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BIOLOGY, MORPHOLOGY, AND TAXONOMY OF AQUATIC ORGANISMS

Two New Species of Free-Living Nematodes (Nematodes, Monhysterida) from the Mouth of the Cấm River, Vietnam

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Abstract—Illustrated descriptions of two new free-living nematodes found in the Cấm River mouth in Vietnam are given. *Eumonhystera rivalis* sp. n. is morphologically similar to *E. dispar* (Bastian, 1865); *E. sudanensis* Zeidan et al., 1989; and *E. maxima* Gagarin, 1996, but differs by the presence of biocrystal-like bodies, amphid fovea located closer to anterior body end, and longer cephalic setae. *Daptonema borealis* sp. n. is similar to *D. vicinus* (Riemann, 1966); *D. aquedulcis* (Gagarin, 1987); and *D. salinae* Gagarin, Gusakov, 2014, but has shorter body, shorter cephalic setae, and spicule size.

Keywords: Cấm River, free-living nematodes, new species, *Eumonhystera rivalis* sp. n., *Daptonema borealis* sp. n., Vietnam

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INTRODUCTION

The study of the fauna of free-living nematodes in the water bodies and watercourses of Vietnam began about 15 years ago in connection with the compilation of a data bank on the aquatic fauna of Vietnam. Currently, large-scale research into the fauna of nematodes is being conducted. Data on the fauna of free-living nematodes in mangroves and in the mouths of some rivers have been published in a number of articles (Gagarin, 1987; Tchesunov and Nguyen, 2010; Nguyen et al., 2011; Gagarin, 2018).

This study aims to provide an illustrated description of two new species of free-living nematodes.

MATERIALS AND METHODS

Twenty samples of nematodes were collected in May 2016 by colleagues from the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam) in the Cấm River mouth. Samples were taken from a boat using a bottom cylindrical core; the nematodes in the samples were isolated by centrifuging in the salt solution LUDOX-TM 50. The nematodes were defined and measured under an MBB-1 light microscope (Seinhorst, 1959). A Nikon Eclipse 80i light microscope equipped with a DIC contrast, a Nikon DS-Fil digital camera, and a PC equipped with NIS-Elements D 3.2 software for analysis and documentation were used for measuring, species definition, photographing, and drawings of the nematodes.

Abbreviations: *a*, ratio of body length to its maximal width; *b*, ratio of body length to pharynx length; *c*, ratio of body length to tail length; *c*, ratio of tail length to body width in the anus or cloaca area; *L*, body length, μm; *V*, the ratio of the distance from vulva to the anterior end of the body to the body length, %.

RESULTS

Species description. Order Monhysterida Filipjev, 1929. Family Monhysteridae de Man, 1876. Genus *Eumonhystera* Andrassy, 1984.

Eumonhystera rivalis Gagarin sp. n. (Figs. 1, 2).

Material. Holotype ♂, slide no. Vu 2.2.16, paratypes: 3♂♂, 2♀♀. The holotype preparation has been deposited in the collection of the Museum of Nature of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam); preparations of paratypes are in the collection of nematodes of the Department of Nematology of the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).

Location. Northern Vietnam, Hai Phong Province, Cấm River mouth, depth 1 m, water salinity 12–15‰, sandy bottom sediments. Coordinates: 20°40′22″–20°40′57″ N, 106°42′48″–106°42′58″ E.

Description. The morphometric characteristics of the holotype and paratypes are given in Table 1.

Males. Small, rather thick worms. The anterior end of the body is narrowed. The width of the labial area is 3–4 times narrower than the diameter of the body in the cardium area. The cuticle is smooth.

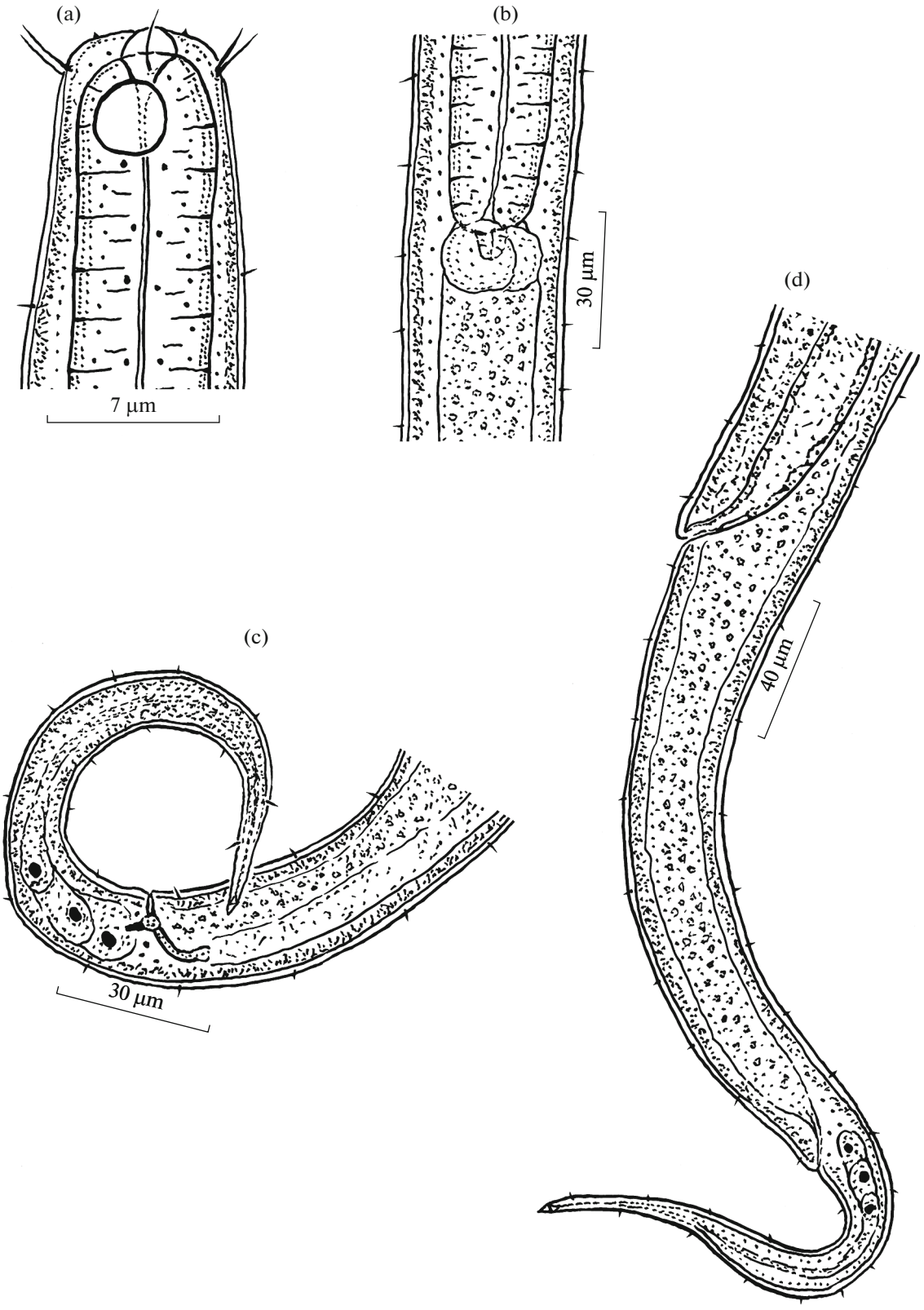

Fig. 1. Details of the structure of *Eumonhystera rivalis* sp. n .: (a) head, male; (b) body in the cardium area; (c) posterior end, male; and (d) posterior end, female.

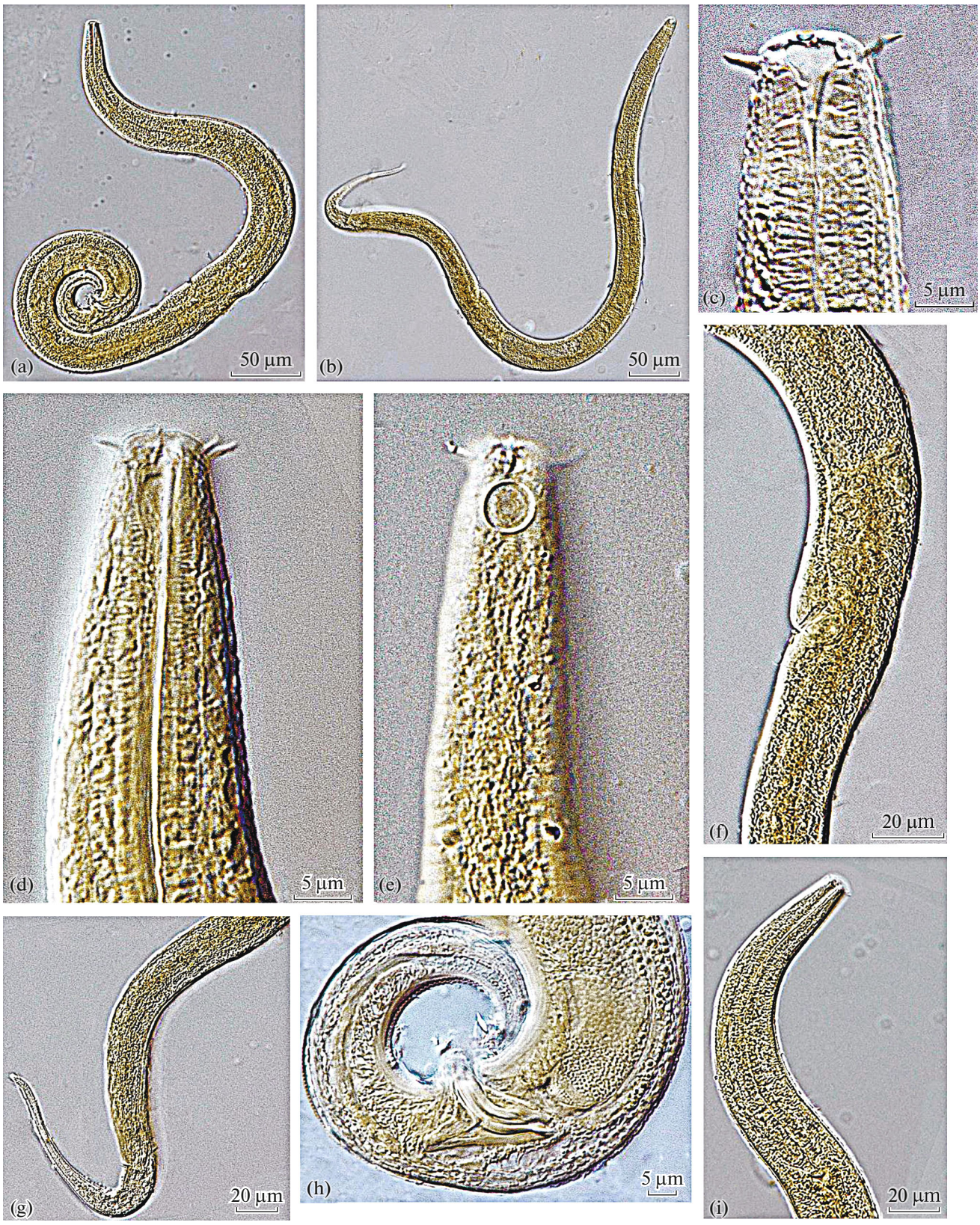

Fig. 2. Micrographs of *Eumonhystera rivalis* sp. n. (a) general view, male; (b) general view, female; (c) head, male; (d, i) anterior end, male; (e) anterior end, female; (f) body in the vulva area; (g) posterior end, female; and (h) posterior end, male.

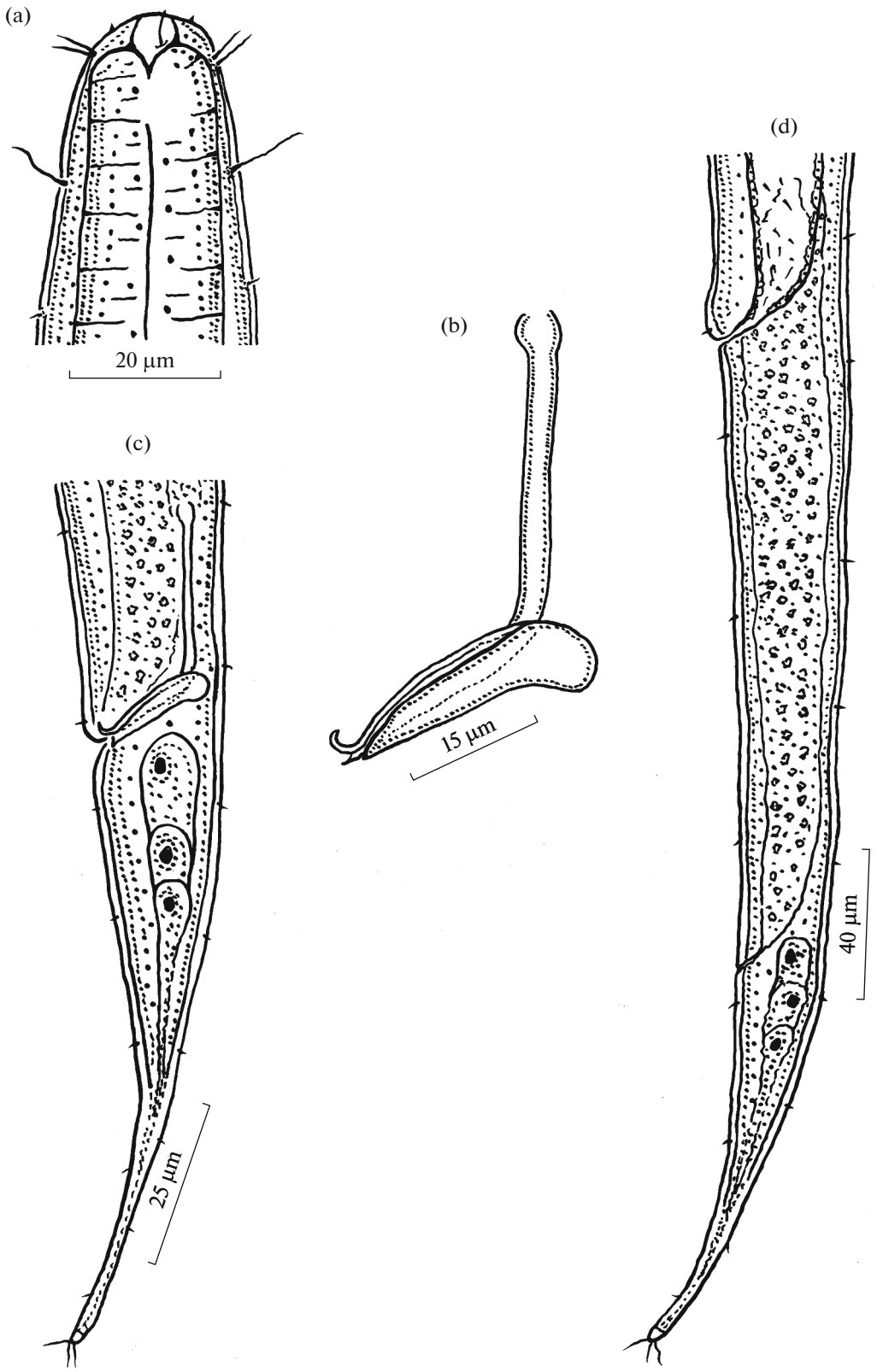

Fig. 3. Details of the structure of *Daptonema borealis* sp. n.: (a) head, male; (b) spicules and gubernaculum; (c) posterior end, male; and (d) posterior end, female.

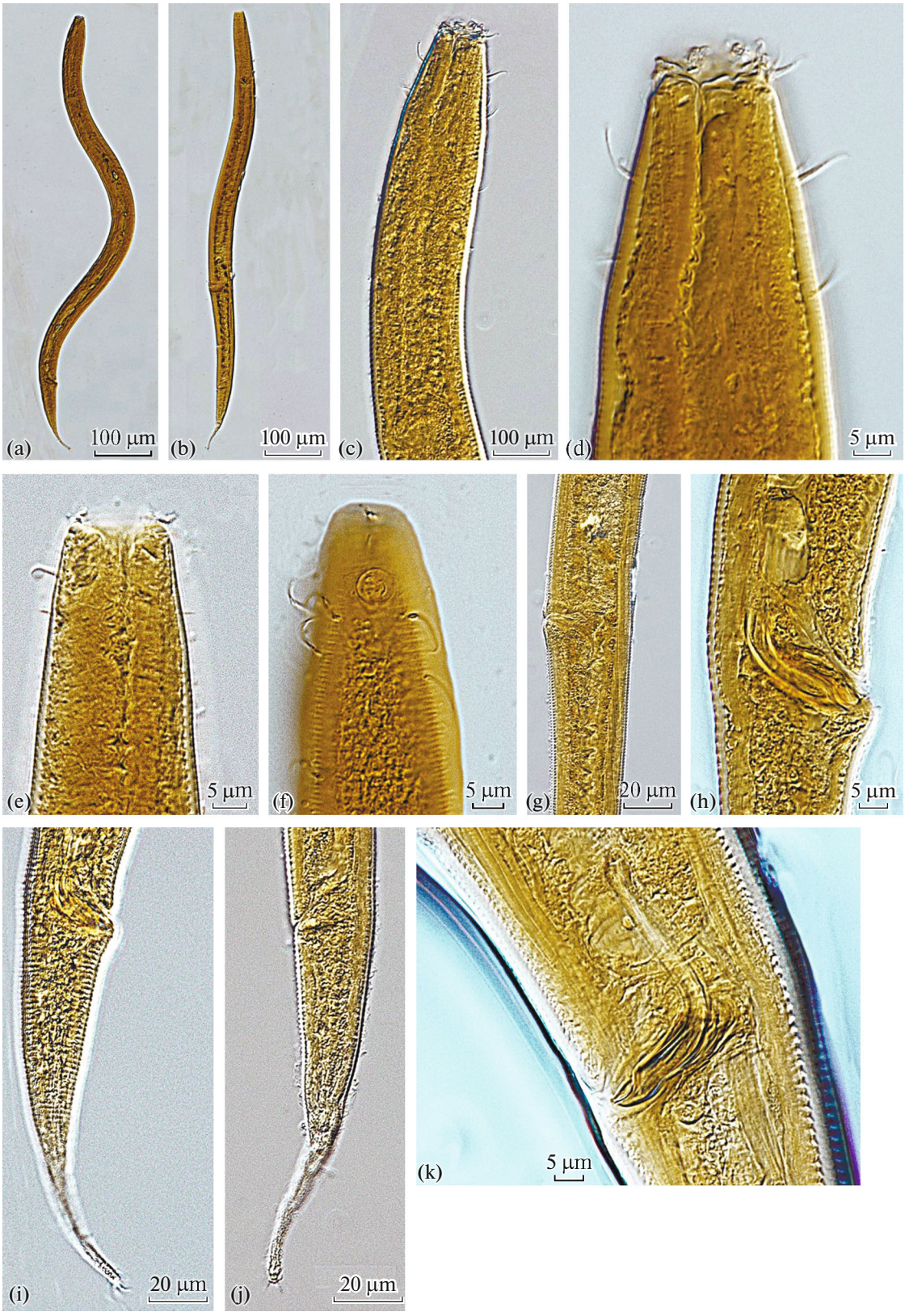

Fig. 4. Micrographs of *Daptonema borealis* sp. n.: (a) general view, male; (b) general view, female; (c) anterior end, male; (d, f) anterior end, male; (e) head, female; (g) body in the vulva area; (h, k) body in the cloaca area; (i) tail, male; and (j) tail, female.

Table 1. Morphometric characteristics of *Eumonhystera rivalis* sp. n.

Sign	Holotype ♂	Paratypes			
		3 ♂♂		2 ♀♀	
		min–max	mean	♀	♀
L, μm	650	666–717	680	768	619
a	19	23–27	25	23	28
b	5.4	4.7–5.0	4.9	6.5	5.4
c	8.7	7.2–8.1	7.6	6.7	6.4
c'	3.7	4.3–4.5	4.4	5.6	7.1
V, %	–	–	–	64.6	59.6
Width, μm:					
labial area	8.5	8.0–9.0	8.5	10	9
body (middle part)	34	27–29	28	34	22
body (anus area) or cloaca	20	20–22	21	20	14
Length, μm:					
head setae	3.5	3.0–4.0	3.5	4.0	4.0
pharynx	121	136–144	140	119	114
tail	75	88–92	89	114	97
spicules (along the curve)	26	26–29	27	–	–
dorsal process of gubernaculum	10	10–12	11	–	–
Distance, μm:					
from amphid fovea to the anterior end of the body	4.0	4.0–5.0	4.5	4.5	5.0
from pharynx end to vulva	–	–	–	377	255
from vulva to anus	–	–	–	158	153
from pharynx end to cloaca	454	425–486	451	–	–

Somatic setae are short and sparse. Biocrystal-like bodies are small and numerous. Six internal papilla-shaped sensillae. Six external labial sensillae and four head sensillae have the shape of fine bristles, both of their circles close together. Length of bristles is approximately equal to half the width of the labial area. Amphid fovea are arranged in a circle whose diameter is half the diameter of the body at this level; they are located 4–5 μm from the anterior end of the body. Cheilostoma is small; its walls are smooth. Pharyngostoma has a shape of a small funnel; its walls are weakly cuticulized. Pharynx is muscular, equally thickened along its entire length. Cardium is small; it protrudes into the lumen of the middle intestine. Renette and its excretory pore were not found. One straight testis. The spicules are small, strongly curved, headed. Their length exceeds the body diameter in the cloaca area 1.3 times. Gubernaculum with a dorsal process. Precloacal supplemental organs are absent. The tail is slender, elongated-conical. The caudal glands and spinneret are well developed.

F e m a l e s. Similar to males by general morphology, the cuticle structure, and the anterior end of the body. The cuticle is smooth. Biocrystal-like bodies are small. Internal labial sensillae have the shape of papillae. External labial sensillae and head sensillae have the shape of fine bristles, their circles are located very close to each other. Amphid fovea are circular, located 4.5–5.0 μm from the anterior end of the body. Pharyngostoma has the shape of a small funnel; its walls are weakly cuticulized. Pharynx is muscular, equally thickened along its entire length. Cardium is small, muscular. Rennet and its excretory pore were not found. One ovary, anterior, straight, without bend, located to the right of the middle intestine. Oocytes are numerous. The vulva is postequatorial, has the shape of a transverse fissure; vulvar flaps are not sclerotized. The uterus is extensive. Vulvar glands and the posterior uterus were not found. Distance from vulva to anus exceeds the tail length 1.4–1.6 times. The tail is slender, elongated-conical. The caudal glands and spinneret are well developed.

D i f f e r e n t i a l d i a g n o s i s. Currently, the genus *Eumonhystera* includes 49 species, of which six were found in Vietnam (Gagarin, 2018; Bezerra et al., 2019).

In females of most species of this genus, the distance from the vulva to the anus is smaller than the tail

length. Only three species, *E. dispar* (Bastian, 1865); *E. sudanensis* Zeidan et al., 1989; and *E. maxima* Gagarin, 1996, have a tail that is which shorter than the distance from vulva to anus. Females of *E. rivalis* sp. n. differ from these three species by the presence of biocrystal-like bodies (compared species do not have these) and by the closer location of the amphid fovea to the anterior end of the body (at a distance of ~0.5 of the labial area width; in the compared species, 1.5–2.5 labial area width); the head setae are longer (their length is ~40–44% of the labial area width, versus 17–30% in the compared species) (Zeidan et al., 1989; Andrássy, 2005).

Males in the species of the genus *Eumonhystera* are very rarely found; they are known only for seven species of this genus. In males of four species, gubernaculum is shaped like a short plate or groove; in males *E. similis* (Bütschli, 1873); *E. borealis* Turpeenniemi, 1997; and *E. vulgaris* (de Man, 1889), it has a dorsal process similarly to that in *E. rivalis*. Male *E. rivalis* sp. n. has biocrystal-like bodies, in contrast to the males of the three species lacking this one. In addition, there are differences in morphometric characteristics. The new species differs from *E. similis* males by a longer body (L = 650–717 μm versus L = 400–600 μm in *E. similis*), a shorter and less slender tail (c = 7.2–8.7, c' = 3.7 –4.5 versus c = 4.5–5.5, c' = 7–9 in *E. similis*), amphid fovea located closer to the anterior end of body (at a distance of ~0.5% of the labial area width versus 2.0–2.6 of the labial area width in *E. similis*), and longer spicules (their length is 26–29 μm, versus 18–23 μm in *E. similis*) (Andrássy, 2005). The males of the new species differ from the males of *E. vulgaris* by a relatively shorter tail (c = 7.2–8.7 versus c = 5.9–6.0 in *E. vulgaris*), amphid fovea located closer to the anterior end of the body (at a distance of ~0.5% the labial area width versus 1.1–1.3 of the labial area width in *E. vulgaris*), and shorter spicules (their length is 26–29 μm, versus 43–45 μm in *E. vulgaris*) (Hernandez and Jordana, 1988). From *E. borealis* males, *E. rivalis* males differ by a longer body (L = 650–717 μm versus L = 290–400 μm in *E. borealis*), a shorter and less slender tail (c = 7.2–8.7, c' = 3.7–4.5 versus c = 4.4–4.8, c' = 9.3–10.8 in *E. borealis*), and longer spicules (their length is 26–29 μm versus 13–14 μm in *E. borealis*) (Turpeenniemi, 1997).

Etymology. The species name means riverine or inhabiting the rivers.

Family Xyalidae Chitwood, 1951

Genus *Daptonema* Cobb, 1920

Daptonema borealis Gagarin sp. n.

Material. Holotype ♂, slide no. PL 3.1.7, paratypes: 3 ♂♂, 1 ♂. Holotype and two paratype (1 ♂ and 1 ♀) preparations are deposited in the collection of the Museum of Nature of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam). Other preparations of paratypes (2♂) are deposited in the collection of nematodes of the Department of Nematology of the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).

Location. Northern Vietnam, Hai Phong Province, the Cấm River mouth, depth 1.5 m, water salinity 1.5‰, sandy bottom sediments. Coordinates: 20°48′84″ N, 106°55′02″ E.

Description. The morphometric characteristics of the holotype and paratypes are given in Table 2.

Males. Relatively thick worms of medium length. The anterior end is narrowed. The width of the labial area is ~2.5 times less than the width of the body in the cardium area. The cuticle is finely annulated. The cuticle thickness in the middle part of the body is 1.0–1.5 μm. Somatic setae are short and sparse. Six internal labial sensillae have the shape of papillae. Six external labial sensillae and four head sensillae have the shape of fine setae 5.0–5.5 μm in length arranged in one circle. No sub-cephalic setae were found. Six to eight cervical setae, 7.0–8.5 μm in length, arranged in one circle and located in 20–22 μm from the anterior end of the body. The amphid foveae are circular, 5.0–5.5 μm in diameter, located 7.0–7.6 μm from the anterior end of the body. Cheilostoma is small. Pharyngostoma is also small; its shape resembles a small funnel. Pharynx is muscular, equally thick along its entire length. The cardium is short, protrudes into the lumen of the middle intestine, and is surrounded by three rounded glands. Renette and its excretory pore were not found. Spicules are thin, headed, curved at almost right angles. Their length exceeds the body diameter in the cloaca area 1.5–1.6 times. Gubernaculum is complex, with a small caudal process. Its main body encloses the apical ends of the spicules like a sleeve. In addition, the main body of gubernaculum has two thin ventral processes ending as clawlike formations. Pericloacal supplementary organs were not found. The tail is slender, elongated-conical. Caudal setae are short. The caudal glands and spinneret are well developed. There are three terminal setae at the tip of the tail.

Female. Similar to males by general morphology, cuticle structure, and anterior end of the body. The cuticle is finely annulated. Internal labial sensillae have the shape of papillae. External labial sensillae and head sensillae have the shape of fine setae 5.0 μm in length arranged in one circle. Subcephalic setae 8-μm-long, located 18 μm from the anterior end of the body. Amphid fovea 5.0 μm in diameter, located 7.0 μm from the anterior end of the body. Cheilostoma is small. Pharyngostoma has the shape of a small funnel. Pharynx is equally thick along its entire length. The cardium is surrounded by three rounded glands. The ovary is single, anterior, straight, and located to the left of the middle intestine. Oocytes are numerous. The vulva is postequatorial and has the shape of a transverse fissure. The vulvar flaps are not sclerotized. The vagina is short, with thin walls. The uterus is extensive, filled with numerous sperm. Posterior

Table 2. Morphometric characteristics of *Daptonema borealis* sp.n.

Sign	Holotype ♂	Paratypes		
		3 ♂♂		1 ♀
		min–max	mean	
L, μm	801	796–887	842	724
A	20	18–24	20	19
B	6.2	6.8–7.1	6.9	6.9
C	6.3	6.7–7.3	6.9	6.9
c'	4.5	3.6–4.0	3.8	4.4
V, %	–	–	–	62.7
Width, μm:				
labial area	17	16–19	18	17
body (middle part)	41	38–48	43	38
body (anus area or cloaca area)	32	29–34	32	24
Length, μm:				
head setae	5	4.5–5.2	4.8	5.0
pharynx	129	112–128	120	105
tail	128	116–122	119	105
spicules (along the curve)	50	50–52	51	–
gubernaculum	18	18–21	20	–
Distance, μm:				
from amphid fovea to the anterior end of the body	7.5	7.0–7.6	7.3	7.0
from pharynx end to vulva	–	–	–	349
from vulva to anus	–	–	–	165
from pharynx end to cloaca	547	568–637	603	–

uterus and vulvar glands were not found. The tail is slender, elongated-oval, its length 1.6 times shorter than the distance from the vulva to the anus. The caudal glands and spinneret are well developed. Terminal setae are present on tail.

Differential diagnosis. At present, the genus *Daptonema* Cobb, 1920 includes 117–136 valid species. In the water bodies of Vietnam, 22 species of the genus were found (Riemann, 1966; Fonseca, Bezerra, 2014). Such a large number of species in the genus is explained by the diversity of the spicular apparatus in males. According to the structure of the spicular apparatus of *D. borealis* sp. n., it is similar to *D. vicinus* (Riemann, 1966); *D. aquaedulcis* (Gagarin, 1987); and *D. salinae* Gagarin, Gusakov, 2014. It differs from *D. vicinus* by a shorter body (♂♂ *L* = 796–887 μm versus ♂♂ *L* = 1160–1180 μm in *D. vicinus*), shorter head setae (5-μm – long, or 25–28% of the labial area width versus 11 μm and 70% of the labial area in *D. vicinus*), and longer spicules (their length is 50–52 μm versus 34–37.5 μm in *D. vicinus*) (Riemann, 1966). The new species differs from *D. aquaedulcis* by a shorter body (*L* = 1400–1650 μm in *D. aquaedulcis*), shorter head setae (5 μm in length, 25–28% of the labial area width versus 10.5–12.0 μm and 50–52% of the labial area width in *D. aquaedulcis*) and shorter spicules (in *D. aquaedulcis*, their length is 67 μm) (Gagarin, 1987). The new species differs from *D. salinae* by a slender body (*a* = 18–24 versus *a* = 11–16 in *D. salinae*), a relatively shorter tail (*c* = 6.7–7.3 versus *c* = 5.3–5.7 in *D salinae*), shorter head setae (their length is 5 μm or 25–28% of the labial area width versus 8.8–10.0 μm or 45–67% of the labial area width in *D. salinae*), and longer spicules (their length is 50–52 μm versus 41–42 μm in *D. salinae*) (Gagarin and Gusakov, 2014).

Etymology. The species name means eastern or from the east.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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