

A NEW NEMATODE SPECIES (DORYLAIMIDA: APORCELAIMIDAE) FROM INDIA WITH A REVISED KEY TO THE SPECIES

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Article History: Received 12th December 2024; Accepted 23rd January 2025; Published 31st January 2025

ABSTRACT

Few numbers of soil-inhabiting nematodes were observed to represent some differences from the present valid species of the genus *Makatinus* Heyns, 1965 under the family Aporcelaimidae Heyns, 1965. Hence a new nematode species is being proposed and described as *Makatinus digicaudatus* sp. nov. The proposed new species is characterized by its large stout body, constricted lip region, strong odontostyle, large aperture occupying more than half (59.3%) of the odontostyle length, well and equally developed anterior and posterior female genital system, long ovaries, short tail, convex-conoid with a small hyaline digitate projection and short prerectum in female. *Makatinus digicaudatus* sp. nov. resembles *M. capensis* Heyns, 1965, *M. crassiformis* (Kreis, 1924) Andrassy, 1986 and *M. punctatus* Heyns, 1965 and can be distinguished from all other species of the genus by its long body and tail shape except *M. punctatus*. A key has been provided based solely on the female of the species.

Keywords: Description, *Makatinus digicaudatus* sp. nov., New species, Taxonomy.

INTRODUCTION

One female and three juvenile nematode specimens were collected from the soils of unidentified grass near aquatic habitat in Odisha state (Latitude 19°39.978N and Longitude 85°15.700E), India. Among the other nematode population in this soil sample, these few specimens showed some interesting characters. Taxonomic observation revealed the existence of a species new to science under the genus *Makatinus* Heyns, 1965. In the present contribution, *Makatinus digicaudatus* sp. nov. is being described as the 10th. species of the genus. The genus *Makatinus* was established to include two new species *M. punctatus* as the type and *M. capensis* from South Africa (Heyns, 1965). Five valid species under *Makatinus* was considered with the addition of a new species *M. aquaticus* from Spain and *Dorylaimus tritici* Thorne & Swanger, 1936, described from United Kingdom, was renamed as *Makatinus goodeyi* on the basis of male specimens only (Jiménez Guirado, 1994). *Dorylaimus simus* Andrassy, 1958, described from Bulgaria, was transferred to *Makatinus* as a new combination and 8 valid species under the genus was

considered (Andrassy, 2002). From India, *M. heynsi* Ahmad & Ahmad, 1992, *M. micropunctatus* Ahmad & Ahmad, 2004 and *M. siddiqii* Gantait, Bhattacharjee & Chatterjee, 2011 have been described. Among these 3 Indian species, Peña-Santiago & Varela (2017) considered *M. siddiqii* as *species inquirenda* and they opined that the validity and status of *M. siddiqii* and *M. simus* require further confirmation. Based on the light and scanning electron microscopic observation, Varela *et al.* (2017) synonymized *M. macrostylus* Shaheen & Ahmad, 2004, described from Costa Rica, with *M. crassiformis* (Kreis, 1924) Andrassy, 1986, reported from Surinam due to similarities in their morphometrics. The species of *Makatinus* are distributed in South Africa, Surinam, India, Sri Lanka, United Kingdom, New Zealand, Spain, Hungary and Costa Rica. In the present communication 9 valid species have been listed apart from the newly proposed species under the genus, although Peña-Santiago & Varela (2017) considered 8 valid species because they did not include *M. micropunctatus* Ahmad & Ahmad, 2002 described from India in the list of valid species.

List of valid species:

1. Type species: *Makatinus punctatus* Heyns, 1965
2. *M. aquaticus* Jiménez Guirado, 1994
3. *M. capensis* Heyns, 1965
4. *M. crassiformis* (kreis, 1924) Andrassy, 1986
= *Dorylaimus crassiformis* kreis, 1924
= *M. macrostylus* Shaheen & Ahmad, 2004
5. *M. goodeyi* Jiménez Guirado, 1994
= *Dorylaimus tritici apud* Thorne & Swanger, 1936
6. *M. heynsi* Ahmad & Ahmad, 1992
7. *M. micropunctatus* Ahmad & Ahmad, 2002
8. *M. minor* (Loos, 1945) Ahmad, 1997
= *Aporcelaimus minor* Loos, 1945
9. *M. silvaticus* Ahmad, Sturhan & Wouts, 2003

MATERIALS AND METHODS

Soil sample of about 500 gm. was collected with the help of a hand-shovel. At the time of collection, the collection data were recorded and the geographical coordinates of that particular sampling site was measured by a GPS (Garmin GPS72H). To avoid dehydration, sample was stored in a transparent polythene bag and its open end was tied with a rubber band with few minute holes on the bag to reduce the hygroscopic pressure. The collected soil samples were processed by Cobb's sieving and decantation technique (Cobb, 1918) followed by modified Baermann funnel technique (Christie & Perry, 1951) for extraction of nematodes. The extracted nematodes were killed and fixed instantly in their characteristic body posture by Seinhorst's method in hot Formaldehyde-acetic acid (f. a. 4:1) solution (Seinhorst, 1966). These were preserved in the same solution with appropriate labels. The preserved specimens were picked one by one and were transferred in a cavity block containing glycerine-alcohol solution. The cavity block containing nematodes was kept in a desiccator at least for 3 weeks. After complete dehydration, the specimens were mounted in anhydrous glycerin and sealing was done by paraffin wax (De Maeseneer & d'Herde 1963) to make permanent slides. Then they were observed under a compound DIC microscope (Nikon Eclipse Ni), measured, photographed by digital camera and was drawn by drawing tube attached to the same microscope.

Systematic Account

Phylum Nematoda Rudolphi, 1808 (Lankester, 1877)
Order Dorylaimida Pearse, 1942
Suborder Dorylaimina Pearse, 1936
Superfamily Dorylaimoidea De Man, 1976
Family Aporcelaimidae Heyns, 1965

Subfamily Aporcelaiminae Heyns, 1965

Genus *Makatinus* Heyns, 1965

Makatinus digicaudatus sp. nov.

RESULT AND DISCUSSION

Makatinus digicaudatus sp. nov.

(Figures 1 and 2)

Description: The measurements given hereafter are based on female holotype and juveniles.

Dimensions: Shown in Table I.

Female: Body ventrally curved to assume a 'C' shape when fixed, tapering towards both extremities. Cuticle consists of two layers, the outer layer with fine transverse striations, without criss-cross lines, punctuations not clearly perceptible, almost uniformly thick throughout the body except on tail, 5 µm thick at anterior end at the level of odontostyle, 7 µm at mid-body and 11 µm on ventral side of tail. Anterior end tapering, lip region about 1/5th of neck base diameter, slightly wider than adjacent body, 1.08 times the adjacent body, 8.5 µm high and 27 µm wide, weakly separated from adjacent body by weak constriction, lips amalgamated, anterior margin almost truncate with minor protrusions. Amphid not clearly visible, at about 15.5 µm from the anterior end. Odontostyle robust with thick walls on dorsal and ventral sides, longer than lip region, about 1.1 lip region-widths long or 5.3 times the width of odontostyle, aperture large, occupying 1/1.6 or 59.3% of the odontostyle length. Guiding ring not well marked 15.5 µm from anterior end. Odontophore simple rod-like, 1.4 times the odontostyle length. Pharynx strongly muscular, about ¼ th. (1/4.6) or 13.4% of the body length, gradually expands. Expanded portion of pharynx more than half, 57.2% of the pharyngeal length or 3.5 times the neck base-width long. Pharyngeal gland nuclei are inconspicuous and their positions could not be measured. Nerve ring at 292 µm from anterior end or at 35.7% of pharynx length. Cardia short, conoid, wide at pharyngo-intestinal junction and then narrow, 27 µm long or about one-fifth (1/4.8) of neck base width. Female reproductive system didelphic-amphidelphic, with both genital branches equally well developed, anterior branch of gonad longer than posterior. Ovaries large, reflexed back near to vulva, posterior ovary longer than anterior ovary, oocytes arranged in a single row except at the growth region. Anterior ovary 427 µm and posterior ovary 486 µm long. Oviduct long, tubular, joining the ovary sub-terminally. Anterior oviduct and sphincter at the junction of uterus-oviduct junction not clear due to the presence of two large intra-uterine eggs, 288 µm long. Posterior oviduct 307 µm long, indistinct sphincter separating oviduct from uterus. Uterus muscular, anterior uterus 288 µm and posterior uterus 119 µm long. Anterior intra-uterine eggs oval in shape, measuring 159-166 µm X 97.5-100 µm. Vulva transverse, equatorial, at 49.2% of the body. Vagina extending inward slightly less than half or 45.6% of the corresponding body width. The length of *Pars proximalis*

vagina 51 μm , *pars Refringens vaginae* 15 μm , two sclerotized pieces almost triangular, combined width of *pars Refringens vaginae* 20 μm and the length of *pars distalis vagina* 3 μm . Prerectum 2.7 anal body widths or 1.8 times the rectum length. Rectum 1.5 anal body widths long. Tail short, convex conoid ending in a small hyaline digitate projection (4.5 μm long) at the terminus, 0.6 anal body-widths long.

Juvenile (J4): Morphologically similar to female except smaller body and absence of female reproductive system and differences in other measurements. Replacing odontostyle is longer, 1.03-1.23 times the functional style. Morphologically tail exactly the same as in female.

Table I. Morphometrics of *Makatinus digicaudatus* sp. nov. (All measurements are in μm except L and body ratios, L in mm).

| Characters | Holotype female | Juveniles |
|--------------------------------|-----------------|-------------|
| n | | 3 |
| L | 3.83 | 1.90-2.45 |
| a | 25.3 | 23.1- 24.7 |
| b | 4.6 | 73.4-96.2 |
| c | 123.5 | 53.5-53.7 |
| c' | 0.6 | 0.6-0.7 |
| V% | 49.2 | - |
| G ₁ % | 26.9 | - |
| G ₂ % | 23.8 | - |
| Odontostyle length | 32.0 | 26.0 |
| Replacing Odontostyle | - | 27.0-32.0 |
| Odontophore length | 45.0 | 40.0-44.0 |
| Odontostyle aperture | 19.0 | 16.0-20.0 |
| Odontostyle width | 6.0 | 4.5-5.0 |
| Guiding ring from anterior end | 15.5 | 9.0-11.0 |
| Nerve ring from anterior end | 292.0 | 134.0-187.0 |
| Maximum body width | 151.0 | 77.0-106.0 |
| Body width below lip region | 25.0 | 23.0-28.0 |
| Body width at neck base | 132.0 | 700-106.0 |
| Body width at vulva | 151.0 | - |
| Pharynx length | 817.0 | 547.0-684.0 |
| Expanded part of pharynx | 468.0 | 290.0-398.0 |
| Length of cardia | 27.0 | 22.0-25.0 |
| Length of anterior gonad | 1034.0 | - |
| Length of posterior gonad | 912.0 | - |
| Length of vagina | 69.0 | - |
| Anterior end to vulva | 1888.0 | - |
| Tail length | 31.0 | 25.5-26.0 |
| Anal body width | 49.0 | 35.0-41.0 |
| Length of prerectum | 134.0 | 87.0-96.0 |
| Length of rectum | 74.0 | 48.0-64.0 |

n = Number of Specimens, L = Body length, a = Body length/Maximum body width, b = Body length/Length of pharynx, c = Body length/Tail length, c' = Tail length/Body width at anus, V = Position of vulva in body in percentage (Position of vulva from anterior end/Body length)x100, G₁

= Length of Anterior gonad in percentage in respect of body length (Length of Anterior gonad/Body length)x100, G₂ = Length of Posterior gonad in percentage in respect of body length (Length of Posterior gonad/Body length)x100.

Table 2. Morphological and morphometric comparison between females of *Makatinus digicaudatus* sp. nov. and the closely related species (All measurements are in μm except L and body ratios, L in mm).

| Characters | <i>M. digicaudatus</i> sp. nov. | <i>M. capensis</i> | <i>M. crassiformis</i> | <i>M. punctatus</i> |
|------------|---------------------------------|--------------------|------------------------|---------------------|
| L | 3.83 | 3.61-3.77 | 2.45-6.04 | 2.40-3.17 |
| a | 25.3 | 32-34 | 20-34 | 36-42 |

| | | | | |
|-------------------|---------------------|------------|-------------------------|---------------------|
| <i>b</i> | 4.6 | 4.4-5.0 | 3.6-5.8 | 4.2-4.4 |
| <i>c</i> | 123.5 | 103-127 | 71- 183 | 84-99 |
| <i>c'</i> | 0.6 | 0.5-0.6 | 0.3-0.7 | 0.7 |
| V% | 49.2 | 48-50 | 37-63 | 56-59 |
| Position of vulva | Equatorial | Equatorial | Pre- to post-equatorial | Post-equatorial |
| Odontostyle | 32 | 24-28 | 30-40 | 25-27 |
| Pharynx | 817 | 754 | 769-1161 | 729 |
| Tail terminus | digitate projection | Round | Round | digitate projection |
| Prerectum | 134 | - | 107-190 | - |
| Anal body width | 49 | 50 | 72-101 | - |
| Tail | 31 | 30 | 22-49 | 31 |

Comparison with other genera: The morphological features of the present specimens like cuticle, odontostyle and its aperture and the tail in particular strongly supports its inclusion under the family Aporcelaimidae Heyns, 1965 and the subfamily Aporcelaiminae Heyns, 1965. The genus *Makatinus* comes close to the genera *Aporcelaimus* Thorne and Swanger, 1936 and *Aporcelaimellus* Heyns, 1965. The females of these three genera can be differentiated from each other on the basis of morphology of lip region, the nature of cuticle and the aperture of odontostyle (Peña-Santiago and Varela, 2017). In *Aporcelaimus*, the cuticle features criss-cross lines, the lips are separate, and the lip region is distinctly set off by a deep constriction, with the odontostyle aperture occupying significantly more than half of its length. This indicates that the present specimens do not belong to *Aporcelaimus*. On the other hand, in *Aporcelaimellus*, the generally smaller body length, the lip region offset by deep constriction and angular lips, along with a three-layered cuticle, visible lacuna at the tail tip suggests that the present specimens cannot be classified as *Aporcelaimellus*. Hence, on the basis of emended diagnosis provided by Peña-Santiago and Varela, 2017, the present specimens have been included under the genus *Makatinus*.

Differential diagnosis and relationship: The female of *Makatinus digicaudatus* sp. nov. can be diagnosed and characterized by its large stout body, weakly constricted lip region separated from the adjacent body, large aperture occupying more than half of the odontostyle length, fairly long pharynx, well and equally developed anterior and posterior female genital system, long ovaries, tail short, convex conoid both dorsally and ventrally with a hyaline short straight digitate projection at the tip of the tail in female and juveniles. Short prerectum in female.

Makatinus digicaudatus sp. nov. comes close to *M. capensis* Heyns, 1965, *M. crassiformis* (Kreis, 1924) Andrásy, 1986 (= *M. macrostylus* Shaheen & Ahmad, 2004) and *M. punctatus* Heyns, 1965 and can be differentiated from all other species of the genus by its long body and tail shape except *M. punctatus*. In *M. microstylus* Ahmad & Ahmad, 2002 female tail is also with a dorsally arcuate digitate projection but the body length is significantly shorter than *M. digicaudatus* sp. nov. A

comparison of morphology and morphometrics of females between *Makatinus digicaudatus* sp. nov. with the closely related species has been shown in table 2.

The proposed new species can be differentiated from female of *M. capensis* in having a stouter body evident from less a-value, longer odontostyle and pharynx and shorter convex conoid tail with a digitate terminus (in the female of *M. capensis*, a = 32-34, odontostyle=24-28 μ m, pharynx = 754 μ m, tail round). The females of *Makatinus digicaudatus* sp. nov. can be distinguished from *M. crassiformis* (= *M. macrostylus*) in having short odontostyle, pharynx and pharyngeal expansion (although within the range in *M. crassiformis*), an equatorial position of vulva, shorter prerectum, less anal body diameter and digitate projection in tail (in the female of *M. crassiformis*, odontostyle = 30-40 μ m, pharynx = 769-1161 μ m, pharyngeal expansion = 429 – 661 μ m, vulva pre- to post-equatorial, V= 37-63%, prerectum = 107-190 μ m, anal body diameter = 72-101 μ m; in the female of *M. macrostylus*, pharynx = 801-959 μ m, pharyngeal expansion = 519 – 607 μ m, vulva pre-equatorial, V= 38-47%, prerectum = 132-178 μ m, anal body diameter = 72-101 μ m, tail round). The female of the present new species is morphologically similar but can easily be distinguished from *M. punctatus* in having a long and stout body evident from a-value, longer odontostyle and pharynx and equatorially located vulva (in the females of *M. punctatus*, L= 2.40-3.17mm, a= 36-42, odontostyle= 25-27 μ m, pharynx= 729 μ m, vulva post-equatorial, V= 56-59%).

Type specimens: Holotype female along with one juvenile on the same slide, 2 juveniles on two different slides. Deposited in the National Zoological Collection, Zoological Survey of India, Kolkata (formerly Calcutta), India.

Type habitat and locality: Collected by the first author from soil around the roots of unidentified grass on the bank of Chilika Lake, Krishna Prasad Garh (Latitude 19°39.978N and Longitude 85°15.700E), Puri district, Odisha state, India.

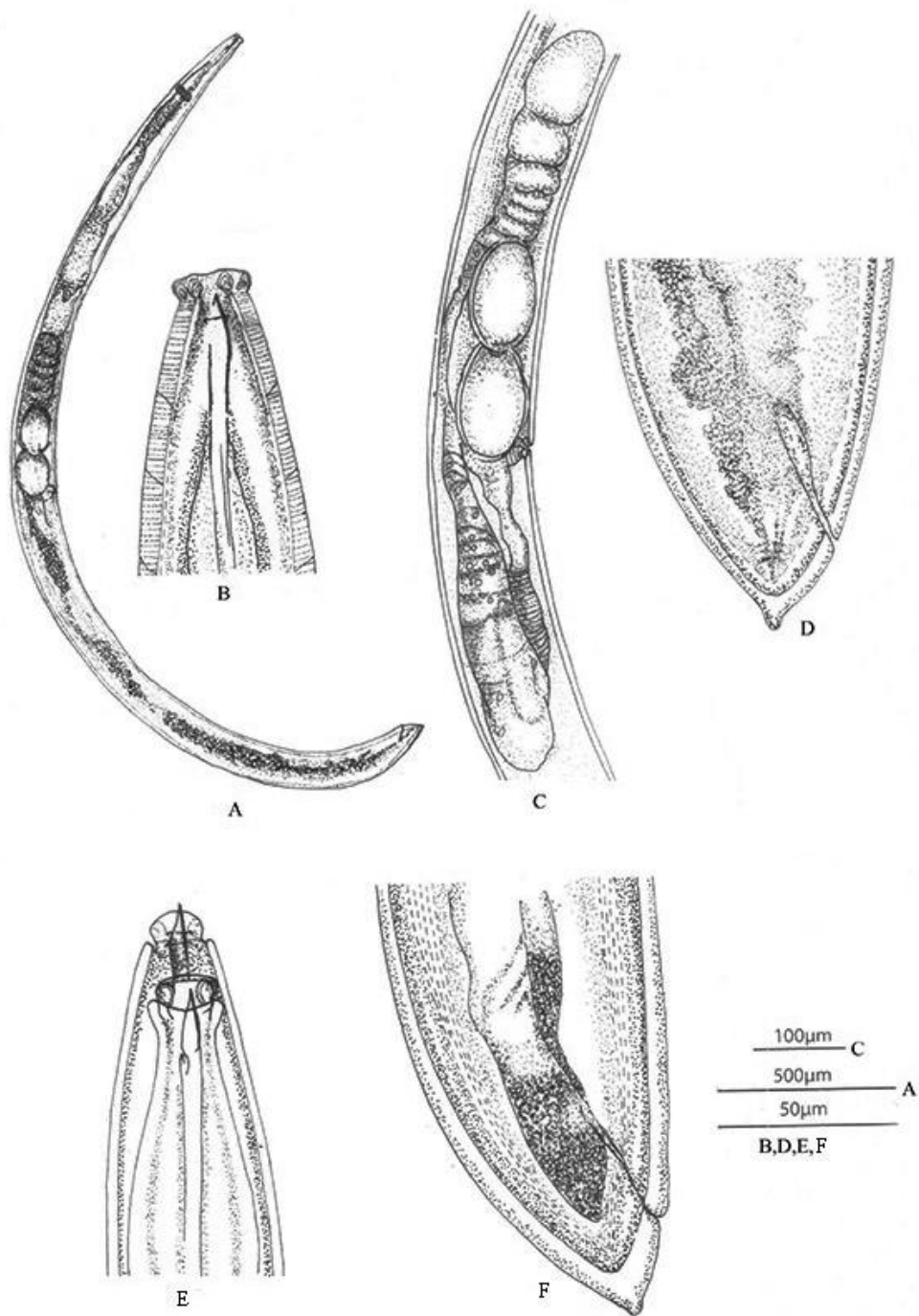


Figure 1. A – F. *Makatinus digicaudatus* sp. nov. **Female.** A. Entire body, B. Anterior end showing odontostyle, C. Female reproductive system, D. Tail. **Juvenile.** E. Anterior end showing functional and replacing odontostyle, F. Tail.

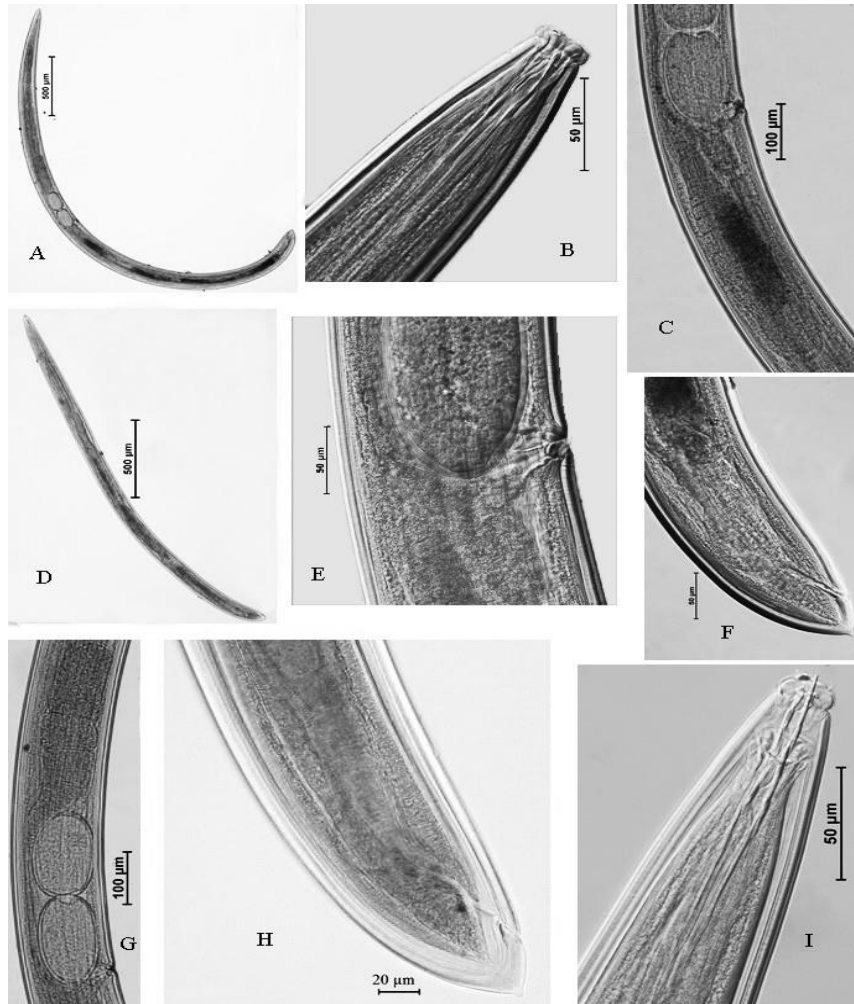


Figure 2. A – I. *Makatinus digicaudatus* sp. nov. **Female.** A. Entire body, B. Anterior end showing odontostyle, C. Posterior gonad, E. Vulva, F. Posterior end showing Tail, G. Anterior gonad. **Juvenile.** D. Entire body, H. Tail, I. Anterior end showing functional and replacing odontostyle.

Etymology: The new species has been named after its characteristic digitate tail terminus in female.

Key to the identification of valid species based on the female:

(*Makatinus goodeyi* is not included because female is unknown and not yet reported). Modified after Andrassy (2002) and Peña-Santiago & Varela (2017).

1. Length of female below 3 mm (1.38-2.72 m).....2
 - Length of female mostly over 3 mm (2.40 – 3.92 mm).....6
2. Female tail with digitate (peg-like) projection, directed towards dorsal side, the smallest species (L = 1.38-1.43 mm)*M. microstylus*
 - Female tail without any projection, Length over 1.5 mm.....3
3. Vulva almost equatorial to post-equatorial (V= 46-59%)4

- Vulva equatorial (V = 49-53%)5
4. Position of vulva varies in species, either equatorial (V=50-51%) or post-equatorial (V= 54-59%), stout body (a = 24-35)*M. aquaticus*
 - Vulva equatorial (V = 46-52%), less stout body (a = 37-43)*M. heynsi*
5. Prerectum long (88-180 µm), Longer species (L = 2.35-2.72 mm) *M. silvaticus*
 - Prerectum short (73-86 µm), smaller species (L = 1.76-2.24 mm)*M. minor*
6. Tail round without any digitate projection7
 - Tail convex-conoid with terminal digitate projection.....8
7. Position of vulva varies in species, pre-equatorial to post equatorial, (V= 37-63%), length of tail also varies from short to long (26-49 µm).....*M. crassiformis*

- Vulva equatorial (V = 48-50%), tail short (37 µm)*M. capensis*
- 8. Vulva post-equatorial (56-59%), L=2.40-3.17 mm, odontostyle 25-27 µm.....*M. punctatus*
- Vulva equatorial (V=49.2%), L= 3.83 mm, odontostyle 32 µm.....*M. digicaudatus* sp. nov.

Remark: The specimens showed strong morphological inclination towards the genus *Makatinus* with reference to its cuticle, lip structure, odontostyle and caudal region under the family Aporcelaimidae and could not be placed under any other genus of the family. Moreover, the specimens are clearly distinguishable from any other species of the genus as discussed under diagnosis and relationship. This is worthy to mention that in the present communication an identification key to the species of the genus *Makatinus* has been provided only on the basis of female characters because identification keys are available depending on both male and female and sometimes only with the male characters (Andrássy 2002, Peña-Santiago & Varela 2017). In the latter case it is difficult to identify a species with only female specimen available.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Dhriti Banerjee, Director, Zoological Survey of India for providing all sorts of facilities, constant inspiration and encouragement to carry out the work and to publish the result.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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