## BIOLOGY, MORPHOLOGY, AND SYSTEMATICS OF HYDROBIONTS

# New Genus and Two New Species of Free-Living Nematodes (Nematoda, Monhysterida) from Artificial Reservoirs in Vietnam

V. G. Gagarin\*

Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Nekouzskii raion, Yaroslavl oblast, Russia \*e-mail: gagarin@ibiw.yaroslavl.ru

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Abstract—Descriptions and illustrations are presented for a new genus and two new species of free-living nematodes found in sediments of four artificial reservoirs for shrimp aquaculture. *Paradaptonema* gen. n. is morphologically close to *Daptonema* Cobb, 1920, but it has three glands around cardia that are absent in *Daptonema*, as well as a peculiar structure of the reproductive apparatus in males. *Paradaptonema securum* gen. n., sp. n. is characterized by a finely annulated cuticle, relatively long external labial setae, the presence of a post-vulvar cell, and a peculiar shape of spicules and gubernacula. *Thalassomonhystera longisoma* sp. n. is morphologically close to *Th. minor* Gagarin, Nguyen Thi Thu, 2008 and *Th. elegans* Gagarin, Nguyen Vu Thanh, 2013, but differs from them in the length and slenderness of the body, as well as in the size of the external labial bristles and spicules.

*Keywords*: Vietnam, artificial reservoirs for strips aquaculture, free-living nematodes, *Paradaptonema* gen. n., *Paradaptonema securum* gen. n., sp. n., *Thalassomonhystera longisoma* sp. n

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## INTRODUCTION

The study of the fauna of free-living nematodes in the water bodies and watercourses in Vietnam began about 15 years ago in connection with the compilation of a database on the fauna of hydrobionts of water bodies and watercourses in Vietnam. In recent years, large-scale studies on the fauna of this group of worms have been conducted in Vietnam. Information on their fauna in fresh water bodies and mangroves is summarized in the works of Gagarin (Gagarin, 2017) and Gusakov (Gusakov, Gagarin, 2017).

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

### MATERIALS AND METHODS

Twelve samples of nematodes were taken in June 2014 by colleagues from the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam) in the sediments of the four artificial reservoirs for shrimp aquaculture. The water bodies are located in the Qung Ninh Province on the coast of the South China Sea; the area varies from 1000 m<sup>2</sup> to 12000 m<sup>2</sup>, the bottom with dense thickets of *Halophila beccarii* Ascherso, 1871 and *Ruppia maritime* L., 1953 grass.

Samples were taken at a depth of 0.3-0.7 m using a plastic cylinder with a 3.5-cm diameter and 10-cm

length; they were then washed through a sieve with an  $80-\mu m$  mesh. The samples were fixed with hot ( $60-70^{\circ}C$ ) 4% formaldehyde solution. After they were placed in 200-mL jars, a Ludox-TM 50 solution (1:1) was added; the jars were then centrifuged five times for 40 min. The nematodes were placed in pure glycerin by the Seinhorst method (Seinhorst, 1979), then mounted in a drop of glycerin on a glass slide and sealed with a paraffin wax ring. A Nikon Eclipse 80i light microscope equipped with DIK contrast monitoring accessories, 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 individuals, species definition, photographing, and making drawings.

In the processed samples, new species of nematodes were discovered. The description and illustrations of one genus and two species of nematodes new to science are given below.

#### RESULTS

## Description of the Genus and Species

Order Monhysterida Filabialjev, 1929.

Family Xyalidae Chitwood, 1951.

Genus Paradaptonema Gagarin gen. n.

Diagnosis. Relatively small nematodes. The cuticle is finely annulated. Three circles of head sensillae. Internal labial sensillae have the shape of papillae;

Parameter	Holotype, male	Males, 12 ind.		Females, 5 ind.	
		min–max	mean	min–max	mean
<i>L</i> , μm	562	528-615	565	585-602	594
a	26	23-31	27	24-30	27
b	4.8	4.7-5.9	5.1	5.0-5.7	5.2
с	6.1	5.4-6.4	5.9	5.4-6.6	5.9
<i>c</i> '	5.5	4.9-6.1	5.4	6.1-7.0	6.6
V, %	_	_	—	53.4-69.5	58.2
Width, µm:					
labial area	6.0	5.5-6.0	5.8	5.5-6.5	6.0
body (middle)	22	18-24	21	20-24	22
body (anus/cloaca area)	18	16-19	18	14-16	15
Length, µm:					
external labial setae	4.0	3.5-4.0	4.0	3.5-4.0	3.8
pharynx	117	105-117	111	105-117	114
spicules (along the curve)	30	30-34	32	—	_
gubernaculum	25	25-28	26	_	—
tail	92	90-105	95	93-108	101
Distance, µm:					
from amphid fovea to the ante- rior end of the body	8.0	7.5-8.0	8.0	8.0-9.0	8.5
from pharynx end to vulva	_	_	—	210-243	223
from pharynx end to cloaca	353	330-414	359	—	—
from vulva to anus	_	-	—	140-173	156

 Table 1. Morphometric characteristics of Paradaptonema securum gen. n., sp. n.

Here and in Table 2: a-ratio of body length to its width, b-ratio of body length to pharynx length, L-body length, c-ratio of body length to tail length, c'-ratio of tail length to body width in the anus or cloaca area, V-the ratio of the distance from vulva to the anterior end of the body to the body length.

external labial sensillae and head sensillae have the shape of thin bristles. Stoma has the shape of a wide funnel, without teeth. Fovea amphids in a shape of a circle. Esophagus without basal expansion. The cardia is muscular. Three small glands around the cardia. The testis is one. Spicules are curved, with welldefined heads and an expanded distal region. The gubernacula are paired, have the shape of a doublecurved loop, and are spherically expanded distally. Precloacal supplements are absent. The tail is elongated—conical, with two long terminal setae.

The genus is morphologically close to the genus *Daptonema* Cobb, 1920. It differs by the presence of glands around the cardia (in *Daptonema* species they are absent) and the presence of a peculiar sexual apparatus in males.

## Paradaptonema securum Gagarin sp. n. (Figs. 1, 2).

Material. Holotype  $\mathcal{J}$ , slide no. PL 2.1.15a, paratypes 13 $\mathcal{J}\mathcal{J}$ , 599. Holotype and paratype preparations (3 $\mathcal{J}\mathcal{J}$ , 299) are stored in the collection of the

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Nature Museum of the Vietnam Academy of Sciences and Technologies. The other paratype preparations are stored in the collection of nematodes of the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).

Finding location. Vietnam, Quang Ninh Province  $(20^{\circ}48'18''-20^{\circ}48'84'' \text{ N}, 106^{\circ}53'21''-106^{\circ}55'48'' \text{ E})$ . An artificial reservoir for shrimp aquaculture, depth 0.3–0.7 m, sediments are silty sands, water salinity 12.9–15.3‰. Sampled in June 2014.

D e s c r i p t i o n. The morphometric characteristics of the holotype and paratypes are given in Table 1.

M a l e. Small worms of medium body thickness. The cuticle is finely annulated. The annulation is best seen on the anterior end of the body and on the tail. Somatic setae are rare and short,  $1.0-1.5 \mu m$  long. The labial area is slightly detached from the rest of the body. Internal labial sensillae have the shape of small papillae. External labial sensillae and head sensillae

GAGARIN



**Fig. 1.** Structural details of *Paradaptonema securum* gen.n., sp. n.: (a) head of the male; (b) anterior end of the male body; (c) body, area of the vulva; (d) tail of the female; (e) spicule and gubernaculum; and (f) tail of the male.

have the shape of thin bristles. Six external labial bristles are  $3.5-4.0 \,\mu\text{m}$  long, which is 60-70% of the width of the labial area. Four head setae are  $1.0-1.5 \,\mu\text{m}$  long. Cervical setae not found. Cheilostoma is relatively large. Pharyngostoma has the shape of a wide funnel. The walls of the pharyngostoma are relatively well sclerotized. There are no teeth in the stoma. Fovea amphids have the shape of a circle with a diameter of  $3.7-4.3 \,\mu\text{m}$  and are located at a distance of  $8.3-8.5 \,\mu\text{m}$  from the anterior end of the body, which is 1.3-1.5 times

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**Fig. 2.** Microphotographs of a male (a, c, e, f, g, i, j, k) and female (b, d, h, l) *Paradaptonema securum* gen.n., sp. n. (a, b) general view, (c, d) head, (e) the anterior end of the body, (f, g) body in the cardia area, (h) body in the vulva area, (i, j) body in the cloaca area, and (k, l) tail. Designations: (an) anus, (els) external labial setae, (vu) vulva, (dp) dorsal process of the gubernaculum, (ve) "ventricle," (hb) head bristles, (cg) cardiac glands, (pvc) postvulvar cell, (gub) gubernaculum, (mi) middle intestine, (sp) spicule, (tb) terminal bristle, (t) tail, (ph) pharynx, and (af) amphid fovea.

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the width of the labial area. The pharynx is muscular, slender, only slightly expanding to its base. The cardia is muscular, triangular, and protrudes into the lumen of the middle intestine. Three oval, slightly elongated glands surround the cardia. The middle intestine in its front part forms a well-defined "ventricle" (according to the terminology of Paramonov, 1962).

The testis is one, located to the right of the intestine. Spicules are strongly curved, with large heads. The distal part of the spicule is greatly expanded. The length of the spicules is 1.6-1.8 times of the body width in the cloaca area. The gubernacula are paired, thin, have the shape of a double curved loop, and distally spherically widen. Precloacal supplements are absent. The tail is slender, elongated—conical. The caudal glands are barely distinguishable. At the end of the tail, two terminal setae  $8.0-8.5 \,\mu m$  long.

F e m a l e s. In general morphology, they are similar to males. The cuticle is thin, finely annulated. Cuticle rings are barely visible under a light microscope. Internal labial sensillae have the shape of small papillae. Six external labial sensillae have the shape of thin setae  $3.5-4.0 \,\mu m$  long. Four head sensillae have the shape of short setae 1.0-1.5 µm long. Fovea amphids are in the shape of a circle and are located 9- $10 \,\mu\text{m}$  from the anterior end of the body, which is 1.3 -1.5 times the width of the labial area. The pharyngostoma is relatively large, having the shape of a funnel, the walls of which are sclerotized. The esophagus is muscular, only slightly expanding to its base. The cardia is muscular, protruding into the lumen of the middle intestine. Around the cardia are three rounded oblong glands. The middle intestine in its front part forms a well-defined "ventricle." The length of the rectum is equal to or slightly less than the width of the body in the anus.

The ovary is single, anterior, straight, and relatively long. The vulva is postequatorial, having the shape of a transverse fissure. The labiae of the vulva are not sclerotized and do not go beyond the contours of the body. The vagina is bent to the anterior end of the body, with thin walls; its length is slightly less than the width of the body at this level. A postvulvar cell is present. The posterior uterus and spermatheca were not detected. The anterior uterus is extensive, containing numerous sperm. In one female, one mature egg ( $25 \times$  $15 \,\mu$ m) was found. The tail is slender, elongated—conical. Caudal glands are present, but poorly distinguishable. There are two terminal bristles 8.5-µm-long on the tail.

Etymology. The species name means "calm."

Family Monhysteridae de Man, 1876.

Genus Thalassomonhystera Jacobs, 1987.

*Thalassomonhystera longisoma* Gagarin sp. n. (Figs. 3, 4).

M a t e r i a l. Holotype  $\mathcal{J}$ , slide no. PL 3.1.9, paratypes 1 $\mathcal{J}$ , 699. Holotype and paratype preparations (1 $\mathcal{J}$ , 299) are stored in the collection of the Nature Museum of the Vietnam Academy of Sciences and Technologies; the other paratype preparations are in the collection of nematodes of the Institute of Ecology and Biological Resources of the Vietnam Academy of Sciences and Technologies (Hanoi, Vietnam).

Finding location. Vietnam, Quang Ninh Province. Coordinates:  $20^{\circ}48'18''-2^{\circ}48'84''$  N,  $106^{\circ}53'21''-106^{\circ}55'48''$  E. An artificial reservoir for shrimp aquaculture, depth 0.3–0.7 m, sediments are silty sand, water salinity 12.9–15.3‰. Sampled in June 2014.

D e s c r i p t i o n. The morphometric characteristics of the holotype and paratypes are given in Table 2.

M a l e. Thin worms of medium size. The cuticle is thin, finely annulated. The annulation of the cuticle is barely noticeable. No somatic setae found. The labial area is barely isolated from the body. Internal labial sensillae have the shape of small papillae. Six external labial sensillae have the shape of thin bristles, 3.5-µmlong, which is 77-88% of the width of the labial area. Four head setae 1.0-µm-long. Cervical setae not found. Fovea amphids in the shape of the circle and are located at a distance of 6.0-6.5 µm from the anterior end of the body, which is 1.4-1.5 times of the width of the labial area. The cheilostoma is extensive; its walls are relatively well sclerotized. Pharyngostoma has a shape of a shallow funnel. No teeth were found in the stoma.

The pharynx is slender, muscular, slightly expanding to its base. The cardia is muscular and protrudes into the lumen of the middle intestine. No glands were found around the cardia. The middle intestine does not form a "ventricle" at its beginning. Renette, its ducts, and excretory pore were not found.

The testis is singular, anterior, straight, and located to the right of the middle intestine. Spicules are strongly bent (almost at a right angle). Their length is 1.2 times of the body width in the cloaca area. The gubernaculum is relatively short, with a dorsal process. The tail is long, gradually tapering; its posterior end is thin. Caudal glands are poorly expressed. Spinneret is porous. No terminal setae.

F e m a l e s. Generally, the morphology is similar to that in males. The cuticle is thin, finely annulated. Somatic setae absent. The labial area is barely isolated from the body. Internal labial sensillae have the shape of small papillae. Six external labial bristles,  $3.0-3.5 \,\mu\text{m}$  long. Four head setae barely visible,  $1.0-\mu\text{m}$ -long. Fovea amphids have the shape of a circle located  $6.5-7.5 \,\mu\text{m}$  from the anterior end of the body. The pharynx is slender, muscular, only slightly expanding to its base. The cardia is muscular and protrudes into the lumen of the middle intestine. Glands around the cardia and rennette are not found. The length of the rec-

(a)





Fig. 3. Structural details of Thalassomonhystera longisoma sp. n.: (a) head of the male, (b) anterior end of the male body, (c) body in the vulva area, (d) tail of the female, and (e) posterior end of the male body.

tum is slightly less than the width of the body in the anus area.

One ovary, anterior, straight. The vulva is preequatorial, having the shape of a transverse fissure. The labiae of the vulva are not sclerotized and do not protrude beyond the contours of the body. The vagina is bent to the anterior end of the body; it has thin walls. No postvulvar cell, posterior uterus, or spermatheca

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**Fig. 4.** Microphotographs of male (a, c, d, e, f) and females (b, g, h) of *Thalassomonhystera longisoma* sp. n.: (a, b) general view, (c) anterior end of the body, (d, e) head, (f) body in the cloaca area, (g) tail, and (h) terminus of the tail. Designations: (hs) head setae, (dp) dorsal process of the gubernaculum, and (che) cheilostoma. Other designations are the same as in Fig. 2.

Dorameter	Holotype, male	Male, 1 ind.	Females, 6 ind.	
Falameter			min–max	average
<i>L</i> , μm	905	899	850-1011	931
a	60	60	53-67	60
b	6.0	6.4	5.7-6.2	6.0
С	6.6	6.8	4.5-5.3	4.9
<i>c</i> '	11.5	11.0	13.8-18.6	17.4
<i>V</i> , %	_	_	56.7-60.6	58.3
Width, µm:				
labial area	4.5	4.0	4.0-4.5	4.3
body (middle)	15	15	15-18	16
body (anus/cloaca area)	12	12	11-13	11
Length, µm:				
external labial setae	3.5	3.5	3.0-3.5	3.5
pharynx	150	141	150-168	156
spicules (along the curve)	14	13	—	_
gubernaculum	5	4	—	_
tail	138	132	165-198	189
Distance, µm:				
from amphid fovea to the anterior end of the body	6.5	6.0	6.0-7.0	6.5
from pharynx end to vulva	_	_	344-422	387
from pharynx end to cloaca	138	132	—	—
from vulva to anus	—	_	168-225	199

 Table 2. Morphometric characteristics of Thalassomonhystera longisoma sp. n.

were detected. There are numerous sperm in the anterior uterus. The tail is slender, long, and divided into two sections. The anterior section is shorter, conical, and the back is thin and whiplike (flagellum). The length of the anterior section is 25-27% of the total length of the tail. Caudal glands are poorly expressed. Spinneret is porous. No terminal setae on tail.

Differential diagnosis. Thalassomonhystera longisoma sp. n belongs to the group of species of the genus Thalassomonhystera, the males of which have a gubernaculum with a dorsal process (Fonseca, Decraemer, 2008). In regard to the tail shape and length, the new species is close to two species of the genus: Th. minor Gagarin, Nguyen Thi Thu, 2008 and Th. elegans Gagarin, Nguyen Vu Thanh, 2013, found in the mangrove sediments in Vietnam (Gagarin, 2017). It differs from the first species by its large body size  $(L = 850-1011 \ \mu m \text{ versus } L = 629-818 \ \mu m \text{ in}$ Th. minor), thinner body (a = 53-67 versus a = 37-46in *Th. minor*), longer external labial setae  $(3.0-3.5 \,\mu\text{m})$ versus 1.2–1.5 µm in *Th. minor*), shorter spicules (13– 14 µm versus 18-22 µm in Th. minor). The new species differs from Th. elegans by a slightly shorter and more slender body ( $L = 850 - 1011 \,\mu\text{m}, a = 53 - 67 \,\text{ver}$ -

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sus  $L = 992-1234 \,\mu\text{m}$  and a = 42-45 in *Th. elegans*), shorter external labial bristles (3.0-3.5  $\mu$ m versus 6.5-8.0 in *Th. elegans*), and shorter spicules (13-14  $\mu$ m versus 16-18  $\mu$ m in *Th. elegans*).

Currently, six valid species of the *Thalassomonhys*tera genus have been found in the reservoirs of Vietnam: *Th. minor* Gagarin, Nguyen Thi Thu, 2008; *Th. tenuis* Gagarin, Nguyen Vu Thanh, 2008; *Th. leptosoma* Gagarin, Nguyen Vu Thanh, 2009; *Th. pygmea* Gagarin, 2012; *Th. elegans* Gagarin, Nguyen Vu Thanh, 2013; and *Th. longisoma* sp. n.

Etymology. The specific name means "longbodied" or "with a long body."

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#### COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests.* The author declares that he have no conflict 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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