-informative characters was 251. Phylogenetic analysis of 18S rDNA data detected two closest sequences to that of *Phasmarhabditis meridionalis* sp. n.: KP017252 obtained for *P. huizhouensis* and KM510210 for *Phasmarhabditis* sp. 'ITD046'. Both these sequences have differed from our sequence in 26 bp while the differences with two sequences obtained from *Phasmarhabditis* collected in Czech Republic were equal to 27 bp. The majority of other *Phasmarabditis* sequences identified as the closest ones with BLAST search differed from the newly found species in 32-37 bp. Under all types of phylogenetic analysis, the 18S rDNA sequences of *P. meridionalis* sp. n. were found clustering together with *P. huizhouensis* from China under strong or medium support (Fig. 4). Surprisingly, it was a 28S rDNA sequence for *Pellioditis* sp. (KJ877242), which was identified with BLAST as the most similar one for the newly described species (32 bp difference). All the *Phasmarhabditis* species differed from the new species from Vietnam in 42-47 bp in this analysis. Still, the phylogenetic analysis demonstrated stable clustering with the low bootstrap support of *P.*

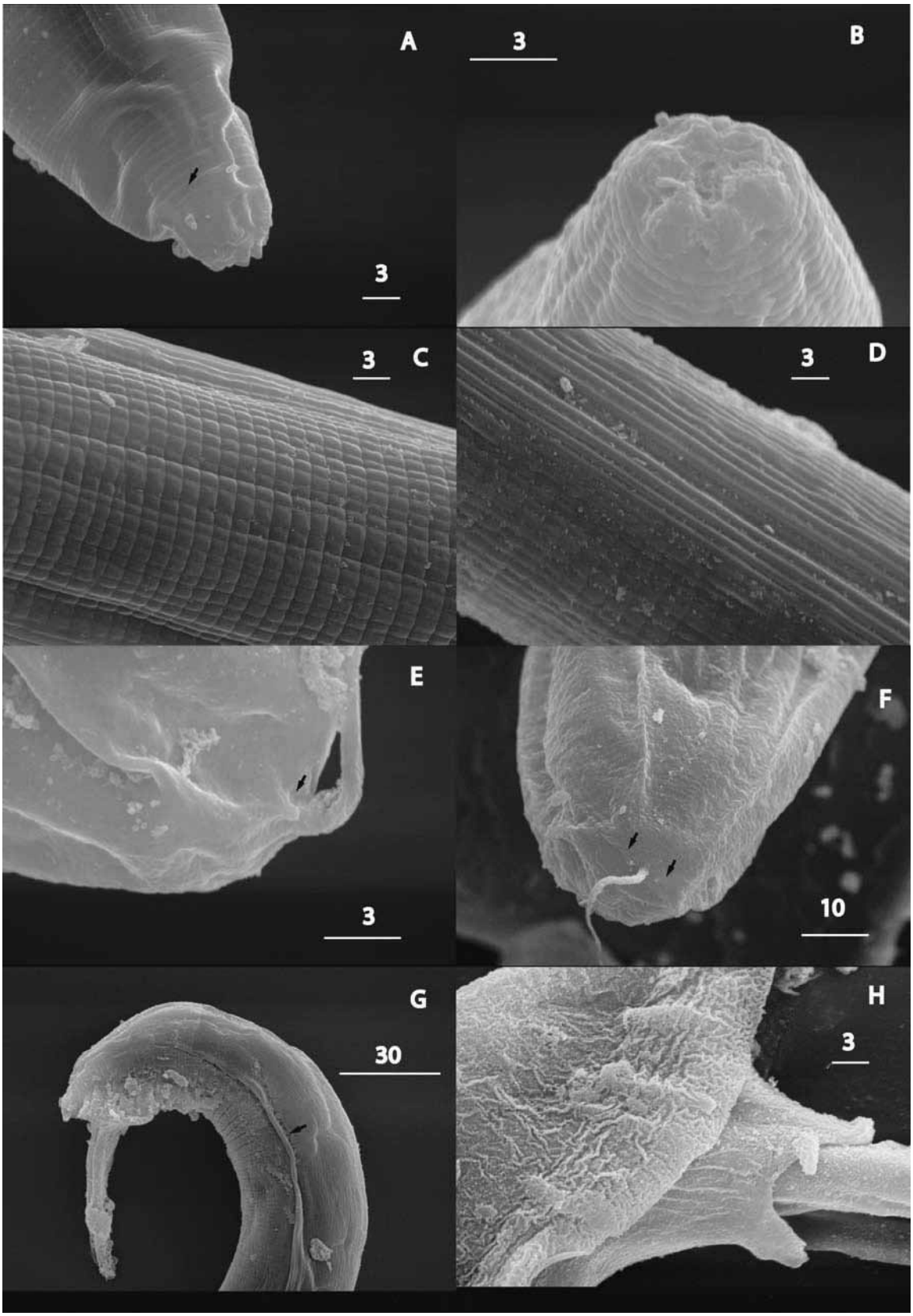

Fig. 3. *Phasmarhabditis meridionalis* sp. n. SEM images. A-D: dauer larva. A, B: head; C: cuticle; D: lateral field; E, F: female tail; G, H: male tail. Arrows indicating amphid (A) and phasmid (E). Scale bars in µm.

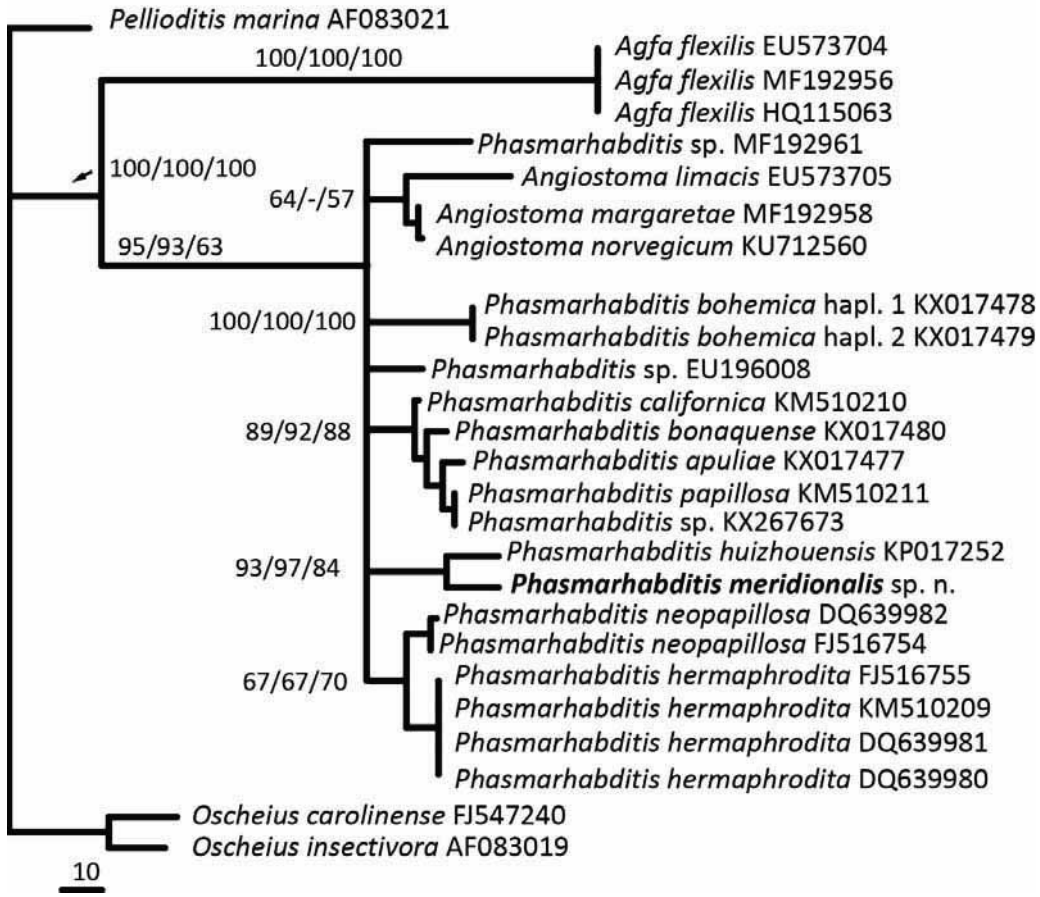

Fig. 4. Phylogenetic relationships of *Phasmarhabditis meridionalis* sp. n. based on 18S SSU rDNA. Bootstrap support values are presented near nodes as MP/NJ/ML. ML analysis (500 bootstrap replications), GTR+G model. For MP and NJ – 1000 bootstrap replications. *Angiostoma* clade with three species was absent in NJ analysis.

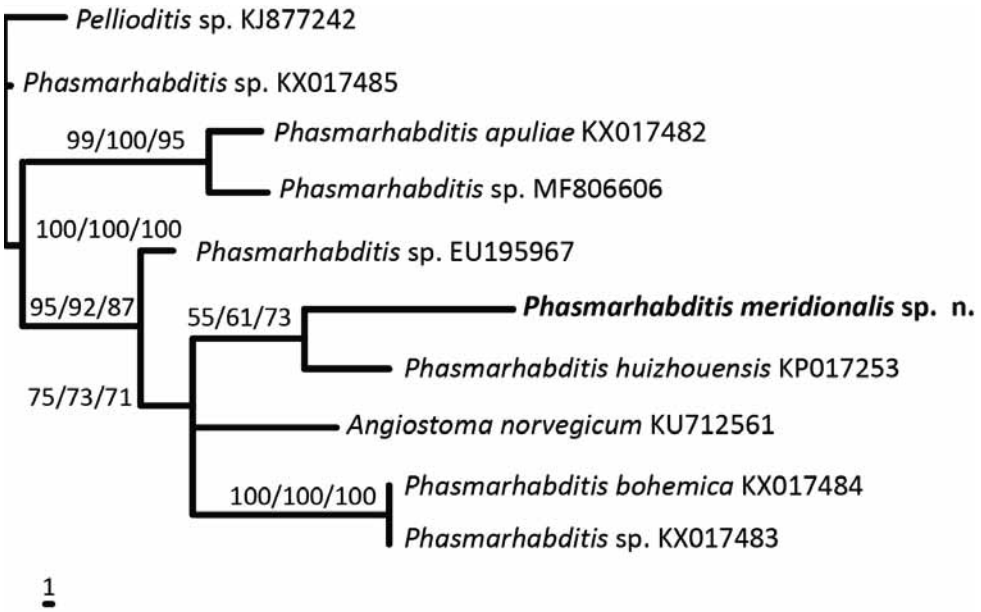

Fig. 5. Phylogenetic relationships of *Phasmarhabditis meridionalis* sp. n. based on 28S LSU rDNA. Bootstrap support values are presented near nodes as MP/NJ/ML. ML analysis (500 bootstrap replications), K2+G model. For MP and NJ – 1000 bootstrap replications.

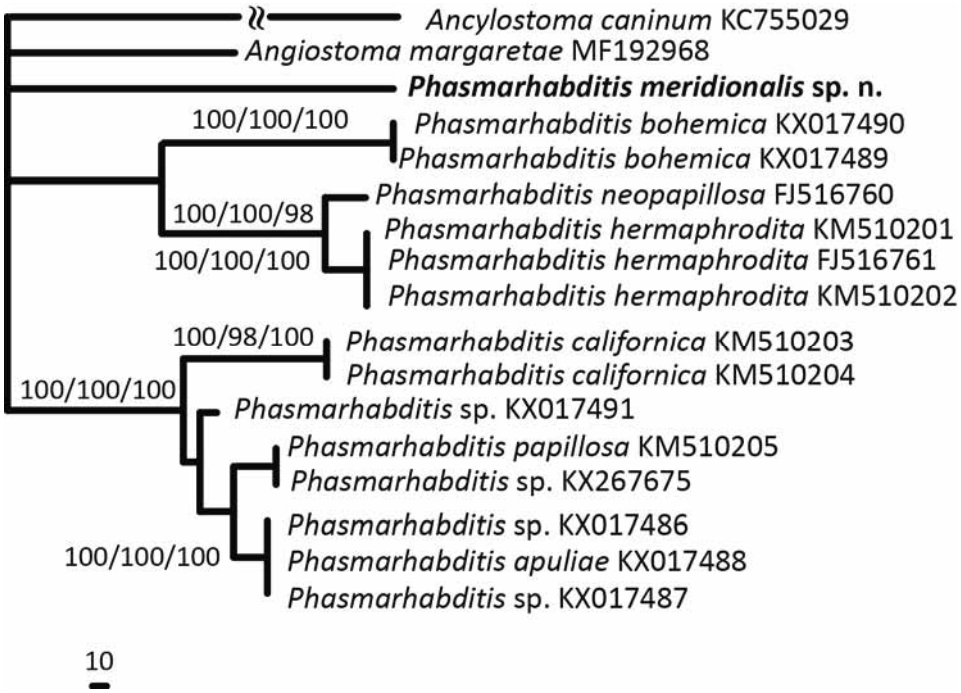

Fig. 6. Phylogenetic relationships of *Phasmarhabditis meridionalis* sp. n. based on ITS rDNA. Bootstrap support values are presented near nodes as MP/NJ/ML. ML analysis (500 bootstrap replications), T93 model. For MP and NJ – 1000 bootstrap replications.

meridionalis sp. n. with *P. huizhouensis* (Fig. 5). The phylogenetic tree based on the analysis of ITS rDNA demonstrated much stronger bootstrap support for the main *Phasmarhabditis* clades (Fig. 6) with corresponding nucleotide differences in 141-152 bp.

DISCUSSION

The morphology of *Phasmarhabditis* species is conservative and there are few traits that proved helpful for distinguishing between the species. *Pellioditis sensu* Sudhaus (Sudhaus, 2011) includes *P. hermaphrodita*, *P. incilaria* (Yokoo & Shinohara in Shinohara & Yokoo, 1958), *P. mairei* (Maupas, 1919), *P. neopapillosa*, *P. papillosa* and *P. tawfiki*. Diagnostic characters given by Sudhaus (2011) for the species of *Pellioditis* nearly perfectly conform with the morphology of those species of *Phasmarhabditis* described after his publication: "**Buccal tube relatively short and wide**; glottoid apparatus with warts; sleeve, median bulb; amphidelphic; female tail cupola-shaped or short conical; **phasmids papilliform and projecting from tail**; bursa well developed, peloderan, anteriorly open, nine GPs, formula 1+1+1+2/1+3+ph, three precloacal rays evenly spaced, **GP 4 and GP 5 very close and staggered**, often positioned precloacally, anterior and posterior dorsal papillae GP 5 and GP 9; **phasmids** posterior to GP 9, **papilliform**; spicules separate, dagger-shaped. **Necromenic** in earthworms, slugs and snails". The only departure from this is in the structure of glottoid apparatus as warts were not reported in all the species (*P. huizhouensis*). Such description of diagnostic characters in relation to *Phasmarhabditis* covers most of its morphology and leaves very little extra to add. However, the characteristics of dauer larvae are generally ignored, possibly owing to the fact that not all species descriptions included descriptions of this stage. We believe that certain morphological characteristics of dauer larvae can provide additional means for differentiation and should be taken into consideration if possible. Similar to infective juveniles of entomopathogenic nematodes of *Steinernema* and *Heterorhabditis* genera, body length, stoma size and a lateral field structure should be considered.

Three loci of ribosomal DNA were studied in the course of the study on *P. meridionalis* sp. n. All these three phylogenetic markers have confirmed the affiliation of the newly found species with *Phasmarhabditis*. In the same time, in two analyses (18S and 28S rDNA) the sequences obtained from *Angiostoma* nematodes formed common clades with the species of *Phasmarhabditis*, while such a pattern

was not observed in the ITS rDNA phylograms. However, in this analysis *P. meridionalis* sp. n. failed to join any of the inner *Phasmarhabditis* clades and formed an independent branch connected to the basal node for all other *Phasmarhabditis* with known ITS rDNA nucleotide data. Unfortunately, ITS rDNA data were not obtained for *P. huizhouensis* from China and thus this aspect could not be compared. Such pattern of phylogenetic links between *Angiostoma* and *Phasmarhabditis* as seen in 18S and 28S rDNA was reported earlier by Nermuť *et al.* (2016a, b, 2017). We can only speculate whether such adherence of the nematodes with prominently different morphology and life style can be explained by the low level of nucleotide differences in these DNA regions in the taxa under study. The differences in ITS rDNA are more prominent, and the majority of *Phasmarhabditis* species are united in well supported clades. However, the support for the basal node for three main clades of *Phasmarhabditis* is negligible resulting in the position of *P. meridionalis* sp. n. outside of these clades (Fig. 6). There is undoubtedly tight phylogenetic relationships of three genera of rhabditids with the trophic relationships with molluscs (*Agfa*, *Angiostoma* and *Phasmarhabditis*). More data are needed, especially for aberrant forms to explain the ambiguity of the 'molecular' gaps between nematodes with such pronounced morphological differences.

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Е.С. Иванова и С.Э. Спиридонов. *Phasmarhabditis meridionalis* sp. n. (Nematoda: Rhabditidae) из наземного моллюска *Quantula striata* (Gastropoda: Dyakiidae) с юга Вьетнама.

Резюме. Новый вид нематод *Phasmarhabditis meridionalis* sp. n. найден в улитке *Quantula striata* в заповеднике Кат Тиен на юге Вьетнама. Нематода характеризуется широкой стомой взрослых особей, куполовидным хвостом с длинным тонким филаментом у самок, тонкими, длинными, слегка выступающими фазмидами у самок и самцами с самыми длинными в пределах рода спикулами (76 (71-83) мкм) с крючковидными дистальными концами. Инвазионные личинки *P. meridionalis* sp. n. длиной 839 (770-912) мкм; латеральные поля взрослых особей представляют узкую полосу со слегка приподнятыми ребрами с каждого края, а на латеральных полях dauer larvae узкая центральная полоса сопровождается с каждого края 4 ребрами. Сделан анализ частичных последовательностей LSU, SSU и ITS участков рибосомальной ДНК. Морфологически и генетически новый вид продемонстрировал родство с единственным другим видом из Азии – *P. huizhouensis* Huang *et al.*, 2015.