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Micoletzkya chinaae n. sp. (Nematoda: Diplogastridae), a potential predacious nematode and *Ektaphelenchus macrobulbosus* (Nematoda: Ektaphelenchinae) isolated from Simao pine in South-western China

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Abstract: Detailed morphology of a new diplogastrid and a known ektaphelenchid species isolated from Simao pine in south-western China were illustrated and described/redescribed. *Micoletzkya chinaae* n. sp. is characterized by a relatively short body length (601-802 μm in female and 505-773 μm in male), undivided cheilorhabdia (forming an entire ring), dimorphic buccal cavity (eury- or stenostomatous), a large claw-like dorsal tooth and a large right subventral tooth in the stoma of eurystomatous form, typical diplogastrid pharynx, didelphic female gonads, nine pairs of genital papillae on male tail region with two ventral pairs (GP1 and GP2) closely associated, a unique gubernaculum morphology, and a long filiform tail in both sexes. The new diplogastrid belongs to the Group 1 category of *Micoletzkya* species *sensu* Massey, 1966, which is characterized by stoma equipped with a large dorsal and a large subventral tooth, and both teeth can cross near the center of the pharynx. The new species can be easily distinguished from other species within this group except for *M. tomea* Massey, 1966 with the long filiform female and male tails. However, it shows great similarities to *Mononchoides* spp., *Koerneria* spp., *Fictor* spp., and *Acrostichus* members in some aspects. More morphological features as well as molecular data of this clade should be available before relationships between and within these genera can be better interpreted. The two large moveable teeth in eurystomatous worms indicate their potentially predacious habits, and re-isolation of this species is necessary. Morphology of south-western Chinese population of *Ektaphelenchus macrobulbosus* (Rühm,1956) Massey, 1974 conforms well to the previous descriptions except for a few minor variations. It is characterized by medium-long female and male bodies (676-791 and 613-685 μm, respectively), three incisures in the lateral field, offset cephalic region, knobless stylet 18-20 μm long, oblong median bulb with posteriorly situated valves, two to three rows of developing oocytes, short postuterine sac, absence of female rectum and anus, two pairs of subventral papillae on the male tail region, a cucullus (apophysis) present on the dorsal distal end of the spicule, and the conoid female and male tails.

Key words: description, redescription, morphology, morphometrics, new species, pine wood nematode, *Pinus kesiya* var. *langbianensis*, SEM, taxonomy, *Micoletzkya*, *Ektaphelenchus*.

During two field surveys for the thoroughness of eradication of pine wilt disease caused by pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Bubrer 1934) Nickle 1970 in Yunnan Province of China at December 2007 and April 2008, two recently described aphelenchoidids (Huang et al. 2011, in revision), a diplogastrid species and an ektaphelenchid were collected from ninety Simao pine *Pinus kesiya* var. *langbianensis* samples. We tried to culture all nematode species isolated using fungal dishes of *Botrytis cinerea*, but the ektaphelenchid failed to multiply on the culture. The diplogastrid could be cultured, although more than one month was required before large numbers of individuals were available. Both Aphelenchoidea (Nematoda: Tylenchina) and Diplogastridae (Nematoda: Diplogastrina) are families containing many poorly described species and species inquirendae (Hunt, 2008; Sudhaus & Fürst von Lieven, 2003). With this proviso in mind, we examined and illustrated the detailed morphology of all the species we obtained. Based on morphological identification, the ektaphelenchid was identified as the known species of *Ektaphelenchus macrobulbosus* (Rühm, 1956) Massey, 1974. The diplogastrid was dimorphic in stoma and the eurystomatous morph bear two large moveable teeth (a claw-like dorsal tooth as well as a right subventral one), which might indicate the possible potential value for biocontrol to plant parasitic nematodes. The stoma characters, together with other morphological evidences (e.g. male genital papillae positioned pattern and gubernaculum morphology) showed the diplogastrid was an undescribed species belonging to the genus *Micoletzkya* Rühm 1960, which was described herein as *M. chinaae* n. sp.

Materials and Methods

Specimens of the diplogastrid and the ektaphelenchid were extracted from a fungal culture of *Botrytis cinerea* and wood chips, respectively, by a modified Baermann funnel technique (Dropkin, 1989). Measurements (except for those mounted on glycerin), drawings and descriptions were made from nematodes killed by heat and mounted in fixative (4% formaldehyde). Measurements were taken using a ZEISS AXioPhot microscope with AxioVision software. Permanent slides were made in glycerin after slowly processing the nematodes to glycerin by the method of Ryss (2003).

For scanning electron microscope (SEM) examination, living adults were heat-relaxed, fixed in 2.5% glutaraldehyde

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in 0.1 M phosphate buffer (PH 7.2), dehydrated through a graded ethanol series, freeze-dried, and sputter-coated with gold-palladium (see Wergin, 1981).

Micoletzkya chinaae[*] n. sp.
(Figs. 1–3)

Measurements: See Table 1.

Description: *Adult:* Cuticle with longitudinal ridges and fine transverse striations. Lateral fields not demarcated. Annules ca 0.8-0.9 μm wide and distance between two longitudinal ridges ca 1.4-1.6 μm at mid-body. Lip region continuous with adjoining body contour. Lips amalgamated with six short papilliform labial sensilla. Four small papilliform cephalic papillae present in males, as typical for other diplogastrid species. Amphidial apertures elliptical in lateral view, located on the base of gymnostom. Buccal cavity dimorphic (steno- and eurystomatous morph) in females, but only stenostomatous males were observed. The buccal cavity of stenostomatous worms narrow, whereas the eurystomatous possess a broad one. Cheilostom wider than long, anterior part undivided, forming an entire ring (see Fig.3 A, B & C). The inner labial walls of cheilostom stave-like occasionally rib-like, formed by cuticularized duplicatures. Gymnostom anisomorphic, ventral wall slightly longer than dorsal wall. Stegostom anisotopic. Eurystomatous worms bear a large claw-like dorsal tooth and a large right subventral tooth, both teeth movable and with a pointed tip. On the stenostomatous morph, no subventral tooth observed and the dorsal tooth not claw-like as that of eury-morph. The dorsal tooth penetrated by the duct of the dorsal gland and can be adducted (see Fig.2 A-3 & A-1). Postdental part cuticularized, outside of subventral wall, usually knob-shaped. The left subventral side of the postdental plate bearing a cuticularized denticle, sometimes protruding into gymnostom with pointed tip especially in steno-morph. Caudally to the dorsal tooth, the dorsal wall of the stegostom heavily cuticularized. The subdorsal sides of the pharyngeal lumen supported by subdorsal struts, the elongations of the heavily cuticularized tissues in the subdorsal stegostom region. An elongated oval shape apodeme sometimes presented on the anterior region of dorsal pharynx, more conspicuous for steno-morph. Anterior part of pharynx (= pro + metacorpus) slightly longer than posterior section (isthmus + basal bulb). Procorpus muscular, stout, occupying half to two-thirds of corresponding body diam. Metacorpus muscular, forming well developed median bulb. Isthmus narrow, not muscular. Basal bulb glandular, pharyngo-intestinal valve difficult to discern. Never ring usually surrounding at or slightly anterior to the middle region of the posterior part of pharynx, extending 76-105 μm from anterior end. Hemizonid sometimes visible, situated posteriorly to nerve ring, ca 85-115 μm from anterior end. Excretory pore position posterior to hemizonid, ventrally located at or anteriorly to the lever of posterior portion of basal bulb, ca 89-122 μm from anterior end.

Female: Body slender, almost straight or slightly ventrally arcuate when heat-relaxed. Gonad didelphic, amphidelphic. Anterior ovary on right of intestine, posterior ovary on left of intestine, both ovaries totally reflexed (= antidromous reflexion). Developing oocytes arranged in one to two rows anteriorly, one row posteriorly with one well-developed oocyte located closely to the oviduct, distal tip of ovary reaching vulva or not. Spermatheca with sperms visible, located at the junction of oviduct and uterus. Two or three eggs at a time were visible in the uterus. Tail long, tapering to a filiform terminus.

Male: Ventrally arcuate, J-shaped when killed with heat. Testis single, on left of intestine, anterior end usually reflexed inwardly (= reflexed to right hand side, between main branch of testis and intestine), spermatogonia arranged in one to two rows in reflexed part, well developed spermatocytes arranged in two or three rows, then mature spermatids arranged in multiple rows in remainder of gonad. Spicules paired (Y-shaped in ventral view), separated, arched, expanded slightly at 25% from anterior end then smoothly tapered to a pointed distal terminus. Manubrium distinguishable as square-shaped attachment to the anterior end. Gubernaculum ca 45-54% of spicule length long, thick proximally with a thin trough-like distal extension, posterior part enveloping the spicule (Fig.1 H). Nine pairs of genital papillae present. GP1-GP3 pre-cloacal pairs, GP1 and GP2 closely associated, ventrally located far anterior to cloacal slit; GP3 subventrally located, slightly anterior to the cloacal slit. GP4 very close to the cloaca. GP5-GP9 post-cloacal pairs, GP5 subdorsally located, GP6-8 ventrally situated closely to one another and just anterior to tail spike, GP9 dorsally situated anteriorly to the tail spike. Tail long, spike, with filiform distal tip. Bursa absent.

Type Host and Locality: The type specimens were collected from a culture of *Botrytis cinerea*. The cultured nematodes were started from those extracted from the xylem of dead Simao pine (*Pinus kesiya* var. *langbianensis*) with no *Bursaphelenchus xylophilus* was detected, in Ruili County of Yunnan Province in China.

Type Material: Slides of holotype male (M.C.m-No.1); paratype males (M.C.m-No.2-9) and paratype eurystomatous females (M.C.f-No.1-3) and paratype stenostomatous females (M.C.f-No.4-6) were deposited in Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China. Other paratype materials: 6 males (T-6092p to T-6097p), 3 eury-females (T-6098p to T-6100p) and 3 steno-females (T-6101p to T-6103p) were deposited in the USDA Nematode Collection, Beltsville, MD, USA.

[*] Patronym in honour of the motherland (P.R. China) of the authors for her first report of the family Diplogastridae.

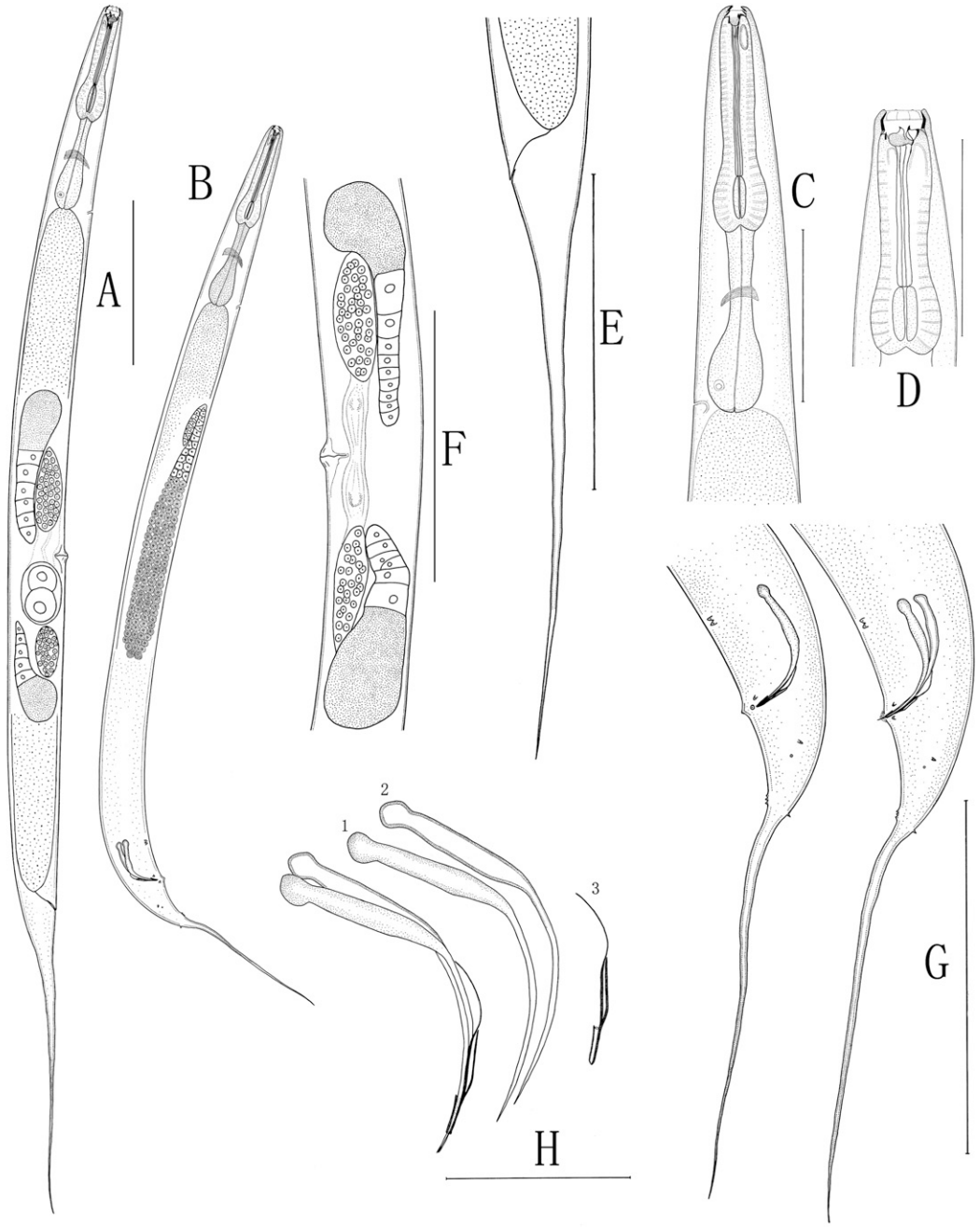

FIG. 1. Drawings of *Micoletzkya chinaae* n. sp. A) Entire view of eurystomatous female. B) Entire view of male. C) Anterior region of male. D) Anterior region of eurystomatous female. E) Female tail. F) Vulva region with two gonads. G) Male tails. H) Spicules and gubernacula (1: spicule in dorsal view; 2: spicule in ventral view; 3: gubernaculum). (Scale bars: A = B = 100 μm; C = 50 μm; D = 70 μm; E, F, G = 100 μm; H = 20 μm.)

DIAGNOSIS AND RELATIONSHIPS: *Micoletzkya chinaae* n. sp. is characterized by relatively short female and male bodies (601-802 μm and 505-773 μm, respectively), undivided cheilorhabdia, dimorphic buccal cavity (eury- or stenostomatous), two large moveable teeth in stoma of eurystomatous morph (claw-like dorsal tooth and right subventral tooth), typical diplogastrid pharynx morphology, didelphic female gonads, nine pairs of male tail genital papillae with the ventral pairs (GP1 and GP2) closely associated, a characteristic gubernaculum shape (thick proximally with a long thin trough-like distal extension), and the long filiform tails of both sexes.

Micoletzkya chinaae n. sp. belongs to the Group 1 category of *Micoletzkya* species (*sensu* Massey, 1966), which is characterized by stoma armed with a large dorsal and a large subventral tooth, both teeth can cross at or near its center. The new species can be distinguished from all species in this group except for *M. tomea* Massey, 1966 by the long filiform tails of both sexes. *M. chinaae* n. sp. is similar to *M. tomea* in stoma teeth morphology (bearing a large claw-like dorsal tooth and a large subventral

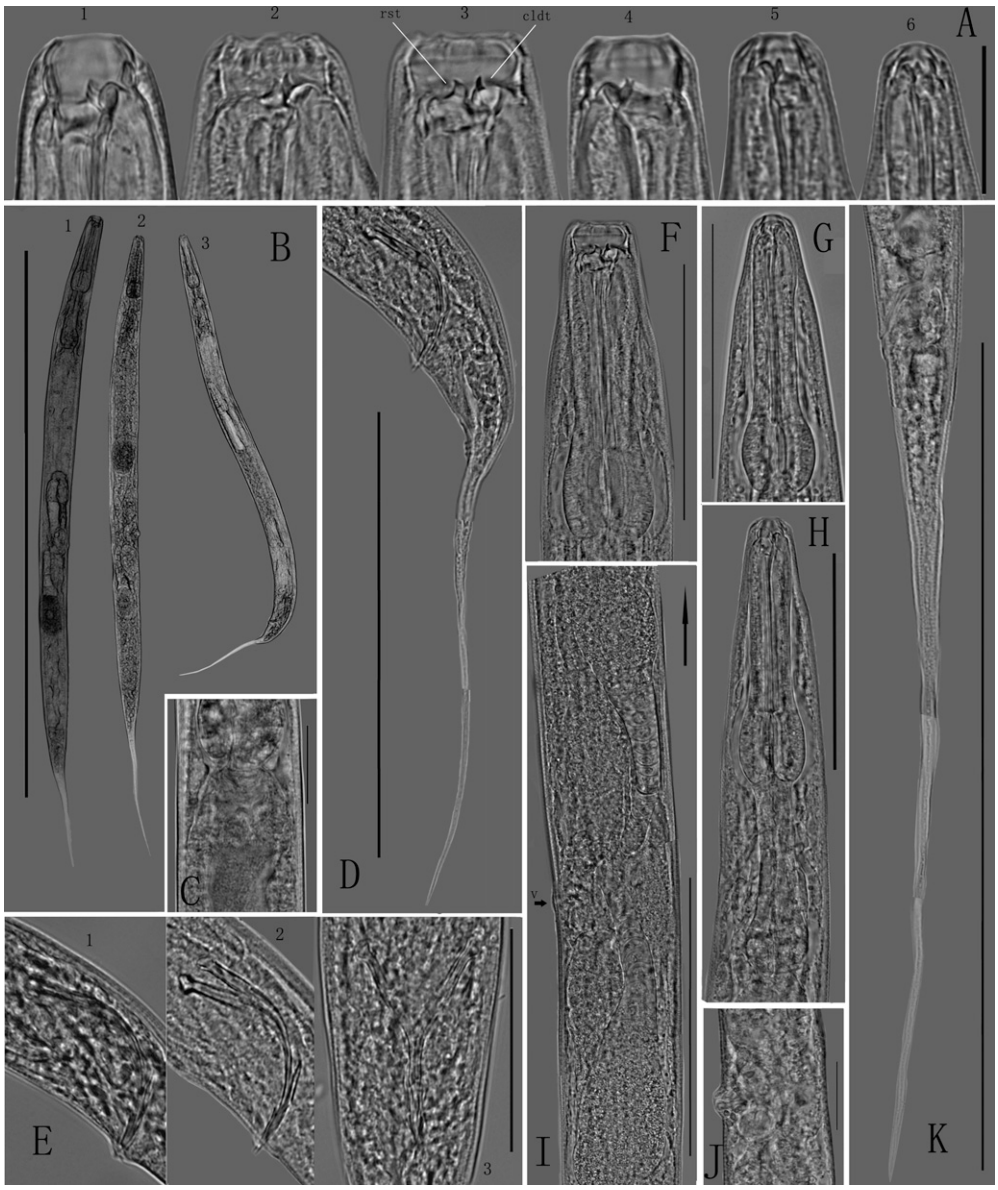

FIG. 2. Light micrographs of *Micoletzkya chinaae* n. sp. (specimens of A 1-3, F, G, H are in left lateral view; specimens of A 4-6 are in right lateral view). A) Variation of stoma with the movement of claw-like dorsa tooth (cldt) and right subventral tooth (rst) (1-4: eurystomatous females; 5: stenostomatous female; 6: male). B) Entire view of worms (1: eurystomatous female; 2: stenostomatous female; 3: male). C) Pharyngo-intestinal valve. D) Male tail. E: Spicules and gubernacula (1, 2: lateral view; 3: ventral view). F) Anterior region of eury-female. G) Anterior region of male. H) Anterior region (pharynx) of steno-female. I) Vulva region with two gonads (v indicates vulva; arrow shows anterior direction). J) Vulva region. K) Female tail. (Scale bars: A, C, J = 20 μm; B = 600 μm; D, I = 100 μm; E = 40 μm; F = 70 μm; G, H = 60 μm; K = 190 μm.)

tooth, and both teeth can cross near the center of oesophagus), typical diplogastrid pharynx, ovaries reflexed, vulva lips protruding, and the filiform female tail shape, but the new species has a conspicuous smaller body size (601-802 μm vs. 850-1510 μm) and a relatively smaller c value (3.6-5.2 vs. 4.3-6). They also can be differentiated by the stoma shape, e.g. the cheilorhabdia overlapping the tip of the prorhabdia in *M. chinaae* n. sp. vs. the prorhabdia overlapping the cheilorhabdia in *M. tomea*.

Micoletzkya chinaae n. sp. is morphologically close to *Mononchoides* spp. in the large claw-like dorsal and right subventral teeth as well as their movements including adduction of the dorsal tooth, and the rugae in cheilostom, but they can be distinguished with cheilorhabdia morphology (forming an entire ring vs. divided) and the telostom shape (knob-shaped outside of subventral wall vs. a wide stegostom-cylinder morph) (Calaway & Tarjan, 1973). Morphology of the large dorsal claw-like tooth and the large right subventral tooth armed in the eurystomate form of *M. chinaae* n. sp. also similar to those of *Fictor* spp. and *Koerneria* spp. However, *M. chinaae* n. sp. can be differentiated from *Fictor* members with the undivided cheilorhabdia shape. Although presence of

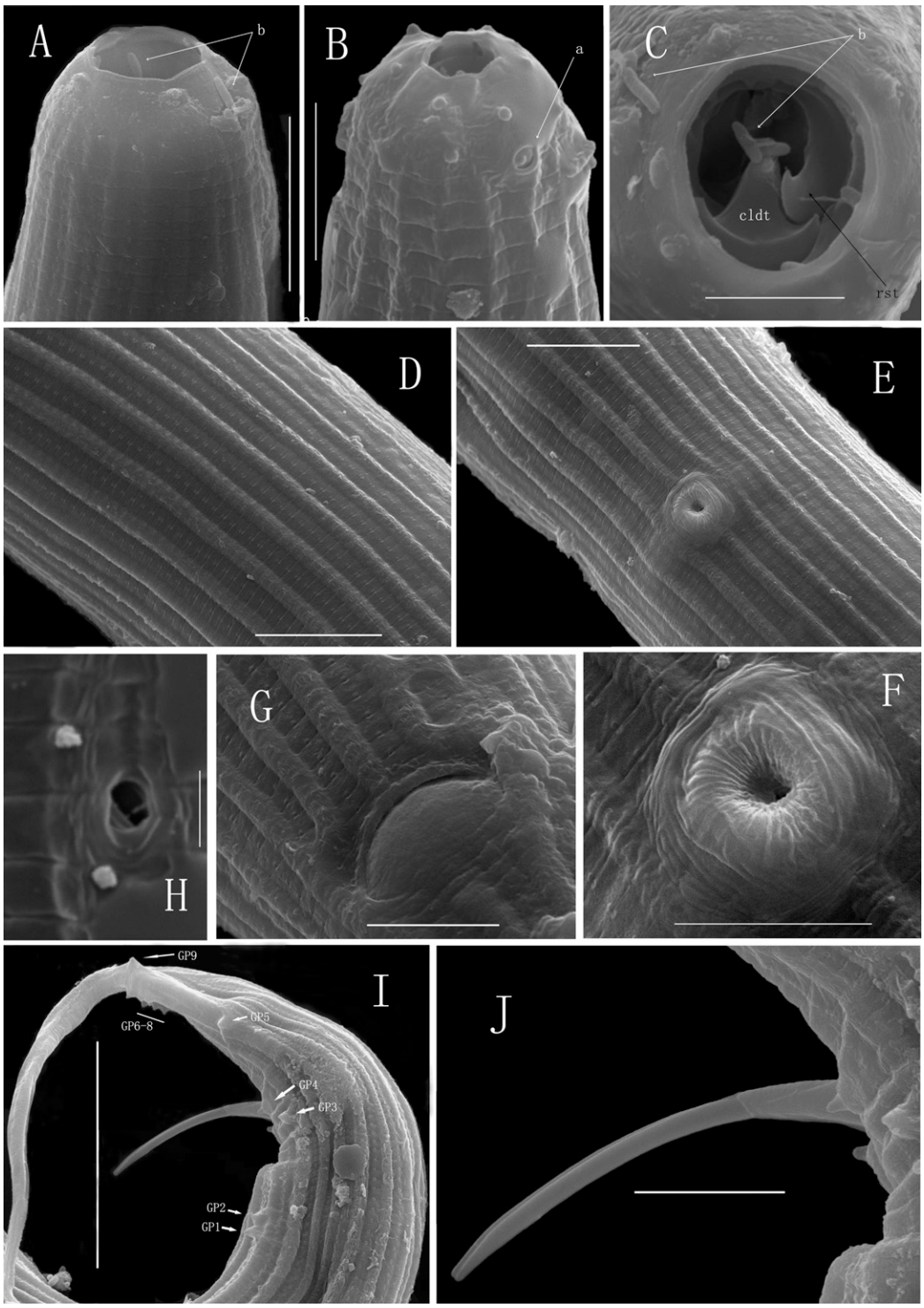

Fig. 3. Scanning electron micrographs (SEM) of *Micoletzkya chinaae* n. sp. A) Head of eurystomatous female (b: bacteria). B) Head of male (a: amphid). C) Frontal view of eurystomatous nematode (b: bacteria; cldt: claw-like dorsal tooth; rst: right subventral tooth). D) Cuticle conspicuously longitudinally striated. E) Vulva region. F) Vulva. G) Anus of female. H) Excretory pore. I) Male tail with 9 pairs of genital papillae (GP1-3: precloacal pairs; GP4: adcloacal pair; GP5-9: postcloacal pairs). J) Spicule with posterior end of gubernaculum. (Scale bars: A, D, E= 10 μm; B, C, F, G, J = 5 μm; H = 1 μm; I = 20 μm.)

apodemes visible sometimes on the anterior pharynx of *M. chinaae* n. sp., according to Sudhaus & Fürst von Lieven (2003) the most important apomorphic character joining the *Koerneria* group was the presence of subventral apodemes in the buccal cavity, which were not presented on *M. chinaae* n. sp. Apodemes on *M. chinaae* n. sp. were not moveable but those for *Koerneria* spp. could be. *M. chinaae* n. sp. resembles to *Acrostichus* spp. in longitudinal cuticular striation, the closely situated male genital papillae GP1 & GP2 and the filiform

TABLE 1. Morphometrics of *Micoletzkya chinaae* n. sp. [all measurements in μm and in the form: mean ± SD (range) coefficient of variation].

Character	Male			Female	
	Holotype (glycerin)	Paratypes (glycerine)	Paratypes (fixed)	Paratypes (glycerin)	Paratypes (fixed)
n	–	13	17	12	17
L	615.3	590.3±50.1 (504.6-664.6)	649.7±63.8 (545.6-773.1)	677.6±54.4 (600.8-791.4)	720.8±51.0 (642.8-801.5)
a	24.6	25.1±1.6 (21.4-27.3)	21.1±2.0 (18.5-24.8)	24.1±1.6 (20.7-26.7)	19.6±2.1 (16.6-23.6)
b	5.7	5.4±0.3 (5.0-6.0)	5.7±0.4 (5.1-6.5)	5.3±0.7 (4.3-6.3)	5.6±0.6 (4.5-6.2)
c	5.4	5.4±0.6 (4.4-6.6)	4.8±0.4 (4.3-6.1)	4.6±0.3 (4.1-5.2)	4.1±0.4 (3.6-4.9)
c'	5.9	5.9±0.6 (4.9-6.9)	6.5±0.7 (4.5-8.0)	8.7±1.1 (6.1-10.3)	9.8±1.3 (7.5-12.5)
V	–	–	–	49.0±1.0 (47.0-50.3)	46.7±1.6 (44.0-49.0)
Stoma length (eury-)	–	–	–	11.5±0.9 (10.3-12.6)	12.1±1.1 (9.5-13.7)
Stoma diam. (eury-)	–	–	–	5.7±1.1 (4.2-6.9)	7.6±2.1 (5.0-12.2)
Stoma length (steno-)	7.2	7.0±0.3 (6.5-7.4)	7.5±0.3 (6.8-7.9)	8.7±0.3 (8.3-9.2)	8.9±0.9 (7.5-10.6)
Stoma diam. (steno-)	3.0	2.0±0.4 (1.5-2.9)	2.6±0.7 (1.9-4.8)	2.6±0.4 (1.9-3.0)	3.8±1.0 (2.7-5.7)
Anterior pharynx length	57.1	58.1±2.1 (55.2-62.8)	61.1±3.2 (56.6-66.0)	70.5±5.8 (63.9-78.4)	71.6±5.3 (64.9-80.3)
Posterior pharynx length	48.7	47.8±3.3 (41.9-53.3)	51.8±3.2 (46.6-58.5)	57.0±5.4 (43.8-66.6)	56.5±6.2 (44.0-68.5)
Post. pharynx. / ant. pharynx.	0.85	0.82±0.04 (0.75-0.92)	0.85±0.04 (0.79-0.89)	0.81±0.07 (0.63-0.88)	0.79±0.06 (0.67-0.90)
Total pharynx length (head to cardia)	108.1	108.5±5.0 (100.5-116.8)	116.6±5.4 (106.8-127.0)	129.5±12.5 (109.8-148.0)	127.7±10.5 (111.0-149.6)
Vulva to anus distance	–	–	–	198.5±18.0 (166.9-234.4)	196.6±21.3 (151.0-232.2)
Vulval body diam.	–	–	–	28.7±2.8 (24.2-34.6)	37.9±3.9 (32.6-44.6)
Anal body diam.	19.1	18.7±1.3 (16.6-21.1)	22.5±2.2 (19.2-26.3)	17.4±3.3 (14.7-27.4)	20.0±2.2 (16.9-24.0)
Tail length	113.8	109.1±5.9 (100.0-116.7)	136.9±10.0 (120.0-151.4)	148.9±13.6 (129.4-168.2)	182.7±17.7 (143.8-211.6)
Spicule length	40.3	40.6±1.0 (39.2-41.9)	43.2±1.7 (39.3-45.7)	–	–
Gubernaculum length	18.7	19.5±1.3 (17.1-20.9)	21.4±1.4 (19.3-24.3)	–	–
Guber. length × 100 / spi. length	46.2	48.0±3.4 (42.2-52.4)	49.5±2.6 (45.0-54.4)	–	–

tails of both sexes, however, they can be easily differentiated by cheilorhabdia structure (undivided vs. separated into six adradial plates), dorsal tooth shape (claw-like vs. thorn-like or dagger-like), and the gubernaculum morphology (small and thin vs. large and massive) (Massey, 1966b; Sudhaus & Fürst von Lieven, 2003).

Ektaphelenchus macrobulbosus (Rühm, 1956) Massey, 1974
(Figs. 4–6)

MEASUREMENTS: See Table 2.

REDESCRIPTION: *Female:* Body slender, C-shaped when killed by heat. Cuticle with fine transverse striae. Three incisures in lateral field. Head set off, lips of unequal size, annulations present. Stylet knobless. Median bulb oblong; valves posteriorly situated. Pharyngeal gland lobe long, extending 112-168 μm from base of median bulb. Excretory pore 37-40 μm posterior to median bulb. Ovary monodelphic, outstretched, not reaching pharyngeal gland lobe. Developing oocytes arranged in two to three rows anteriorly, two to one row posteriorly. Spermatheca often invisible, sperms present when visible. Vulva posteriorly located, flap absent, distance from vulva to terminus is ca 140-171 μm. Postuterine sac short (ca 17-24 μm), no sperm was observed. Rectum and anus absent; intestine terminating in blind sac. Tail conoid, tapering to pointed.

Male: Body slender, ventrally curved when heat-relaxed. Cuticle, lip region, excretory pore, median bulb, stylet and pharyngeal gland lobe similar to female. Testis single, outstretched. Developing spermatocytes arranged in two rows. Spicule with elongate apex and

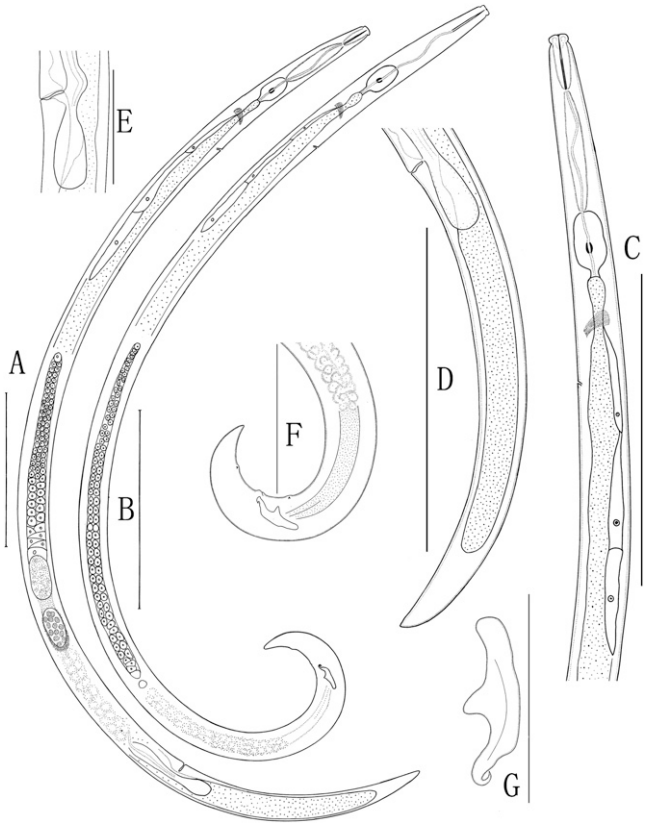

FIG. 4. Drawings of *Ektaphelenchus macrobulbosus*. A) Entire view of female. B) Entire view of male. C) Anterior region of female. D) Posterior region of female. E) Vulva. F) Male tail. G) Spicule. (Scale bars: A, B, C, D = 100 μm; E = 30 μm; F = 50 μm; G = 20 μm.)

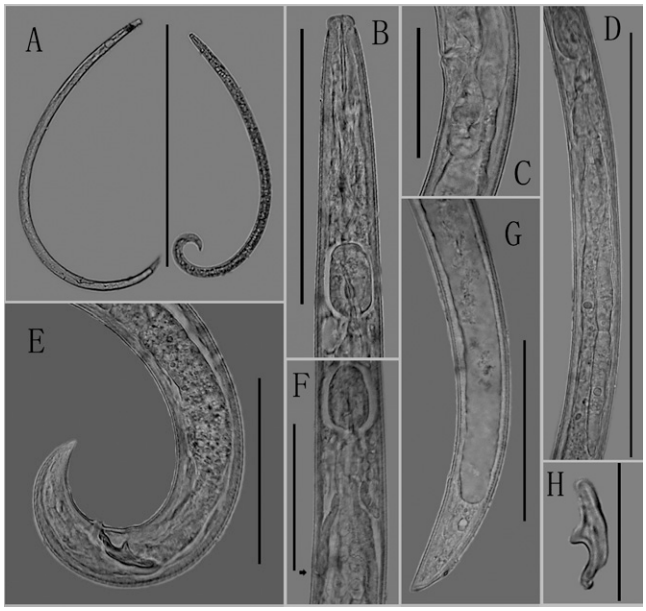

FIG. 5. Light micrographs of *Ektaphelenchus macrobulbosus*. A) Entire view of female (left) and male. B) Anterior region of female. C) Vulva and postuterine sac. D) Pharyngeal gland lobe. E) Male tail. F) Excretory pore. G) Female tail. H) Spicule. (Scale bars: A = 400 μm; B = 70 μm; C = 30 μm; D = 130 μm; E, G = 50 μm; F = 40 μm; H = 20 μm.)

prominent pointed rostrum, a cucullus (apophysis) presented on the dorsal distal end. Gubernaculum absent. Tail conoid, tapering to pointed terminus. Two pairs of subventral papillae present: first pair precloacal, second pair postcloacal. Bursa absent.

HOST AND LOCALITY: The examined specimens were extracted from the xylem of dead Simao pine (*Pinus kesiya* var. *langbianensis*) infested with *Bursaphelenchus xylophilus*, in Wanding County of Yunnan Province, China.

DIAGNOSIS: *Ektaphelenchus macrobulbosus* is characterized by a medium-long female and male body (676-791 and 613-685 μm, respectively), three incisures in the lateral field, knobless delicate stylet, two to three rows in anterior gonad, short postuterine sac, absence of anus and rectum in females, presence of two pairs of male caudal papillae, the characteristic spicule shape (a elongated apex and a cucullus present on the dorsal distal end), and the conoid female and male tails.

REMARKS: The Chinese population of *Ektaphelenchus macrobulbosus* was first recorded by Qin & Pan (2003) from *Pinus massoniana* in south-eastern China, and morphometric comparisons with the original description by Rühm (1956) (unknown type host), and later observations by Massey (1974) (from beetle) and Baujard (1984) (from *P. pinaster*) were first taken by them. In the present study *E. macrobulbosus* was isolated from *P. kesiya* in south-western China, but the morphmetrics agrees well with population in south-eastern China and that remarked by Baujard (1984) except for a larger b value (8.8-10.3 vs. 7.7-9.4 and 7-9). It also conforms to the description of Rühm (1956) except for longer female and male bodies (676-791 μm vs. 520-580 μm in female and 613-685 μm vs. 430-460 μm in male) and a small V value (78-80 vs. 80.6-82). Other morphological characters including lateral field, lip region, stylet, pharynx, gonads, spicule as well as the male caudal papillae positioned pattern are quite similar to the redescription of Baujard (1984) from *P. pinaster* in France, although they show minor variation e.g. the male tail tip morphology (sharper in the latter), which might be the result of the geographical isolation. However, re-isolation and further comparisons involving molecular phylogenetic inferences within these populations are necessary to precisely determine the phylogenetic position of this Chinese population *E. macrobulbosus*.

DISCUSSION

The family Diplogastridae is a taxonomically difficult group. Though Sudhaus & Fürst von Lieven (2003) organized the taxonomic status of this group into 28 genera. most species are poorly described. This Chinese diplogastrid population was designated as a new species. According to the available description data from literature (Massey, 1966), it can be differentiated from *Micoletzkya tomea* only by its small body sizes and nuances concerning the stoma morphology. In addition, many

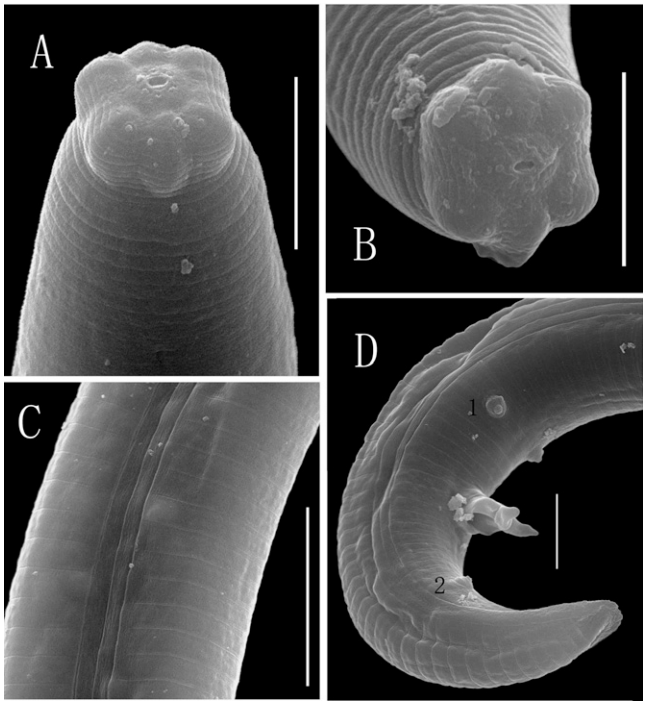

FIG. 6. Scanning electron micrographs (SEM) of *Ektaphelenchus macrobulbosus*. A) Head. B) Lip region. C) Lateral field with 3 lines. D) Two pairs of subventral papillae on male tail (1: precloacal pair; 2: postcloacal pair). (Scale bars: A, B, D = 5 μm; C = 10 μm.)

TABLE 2. Morphometrics of *Ektaphelenchus macrobulbosus* [all measurements in μm and in the form: mean ± SD (range) coefficient of variation].

Character	Females	Males
n	10	8
L	725.7±37.1 (675.5-790.6)	635.8±24.4 (612.8-685.0)
a	35.7±3.0 (31.8-41.0)	36.8±3.7 (31.9-41.3)
b	9.5±0.48 (8.8-10.3)	8.7±0.26 (8.4-9.1)
c	–	19.8±0.70 (18.4-20.7)
c'	–	2.4±0.19 (2.1-2.7)
V	78.7±0.58 (78.0-79.9)	–
Stylet	18.9±0.76 (18.2-20.4)	18.4±0.39 (18.0-19.1)
Median bulb long	18.2±0.80 (17.1-19.7)	17.4±0.84 (16.1-19.0)
Median bulb diam	10.0±0.45 (9.3-10.6)	9.3±0.57 (8.6-10.4)
Pharyngeal gland lobe length	134.1±18.6 (112.2-167.8)	124.2±8.2 (110.7-132.8)
Body diam. (females = VBD; males = GBD)	18.2±1.6 (16.7-22.0)	17.4±2.4 (15.1-20.9)
Spicule length	–	16.5±0.84 (15.8-18.3)
Tail length	–	32.2±2.0 (29.8-35.3)

genera bear some striking resemblances and display close relationships to one another (Calaway & Tarjan, 1973; Fürst von Lieven & Sudhaus, 2000). This diplogastrid species was morphologically placed into the genus *Micoletzkya*, but it showed close similarities to *Mononchoides* clade, *Koerneria* spp., *Fictor* members and *Acrostichus* group. A recently molecular phylogeny of beetle associated diplogastrid nematodes provided a sketch phylogenetic relationship among this group (Mayer et al., 2009). However, more morphological characters as well as molecular data are indispensable before a sound evolutional and phylogenetic tree between and within these genera can be erected. And further detailed comparison morphologically between the new diplogastrid and *M. tomea* is necessary when *M. tomea* is recollected or its male specimens are found in the type locality.

Individuals of *Micoletzkya chinaae* n. sp. were fed on fungal culture dishes in this study, but bacteria were found around the opening of the stoma as well as inside the buccal cavity (see Fig.3 A & C). – They are microbivorous. According to Fürst von Lieven & Sudhaus (2000) and Fürst von Lieven (2008), juveniles of *Koerneria* spp. were at risk to predation by the adults especially when the presence of the eurystomatous morph. Since the two large moveable teeth are also present in eurystomatous nematodes of *M. chinaae* n. sp., the question whether *M. chinaae* n. sp. could have the potential to feed as predators on other nematodes might be posed. And if so, it might have the promise as a biological control agent for pine wood nematode, a destructive but difficult to control parasite of pines. All known *Micoletzkya* members are reported as insect associates (Massey, 1966; Sudhaus & Fürst von Lieven, 2003). With the beetle tunnels observed on the wood sample from which *M. chinaae* n. sp. was extracted, this species might have phoretic relationships with bark beetles. However, the bionomics/predatory lifestyle and mode of transmission to Simao pine of this species need further studies. Phasmids might be located posterior to the anus of female and behind GP5 genital papillae of male, but they were not clearly observed in this study. In addition, more denticles might exist in the left sector of buccal cavity especially in steno-morph, however, it is difficult to observe with the present study methods. According to Fürst von Lieven & Sudhaus (2000), treatment with lactic acid (96%) may provide a better view of pharynx structures. Thus, re-isolation of this species and re-examination of the above features are necessary in the future.

According to previous description of Hunt (1993), the genus *Ektaphelenchus* is distinguished from the genus *Cryptaphelenchoides* by lip morphology (lips of unequal size vs. lips of equal width), and presence (*Cryptaphelenchoides*) or absence (*Ektaphelenchus*) of a cucullus at the distal end of spicule. However, Kaisa (2000) discussed that *Cryptaphelenchoides* should be considered as a synonym of *Ektaphelenchus* as proposed by Massey

(1974), Andrássy (1976), Baujard (1984), and Ebsary (1991). In this study, we confirmed a cucullus at the distal end of spicule of *E. macrobulbosus*. The SEM observations show for the first time the detailed lip shape of *E. macrobulbosus* that the lateral lips are slightly narrower than the other four lips (see Fig.6 A & B), which conforms well to the description of the genus remarked by Hunt (1993). However, since detailed lip morphology is difficult to be observed beyond SEM examination, we agree the revision by Hunt (2008) to transfer *Cryptaphelenchoides* spp. into the genus of *Ektaphelenchus*.

Many nematode groups are reported to vary in sex ratio, e.g. *Seinura* spp. (Triantaphyllou, 1971), in which male occurrence is about 50% in *S. tenuicaudata* (with a sex ratio of approximately 1:1) but only 15-40% and 5-20% in *S. celeries* and *S. oliveirae*, respectively. A lower percentage of males to females in *Ektaphelenchus macrobulbosus* was observed in the present study. Together with the south-eastern population described by Qin & Pan (2003) in which no male was found, the phenomena might indicate males of this species are present less than females. However, only one female was recorded by Massey (1974), and the proportion of sexes was not mentioned by Rühm (1956) and Baujard (1984), so the exact sex ratio of this species needs further detection.

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