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ONE NEW AND TWO UNKNOWN SPECIES OF FREE - LIVING MARINE NEMATODES FROM CANGIO MANGROVE FOREST, HOCHIMINH CITY, VIETNAM

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ABSTRACT: One new and two unknown species of free living marine nematodes belonging to family Oxystominidae are described from Cangio mangrove forest, Hochiminh city of Vietnam. The *Oxystomina paraclavicaudata* sp. nov. is characterized by the long conico-cylindrical tail with claviform tip, the double parallel spicules and the pre-and postvulval papillae. The species Litinium sp1. can be recognized by labial setae and cephalic setae follow the structure 6 + 6 + 4; amphid pear-shaped with slit-like aperture; spicules have a kink at the middle; gubernaculum short, plate-like; two papilliform supplements with short seta; tail rounded with the pore of caudal glands at the end. And Litinium sp2. is characterized by labial setae small or absent, two subcephalic setae at the posterior edge of the amphid; amphid elongate pocket-like with a fringe around the aperture; only one short somatic setae at the base of pharynx. Reproductive system diorchic with short testes; two supplement setae; only two caudal gland cells observed within the tail, the opening is shifted ventrally.

Key words: Oxystomina, Litinium, new and unknown nematode species, Cangio mangrove forest.

I. INTRODUTION

The community of free living nematodes in Vietnam was studied very academic in the North of Vietnam in almost water bodies. But in the South of Vietnam, only a few investigation of free living nematode in mangrove, estuarine, wetland and river were implemented by Doan Canh, Nguyen Vu Thanh (2000); Nguyen Vu Thanh, Lai Phu Hoang, Gagarin (2005); Nguyen Thi Thu, Nguyen Vu Thanh (2004) and Gagarin, Nguyen Vu Thanh (2004, 2005, 2006). In this phylum, most genera of family Oxystominidae were found in Vietnam from the North to the South coastal waters. Four new species for Vietnamese fauna belonging to this family, *Halalaimus* (*Halalaimus*) minor, Halalaimus (Halalaimus) lineatoides, Halalaimus (Tycnodora) luticolus and Halalaimus

(*Halalaimus*) durus were found in Baria - Vungtau province (neighbouring province of the Cangio mangrove forest), by Gagarin and Nguyen Vu Thanh (2004). In this paper, two unknown species of genus *Litinium* and one new species of *Oxystomina* are described.

II. MATERIAL AND METHODS

Samples collection and processes

Samples were collected between 11th and 17th of April 2005 during the dry season in the intertidal zone of the mudflat along a transect from the mangrove forest to the low water level line. Along the transect, 4 stations (stations CG1, CG2, CG3 and CG4) were sampled from the mangrove fringe to the low water line (fig. 1). The Nematode samples were collected

using cores of 3.5 cm diameter (10 cm² surface area) and 30 cm high. The

samples were fixed in 60°C hot 10% formalin solution and gently stirred.

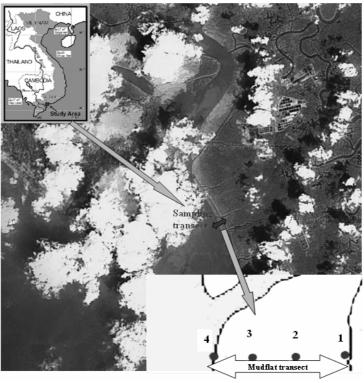


Figure 1. Sampling map

Samples were extracted by flotation with Ludox (specific gravity of 1.18). Samples were then evaporated to anhydrous glycerol after Seinhorst, 1959. Nematodes identified to genus and species level using a high magnification microscope Olympus CH30RF200. Taxonomic classification after De Ley and Blaxter, 2004 and Lorenzen, 1994.

III. DESCRIPTION

1. The genus Oxystomina Filpijev, 1921

Oxystomininae (Smol and Coomans, 2006). Anterior sensilla in three circles: six inner labial papillae indistinct, six outer labial setae, four cephalic setae backwardly positioned (due to elongated neck region). Buccal cavity absent. Amphidial aperture typically oval-shaped, sometimes larger in the male than in the female. Prominent oval cells are scattered throughout the body. Excretory-excretory pore usually conspicuous and sclerotized. Tail clavate.

List of known valid species: Oxystomina acuta Gerlach, 1957; O. affinis Gerlach, 1956; O. alpatovi (Filipjev, 1927) Wieser, 1953; O. alpha Chitwood, 1937; O. antarctica Mawson, 1956; O. asetosa (Southern, 1914) Filipjev, 1921; O. astridae (Jensen 1979) Lorenzen 1981; O. brevicaudata (Kreis, 1929) Gerlach and Riemann, 1974; O. chitwoodi Timm, 1967; O. caspica Tchesunov, 1978; O. clavicauda (Filipjev, 1918) Filipjev 1922; O. cobbi (Filipjev, 1927) Wieser, 1954; O. elegans Platonova, 1971; O. elongata (Butschli, 1874) Filipjev 1922; O. exilis (Cobb, 1920) Filipjev in Kreis, 1926; O. filicauda (Kreis, 1929) Wieser, 1953; O. filicaudata Allgen, 1959; O. greenpatchi Allgen, 1959; O. insulaealbae Filipjev, 1927; O. islandica (De Coninck, 1943) Wieser, 1953; O. mirabilis Allgen, 1959; O. miranda Wieser, nidrosiensis 1953: O. Allgen, 1933; O. novozemelica (Filipjev, 1927) Wieser, 1953; nuda (Filipjev, 1927) Wieser, 1953: O. orientalis Platonova, 1971; O. oxycaudata (Ditlevsen, 1926) Allgen, 1929; *O. pellucida* (Cobb, 1898) Filipjev, 1921; *O. pulchella* Vitiello, 1970; *O. tenuicaudata* Filipjev, 1946; *O. tenuicollis* Allgen, 1959; *O. unguiculata* Stekhoven, 1935; *O. vespertilio* Wieser, 1953.

Oxystomina paraclavicaudata sp. nov. (fig. 1)

Measurements: Table 1.

Holotype: L = 1.6 mm; a = 92; b = 5; c = 14; c' = 8.5; spicule = 25.8 μ m

$$∂1 = \frac{-155}{4.2} = \frac{321}{18} = \frac{M}{13} = \frac{1494}{13}$$
Allotype: L = 1.7 mm; a = 78; b = 3.7; c = 16; c' = 8.7.

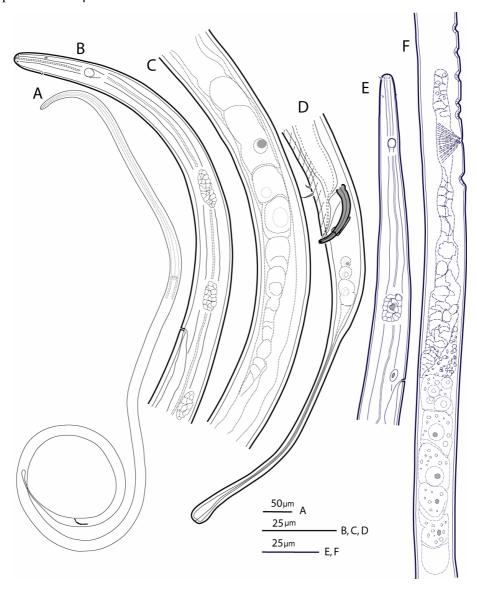


Figure 2. Oxystomina paraclavicaudata sp. nov.

A. Entire male; B. Pharyngeal region of male; C. Female genital system; D. Posterior end of male; E. Head of female; F. Female reproductive system.

Measurement of male and female Oxystomina paraclavicaudata sp. nov

Characters measurements	Male	Female (*)
(In µm, exception the ratio)	n = 1	n = 2
L	1607.5	1668.5 □ 189
A	92	78 □ 51
В	5	3.7 □ 8.6
С	14	16 🗆 7.7
C'	8.5	8.9 □ 11.7
Head dia	4.2	4.1 □ 0
Labial setae	-	0.9 □ 1.5
Cephalic setae	1.1	0.7 □ 0
Amphid: dis. from ant	24	25 🗆 2.5
Length	6.5	6.1 □ 0.28
Width	3.2	3.3 □ 0
c. b. d	7.8	7.8 □ 1.1
Excretory pore: from ant	128.9	136.5 □ 1.8
c. b. d	14.3	15 □ 2
Nerve ring from ant	155	175 □ 2.8
c. b. d	18.5	17 □ 3.4
Pharynx: length	321.4	447 □ 22
c. b. d	12.8	21 🗆 3.5
Max. dia	17.5	21.4 □ 3.7
V%	-	35 □ 5
Vulva: from ant	-	576 □ 10
c. b. d	-	21 🗆 3
Spicule length	25.8	-
Gubernaculum length	7	-
Testis length	112.9	-
Supplement1	4.4	-
Supplement2	1.5	-
Tail length	112.9	107 🗆 24.5
a. b. d	13.3	12 🗆 2.1

Note: Mean \square *SD only in* *.

Description

Male: Nematode is slender, thin and long. Head rounded. Buccal cavity absent. Cuticle smooth and thick (1.8 µm). The labial setae are very minute and very difficult to observe with light microscope, actually the two separate circles are not clearly visible, but are supposed to be present. Four cephalic setae very short, about 1.1 µm positioned at 11 µm from anterior end. Amphid typical for the genus: elongated, with cuticular bordering in the form of a horse-

shoe, located at 24 µm from anterior end, 3.2 um wide and 6.5 µm long. Pharynx slender, 321.4 µm long, enlarged at the base. Nerve ring positioned at 155 µm from anterior end. Epidermal gland cells scattering all over the body, starting in the pharyngeal region. Secretory-excretory gland cell situated at right side in front of the posterior part of pharynx. Excretory pore slightly cuticularized at 128.9 um from anterior end. Cardia triangular leading to a thin walled intestine. Male reproductive

system monorchic, testis short 112.9 μ m long. Spicules 25.8 μ m long, curved, each with two parallel sclerotized lines, giving the appearance of a double spicules. Gubernaculum short (7 μ m long) composed of a caudal plate with lateral round extension distally. Two precloacal setae of unequal length (4.4 μ m (big one) and 1.5 μ m (shorter one)) located at 13.7 μ m (big one) and 16.2 μ m (smaller one) before cloacal opening. The ventral precloacal cuticle can be seen with a lot of parallel muscle fibres. Three caudal glands within the tail. Tail conical cylindrical with clavate tip, 112.9 μ m long, spinneret indistinct, no terminal setae.

Female: Different in shape and size of setae: in one female (paratype specimen), the outer labial setae are long but in the other female (holotype specimen) they are as short as in the male. Cephalic setae similar as in male. Number of epidermal gland cells in pharyngeal region is less than in male. Reproductive system monodelphic, opisthodelphic. Vulva at 35% distance from anterior end. Vagina sphincter well developed, muscle bands near vulva more refractive. Anterior uterus reduced to a short branch. Pre- and postvulval papillae present: one posterior papilla and three to five prevulval papillae. Anal diameter is smaller than in male. Tail similar shape as in male.

Differential diagnosis: This species is characterized by the long conico-cylindrical tail with claviform tip, the double parallel spicules and the pre-and postvulval papillae.

Type Locality and habitat: Khe Nhan, Cangio mangrove forest, silt sediment of an intertidal mudflat Hochiminh city, Vietnam.

Type material: One male and two females. Holotype male on slide number N°: CG1-I2-6 and allotype slide N°: CG1-III9 and paratype slide N°: CG2-III19, deposited at the Institute of Tropical Biology, Hochiminh city, Vietnam.

Discussion: Three specimens belonging to Oxystomina paraclavicaudata sp. nov were found in the samples near mangrove forest edge at high mudflat stations. Within these specimens a difference was observed in the length of the anterior setae between the male (holotype) having minute labial setae and the female (allotype) having longer labial setae, however in

the other female (paratype) the setae were minute as well and both females were identical for the other characteristics; therefore we concluded that the labial setae are probably long in both sexes and easily can be broken. The presence of two short caudal plates with lateral extension distally gubernaculum composed of a caudal plate with lateral round extension distally attached with two parallel spicules is unique in all hitherto described species within the genus Oxystomina. The tail is typical for the genus Oxystomina in having a clavate shaped terminal end, but unique in its long size (length = $112.9 \mu m$ in male). Females have papillar supplements around vulva, which is unique as well within the genus Oxystomina.

In the case there should be a sexual dimorphism in the labial setae and the male has indeed minute setae, then this new species is, based on the minute labial setae, shares this character with many species as O. acuta; O. affinis; O. alpatovi; O. antarctica; O. asetosa; O. caspica; O. chitwoodi; O. cobbi; O. elegans; elongata; O. brevicauda; O. exilis; O. islandica; O. mirabilis; O. miranda; O. novozemelica; O. tenuicaudata; O. unguiculata; O. vespertilio. But the other distinguished characters to be considered that this species has some closed characters such as O. cobbi, O. affinis and O. islandica. But O. cobbi lacks the supplementary setae and has a single spicule and a different tail shape; O. affinis has longer labial and cephalic setae, typical amphid of genus Oxystomina, the same two precloacal supplements in different size, single spicule and supplement but in Oxystomina n. sp double two equal spicule with double short gubernaculum and tail similar typical clavate Oxystomina n. sp. are observed numberous parallel fibre muscle in the ventral precloacal region, two supplements and tail shape as O. islandica but the structure of anterior part is very different even though similar pattern of cephalic setae. Two setae supplement in Oxystomina n. sp. are one short and the other higher but in O. islandica, both setae are equal. Oxystomina n. sp with double spicule when O. islandica was described in single spicule. The structure of anterior part is also very different in labial shape and pharynx.

Etymology: The species is closely referred to known species *clavicaudata*.

2. The genus Litinium Cobb, 1920

Oxystomininae. Sexual dimorphism in the shape of the amphid: in males horseshoe-shaped and in female a round aperture surrounded by heart-shaped fovea, continuing in a distinct canalis. Inner and outer labial sensilla setiform, cephalic setae situated behind the amphid. Buccal cavity absent. Marine.

List of known species:

- 1. Litinium aequale Cobb, 1920;
- 2. L. bananum Gerlach 1956;
- 3. L. parmatum Wieser 1954;

- 4. L. simplex Allgen 1935 (doubtful species);
- 5. L. volutum Gerlach 1962.

Litinium sp1. (fig. 2)

Measurements:

Holotype: 1 ♂, deposited at the Institute of Tropical Biology, Hochiminh city, Vietnam.

$$L=2070~\mu m,~a=74~;~b=10;~c=47;~c'=2;$$
 spicule = $26.8~\mu m$

Cobb formule:

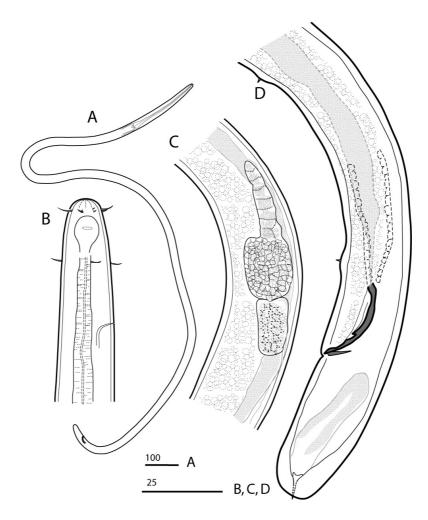


Figure 3. Litinium sp1.

A. Entire male; B. Head region; C. Male system testis; D. Spicule structure with precloacal supplements.

Description:

Male: Body is slender, cylindrical, narrowing towards the two ends, 2070 µm long and maximum width is 27.9 µm. Cuticle smooth and transparent. Head rounded, 8.9 µm wide. Anterior sensilla in 3 circles with pattern of 6 + 6 labial setae and 4 cephalic setae. All labial setae approximately equal to 5.2 µm long. Inner and outer labial are very close to each other. Four cephalic setae 4.4 µm long, located at the posterior side of amphids. The anterior edge of the pear-shaped amphids, slit-like aperture, is situated at 4.8 µm behind the anterior end, amphid is 10.3 µm long and 7.4 µm wide (body width at amphid is 11.4 µm). Buccal cavity very small or absent. Pharynx is 216 µm long, slender and expanded at posterior end. Cardia present, triangular. Nerve ring located at 52% of pharynx length from anterior end. Secretoryexcretory system opens through a pore at 38.9 µm from anterior end where corresponding body diameter get 16.4 µm. Intestine with thin wall. Reproductive system diorchic, testes come close to the base of pharynx. Spicules is not smoothly curve but have a kink at the middle, 26.8 µm long. Gubernaculum short, 8 µm long, plate-like. Two small papilliform supplements with short seta of 1.1 µm long. The first supplement is located at 31.7 µm and the second at 96.7 um anterior to the cloacal opening. Tail rounded with the pore of caudal glands at the terminal end. Small tail tip. Tail length 43.5 µm. The position of the caudal glands is unclear because the cells are indistinct.

Differential diagnosis: Labial setae and cephalic setae follow the structure 6 + 6 + 4. Amphid pear-shaped with slit-like aperture. Spicules have a kink at the middle. Gubernaculum short, plate-like. Two papilliform supplements with short seta. Tail rounded with the pore of caudal glands at the end.

Type locality and habitat: Khe Nhan mudflat, silt sediment intertidal mudflat of Cangio mangrove forest, Hochiminh city, Vietnam.

Type material: one male in slide No.KN1-CG, deposited at the Institute of Tropical Biology, Hochiminh city, Vietnam.

Litinium sp2. (fig. 4)

Measurements:

Holotype: \Diamond .

 $L = 3431 \mu m$; a = 63; b = 9.8; c = 110; spicule 46.5 μm .

Cobb formule

$$\frac{-\ 186\ 349.3\ M\ 3399.8}{13\ 40.7\ 45.2\ 52.5\ 33.3}$$
 $3431 \mu m$

Description

Male: Large body size, long cylindrical, slender, anterior part narrowed, 3431 µm long and maximum 52.5 µm wide. The cuticle is smooth and transparent. Head is continuously rounded, head diameter is 15.7 µm. Labial setae minute, difficult to observe. Four cephalic setae positioned at the middle of amphid (about 2.1 µm long). Two subcephalic setae at posterior border of amphid. Amphidial fovea is elongated pocket-like with a delicate fringe around the round aperture. Amphid is positioned at 6.1 µm from anterior end and is 12 µm long and 5.2 µm wide. Body diameter at the level of the amphid is 15.9 µm. Buccal cavity minute. Pharynx is typical for the genus gradually expanding towards the posterior part. Pharynx length 349.3 µm. Short somatic setae only one at the base of pharynx. Secretory - excretory system with big ventral gland cell located at the expanded pharynx - cardia region and; pore at 48 µm distance from anterior end. Nerve ring located at 186 µm from anterior end. Cardia is triangular. The intestine is very large and thin walled. Reproductive system is diorchic. Testes paired, opposite and outstretched, testis is very short compared with total body length (109.6 µm long). Sperm cells present. The spicule is 46.3 µm long, ventrally curved. Gubernaculum short, triangular, distally pointed and proximal - lateral widened, length 24.3 µm. Two preanal supplements located immediately anterior to cloacal opening: at 5.9 µm and the other is located at the level of spicules capitulum at 37.3 µm. The two precloacal setae are equal (4.4 µm). Anal diameter about 33.3 µm. Tail short, hemispherical 31.2 µm long, without caudal setae; c' = 0.94. Caudal gland observed with two clear big cells located within the tail, the opening is shifted ventrally.

Differential diagnosis: Labial setae small or absent, two subcephalic setae at the posterior edge of the amphid. Amphid elongate pocketlike with a fringe around the aperture. Only one short somatic setae at the base of pharynx. Reproductive system diorchic with short testes. Two supplement setae. Only two caudal gland cells observed within the tail, the opening is shifted ventrally. Tail short, hemispherical.

Discussion: The genus Litinium has been erected to accommodate the type species Litinium aequale described by Cobb (1920) and found in Florida. To this genus only 4 more species have been described up to now: L. aequale Cobb 1920, L. parmatum Wieser 1954, L. bananum Gerlach 1956, L. volutum Gerlach 1962 and L. simplex Allgen, 1935, regarded as a doubtful species by Lorenzen (1981, 1994).

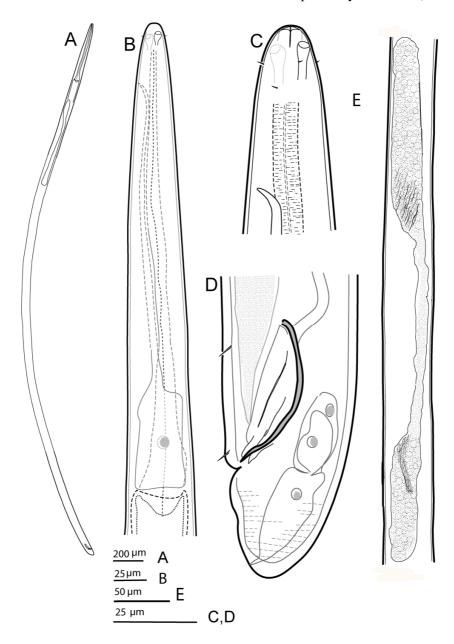


Figure 4. Litinium sp2.

A. Whole body; B. The secretory - excretory cell at base of pharynx; C. The head; D. Tail.

The description of the type species Litinium aeguale Cobb 1920 is based on one female only. In 1958 Gerlach described a male from the coastal ground water of Madagascar as belonging to L. aequale, mainly based on the similar tail shape (hemi-spheroid) and similar labial setae, however Cobb did not mention nor draw the cephalic setae which are present in the male described by Gerlach (1958) and there is a difference in the shape of the amphid. In 1962, L. volutum was described, based on one male specimen, by Gerlach from the Malediven and he concludes that L. volutum closely resembles the type species and also L. volutum could be the male of L. aequale, therefore he concludes that the assignment of the male of L. aequale he described in 1958 from Madagascar is uncertain. However the male of L. volutum is different from the male of Madagascar by the ornamental shape of the amphid, for this reason the author decided to describe the species as a new one. An additional difference is the four preanal papillae, whereas L. aequale only has two.

This resulted in the differential diagnostic characters mentioned in Warwick et al., (1998): *Litinum* can be recognized by horse-shoe shaped amphid in the males and by round aperture surrounded by heart-shaped fovea in females continuing in a distinct canalis and a hemispherical tail.

In Vietnam, the genus *Litinium* was found in limited number in estuaries of coastal regions as Halong Bay, Haiphong, Nhatrang, Quynhon, Danang... However, this genus is hitherto only observed in shallow water bodies (less than 25 m deep) and not in the deep water in Vietnam (Nguyen Vu Thanh, personal communication).

The Litinium sp1. is close to Litinium bananum Gerlach, 1956 regarding the ratio's "a", "b" and "c", the pattern of anterior setae but the distance from labial setae to amphid and from amphid to cephalic setae is different, the pear shape of the amphid, although anteriorly not open in L. bananum; however the tail of Litinium sp1. is much shorter than in L. bananum. Compared to the other hitherto described species, Litinium sp1. is differentiated

by its pear-shaped amphid with slit-like aperture (distinguished from *L. volutu* Gerlach 1962; *L. parmatum* Wieser 1954; *L. aequale* Cobb, 1920 and *Litinium* sp2.). Compared to *L. aequale*, *Litinium* sp1. has shorter supplement setae. The anterior part of *Litinium* sp1. looks quite similar to *Thalassoalaimus pirum* but differs in type and number of supplements as well as in the tail and the copulatory structure.

The new species *Litinium* sp2. can be considered quite differently from the four other species by the elongate pocket-like amphid with a delicate fringe around the round aperture, as well as by the pattern of the anterior setae and the supplements. This species has a big swollen ventral gland at the base of pharynx but the conjunction to excretory pore is difficult to observe, and by the hemispherical tail with well developed muscles. These characters bring *Litinium* sp2. unique from the rest of hitherto species of the genus.

However, according to the differential characters of Thalassoalaimus (buccal cavity absent, 10-12 cephalic setae, four subcephalic setae, amphid pocket like, precloacal papillae) and Litinium (the same character with Thalassoalaimus but male amphid horse-shoe shaped) in Warwick et al. (1998), Litinium sp2 well belong to the genus as Thalassoalaimus because of similarity in shape of amphid (similar as in T. spirum) and the position of labial and cephalic setae even different posterior part (amphid with slit-like aperture distinguished from L. volutum, L. parmatum, L. aequale). When consider about species Thalassoalaimus pacificus Murphy, 1965 showing that the position cephalic setae and amphid is quite similar as well as testis but this species have seven preanal genital setae instead of two long supplement seta as Litinium sp2., the shape of tail also different.

Type locality and habitat: Khe Nhan mudflat, silt sediment of Cangio mangrove forest Cangio mangrove forest, Hochiminh city, Vietnam.

Type material: One male in slide No.KN2-CG.

Key to known species of the genus Litinium Cobb, 1920

1.	Tail shape conical, c' = 4.2
	Tail shape hemispherical-conoid, $c' \cong 1$, spinneret terminal
	Tail shape cylindrical, hemispherical tail end
2.	$c' \cong 1 \square \square$
	c'>1
3.	Four precloacal papillae, each bearing strong thorn
	Two precloacal papillae bearing a seta
4.	Two precloacal papillae, one situated close to the cloaca, anterior one at level of capitulum of spicule, short cephalic setae
	Two precloacal papillae situated in front of the spicules, long cephalic setae
5.	$c'\cong 2$
	c' ≅ 6-8

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MỘT LOÀI MỚI VÀ 2 LOÀI TUYẾN TRÙNG KHÁC CÒN CHƯA BIẾT TỚI Ở RÙNG NGẬP MẶN CẦN GIỜ, THÀNH PHỐ HỒ CHÍ MINH, VIỆT NAM

NGÔ XUÂN QUẢNG, NGUYỄN VŨ THANH, NGUYỄN NGỌC CHÂU, NIC SMOL, ANN VANREUSEL

TÓM TẮT

Một loài mới và 2 loài khác còn chưa thể xác định được tên khoa học thuộc ngành Tuyến trùng, sống tự do trong rừng ngập mặn Cần Giờ thuộc họ Oxystominidae đã được mô tả là mới đối với khoa học: loài mới Oxystomina paraclavicaudata sp. nov. đặc trưng bởi đuôi thon dài với phần đầu hình trụ, phần sau hình chóp dài với mút đuôi loe rộng, gai sinh dục kép; ở con cái có các nhú ở phía trước và phía sau vulva.

Loài *Litinium* sp1. chỉ có 1 cá thể trưởng thành do đó chưa thể xác định là loài mới, khác biệt với các loài đã biết trong giống bởi các lông cứng trên vùng môi, amphid dạng quả lê với lỗ amphid nằm ngang hình khe hở; gai sinh dục với nút thắt ở giữa, trợ gai ngắn và dẹt. Đuôi tròn với lỗ đổ của tuyến đuôi ở cuối mút đuôi.

Loài khác *Litinium* sp2. cũng chỉ bắt gặp với 1 cá thể trưởng thành do đó chưa thể định loại là loài mới, tuy nhiên nó có các đặc điểm hình thái học hoàn toàn khác với các loài đã biết bởi các lông trên môi ngắn hơn nhiều hoặc không quan sát thấy, 2 lông cứng nằm ở ngay sau lỗ amphid, 1 lông ngắn somatic ở giáp ranh ruột - thực quản. Con đực với cơ quan sinh sản diorchic và có 2 nhú sinh dục dạng lông, lỗ đổ của tuyến đuôi nằm bên phía bụng của mút đuôi.

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