



## Morphological Characterization of *Mesocriconema mamitensis* N. Sp. and A Known Species, *Mesocriconema medani* (Phukan & Sanwal, 1981) Loof & De Grisse, 1989, From Mamit District, Mizoram

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Article History	Abstract
Received: 06 June 2022 Revised: 05 March 2023 Accepted: 11 March 2023	<i>Nematological investigation of the order Tylenchida, super-family Criconematoidea and sub-family Macroposthoniinae revealed the presence of two species of the genus Mesocriconema i.e., Mesocriconema mamitensis n. sp. and M. medani, in the rhizospheres of lemongrass and pineapple, respectively, from Mamit district of Mizoram, India. Mesocriconema mamitensis n. sp. is characterized by body length = 0.34-0.45 mm, stylet length = 37.6-49.6 <math>\mu</math>m, R = 117-120, RV = 7-9, Rvan = 1-2, Ran = 5-7, Roes = 24-32, V = 91.6-93.2 <math>\mu</math>m, and VL/VB = 0.91-1.27 <math>\mu</math>m. Mesocriconema medani is characterized by body length = 0.37-0.47 mm, stylet length = 40.6-46.1 <math>\mu</math>m, R = 117-118, RV = 7-9, Rvan = 1-2, Ran = 5-7, Roes = 26-29, V = 90.4-91.6 <math>\mu</math>m, and VL/VB = 1.2-1.3 <math>\mu</math>m. The morphological characterizations of the two species have been done through light and scanning electron microscopy studies. Morphometrics, line illustrations, and information about their hosts and locality are also provided. In addition to the new species description, this study reports M. medani for the first time in Mizoram, India.</i>
CC License CC-BY-NC-SA 4.0	<b>Keywords:</b> <i>Mesocriconema, Scanning Electron Microscopy, Taxonomy, Tylenchida</i>

### 1. Introduction

The north-eastern region of India comprises eight states, namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura, which are highly rich in faunal diversity. Among them, Mizoram is also one of the biodiversity hotspot regions,

largely covered by hilly areas, extending from 21°56' N to 24°31' N and 92°16' E to 93°26' E. As less exploration of soil nematodes has been done from this region, a survey for Criconematids (Nematoda: Tylenchida) was conducted in the region in October 2019 to fill some knowledge gaps on the diversity of nematodes.

The plant parasitic nematodes (PPN) belonging to the superfamily Criconematoidea, Taylor (1936) are called Criconematids. They are both cosmopolitan and indigenous in occurrence Hyen's (1970) and are mostly ectoparasites to various plants Geraert (1966). They mostly feed on woody perennials, crops and vines with their habits ranging from migratory through semi-sedentary to sedentary ectoparasitism Christie (1959); Thorne (1961); Jenkin & Taylor (1967). They are morphologically readily distinguishable from other PPN by the presence of strong body annuli, lip forming a labial disc, a poorly developed labial region, generally amalgamated pro-metacarpus and pyriform post-carpus which is set off from the intestine. Females are generally used in the identification of species levels. It has a single anteriorly directed genital tract; may have retrorse annuli, scales, spines, or an extra cuticular sheath on the body, and a lateral field differentiation may be present or absent.

The criconematid genus *Mesocriconema* Andrassy (1965) was proposed for the species of *Criconemoides sensu lato* with crenated margins on body annuli. De Grisse & Loof (1965) also proposed to split *Criconemoides* Taylor (1936) into various genera including *Macroposthonia* de Man (1880). However, *Macroposthonia* was placed under the genus *dibium* by Coomans *et. al.*(1990) but, Siddiqi (2000) & Wouts (2006) considered it valid. Later, Loof & De Grisse (1989) discussed the comparative characters of *Criconemoides* and *Macroposthonia*, replaced *Macroposthonia* with its oldest synonym *Mesocriconema* and revalidated the genus *Criconemoides* based on the discussion of Loof & Grisse (1967). Furthermore, Brzeski *et. al.*(2002) also validated the genus *Mesocriconema* and listed 90 valid species Geraert (2010) in this genus. *Mesocriconema* is characterized by the presence of retrorse annuli which can be smooth or uneven or crenate. The lateral field is usually with anastomoses. The second annule is wider than the first, true submedian lobes are present but can be absent exceptionally, the labial plate may be absent or present, the female vulva is an open type with ornamented vulval lip in several species and the tail is short, rounded or truncate but seldom acute.

The study aimed at examining the morphological characterization of a new criconematid species, *Mesocriconema mamitensis* n. sp.. A population of *M. medani* detected in our soil samples was collected during our nematode survey. The species description is based on morphological analyses including light and scanning electron microscopy studies, morphometrics, line illustrations, host information, and geographic distribution of the species.

## 2. Materials And Methods

Soil samples were collected randomly from around the rhizosphere of lemongrass and pineapple, in October 2019 from Ailawng, Mamit district of Mizoram, India. Nematodes were extracted from the soil by Cobb's (1918) sieving and decanting method followed by Baermann's funnel technique Thorne (1961). The extracted nematodes were killed and fixed in warm Formalin-Alcohol or F. A. (4:1) and dehydrated using glycerine-alcohol methods of Seinhorst (1962) and kept in a desiccator for slow dehydration. The fixed specimens were mounted on glass slides in dehydrated glycerine, covered with coverslips and sealed the edges of coverslips using nail polish. Measurement of morpho-anatomical structure and light microscopy images of the specimen were taken using a microscope-mounted camera and software (Nikon Eclipse E200 with VUE2017) and line illustrations were made with the help of the drawing tube, also mounted on the same microscope.

For scanning electron microscopy (SEM), some specimens were fixed in Trump fixative {2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Sorenson buffer (Sodium phosphate buffer at pH = 7.5)}. The fixed specimens were then washed in 0.1 M phosphate buffer (pH=7.5) and subsequently dehydrated in different grades of alcohol. The dehydrated specimens were dried at a critical-drying

point with liquid CO<sub>2</sub> and mounted on stubs with double conductive carbon tape and coated with 25 nm gold. The photographs were taken with a JSM-840EM (JOEL) at 12 kv Singh *et. al.* (2022).

***Mesocriconema mamitensis* n. sp.**

(Figure 1-2; Table 1-2)

**Description:**

Female: Body cylindrical and slightly arcuate upon fixation; both anterior and posterior ends tapering, but anterior end is somewhat conically pointed compared to the posterior end. Lip region consists of two annuli, the first annule is not retrorsed but all other succeeding annuli retrorsed; the second annule is wider than the first annule. Labial plate uninterrupted laterally, trilobed on one side and bilobed on another side, with an I-shaped oral aperture, and four large submedian lobes at four corners of the labial plate. Few anastomoses are present in the mid-body region. Total body annuli 117-120 in number and annuli with posterior smooth margins. Stylet is strong and relatively short with a large rounded basal knob that lies at 15-17 annuli from the anterior end of the body. Oesophagus criconematid type with a length of 37-49 µm. Reproductive system mono-pro delphic, ovary outstretched with a length of 98-119 µm. Spermatheca oval, filled with spermatozoan. Vulva open type and lies at 8<sup>th</sup>-9<sup>th</sup> body annule from the posterior end. The tail is short, conical and slightly pointed with a bilobed terminus.

Male: Not found.

**Type Host and Habitat:** *Cymbopogon* sp. (common name: lemon grass) from Ailawng, Mamit district, Mizoram with longitude and latitude of 23'693443" E & 92'629141" N, respectively.

**Type specimen:** Holotype slide MU/MO/1 *Mesocriconema mamitensis* n. sp., and paratype slides MU/MO/2-8 *Mesocriconema mamitensis* n. sp. have been deposited at the nematode collection center of Parasitology Section, Department of Zoology, Manipur University Canchipur 795003, Manipur, India.

**Etymology:** The species is named after the district Mamit of Mizoram from where it has been recovered.

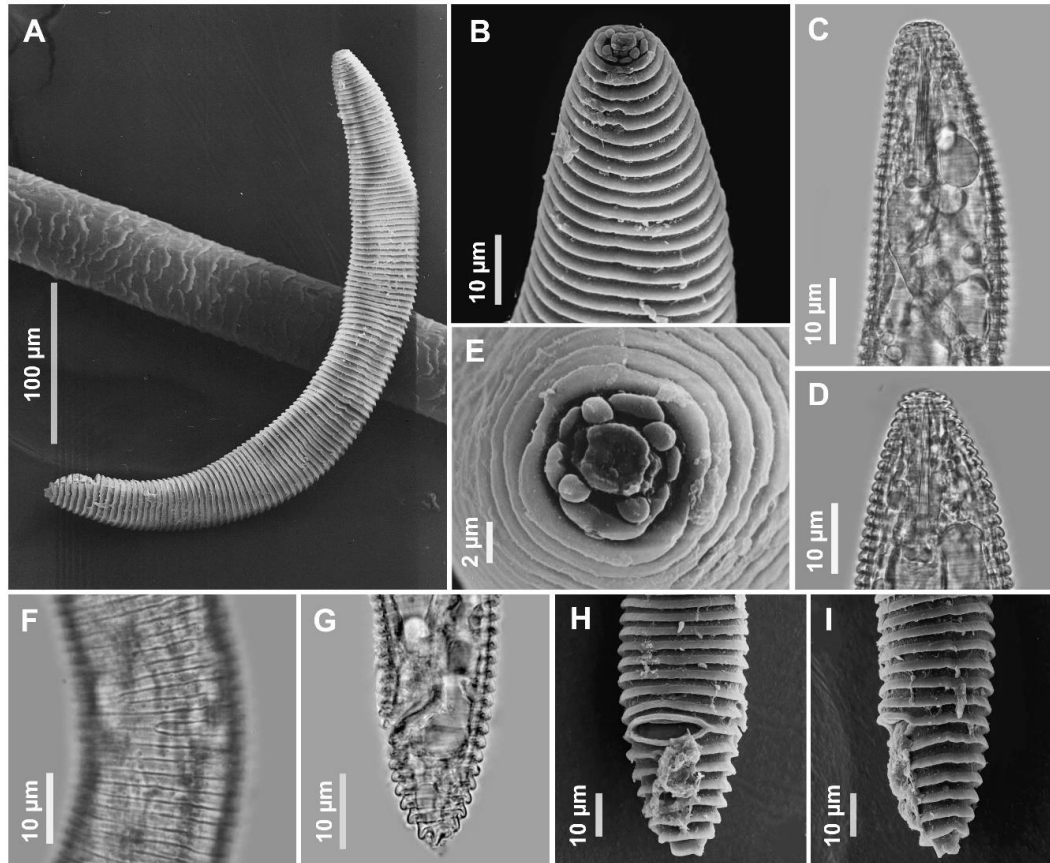


Figure 1. Light and scanning electron microscopy images of *Mesocriconema mamitensis* n. sp. females. A: Total body, B-D: Anterior regions showing lip region, stylet and knobs, E: *En face* view showing labial disc and four submedian lobes, F: Anastomoses of the cuticle, G-I: Posterior region showing open type vulva, conoid tail and bilobed terminus.

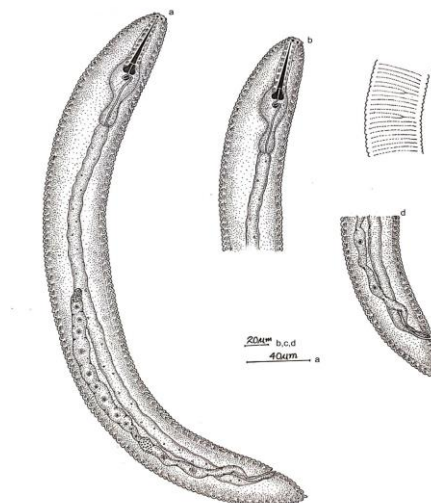


Figure 2. Line illustrations of *Mesocriconema mamitensis* n. sp. females. a: Total body, b: Anterior regions showing lip region, stylet, knobs and oesophagus, c: Anastomoses of the cuticle, d: Posterior region showing open type vulva, anus, conoid tail and bilobed terminus.

**Relationships:**

*Mesocriconema mamitensis* n. sp. has a close morphological resemblance with the following species: *M. curvatum*, *M. denoudenii*, *M. parareedi*, *M. paradenoudenii*, *M. ornicauda* and *M. theobromae*. They all have retrorse body annuli with smooth posterior margins, a labial plate, four distinctly separated submedian lobes, a robust stylet, open type vulva and conical shaped posterior region of the body. *Mesocriconema mamitensis* n. sp. is further similar to *M. curvatum* (Raski, 1952) Loof & De Grisse (1989) in having a lip region with two annuli, 1<sup>st</sup> annule smaller than 2<sup>nd</sup> annule and not set off, similar values of RV, Rvan, Ran, V and VL/VB. However, it differs from *M. curvatum* in having a labial plate uninterrupted laterally, trilobed on one side and bilobed on the other side vs trilobed on each side, rounded submedian lobes vs conical submedian lobes, shorter stylet (37-49 µm) vs longer stylet (47-68 µm), presence of anastomoses vs absence of anastomoses, higher (117-120) vs lower (74-106) number of body annuli, and smooth vulval lip vs anterior vulval lip with two rounded lobes.

**Table 1:** Morphometric data of females of *Mesocriconema mamitensis* n. sp. and *M. medani*. Measurements of lengths, heights, widths and diameters are in µm. All data except that for the holotype are presented as min-max (mean ± stdev.).

Character	Table 1: <i>Mesocriconema mamitensis</i> n. sp.		<i>Mesocriconema medani</i>
	Holotype female	Paratype female	Female
n	1	8	10
Body length (L)	416	340-456 (404 ± 48.1)	370-470 (417 ± 33.5)
a	9.6	7.6-9.6 (8.8 ± 0.8)	10.2-12.3 (11.3 ± 0.7)
b	4.7	4.5-4.9 (4.7 ± 0.1)	4.4-4.8 (4.6 ± 0.1)
c	15.9	13.2-17.5 (15.5 ± 1.7)	12.1-14.4 (13.3 ± 0.7)
c'	0.9	0.8-1.3 (1 ± 0.2)	-
v	92.9	91.6-93.2 (92.5 ± 0.6)	90-91 (91 ± 0.4)
R	117	117-120 (118 ± 1.11)	117-118 (118 ± 0.43)
Roes	28	24-32 (28.2 ± 2.8)	26-29 (27.7 ± 1.08)
Rst	17	15-17 (16 ± 0.7)	15-16 (15.2 ± 0.4)
Rv	9	7-9 (8.2 ± 0.82)	7-9 (8.2 ± 0.82)
Rvan	1	1-2 (0.5 ± 0.9)	1-2 (1.5 ± 0.9)
Ran	7	5-7 (6.2 ± 0.82)	5-7 (6.2 ± 0.8)
VL/VB	1	0.91-1.2 (1.0 ± 0.12)	1.2-1.4 (1.3 ± 0.05)
Stylet	45.3	37.6-49.6 (22.7 ± 1.04)	40.3-46.1 (42.6 ± 2.35)
Oesophagus	82.4	70.5-86.7 (42.9 ± 4.5)	77.5-88.1 (82.8 ± 3.7)
Stylet%L	10.8	10.7-11.0 (10.8 ± 0.1)	9.5-10.9 (10.2 ± 0.5)
Oesophagus%L	19.9	18.2-20.6 (19.5 ± 0.9)	18.7-20.9 (19.9 ± 0.7)
Lip width	9.7	8.7-9.8 (9.3 ± 0.3)	10.5-12.7 (11.3 ± 0.7)
Lip height	2	1.9-2.2 (1.9 ± 0.03)	1.7-2.1 (1.8 ± 0.1)
Knob width	8	7.4-8.5 (8.0 ± 0.4)	7.4-10.1 (8.7 ± 1.1)
Knob height	3.9	2.9-3.9 (3.7 ± 0.2)	3.7-4.9 (4.2 ± 0.4)
Anterior end to			
oesophagus length	86.7	70.5-91.7 (84.3 ± 7.1)	83.2-96.5 (89.7 ± 5.1)
Tail length	26	25.5-26.1 (25.8 ± 0.1)	30.0-32.6 (31.2 ± 0.74)
Max. body width	43.2	43.8-49.8 (44.4 ± 3.9)	-
Anal body diameter	26.1	19.3-29.4 (24.9 ± 4.2)	24.1-26.5 (25.4 ± 1.0)

*Mesocriconema mamitensis* n. sp. differs from *M. denoudenii* (De Grisse, 1967) Loof & De Grisse (1989) in having labial plate laterally uninterrupted with trilobed on one side and bilobed on another side vs one lobed on each side, presence of anastomoses vs anastomoses rare in occurrence, simple anterior vulval lip without lobes vs vulval lip with two projection or lobes, shorter stylet length (37-49  $\mu\text{m}$ ) vs longer stylet length (53-59  $\mu\text{m}$ ), and conoid and pointed tail with bilobed terminus vs conoid tail with gradually tapering to a fine terminus.

*Mesocriconema mamitensis* n. sp. differs from *M. parareedi* (Ebsary, 1981) Loof & De Grisse (1989) in having a labial plate laterally uninterrupted with trilobed on one side and bilobed on another side vs absent, but if present, it is small and one-lobed on each side; a slightly upward head with two annuli vs truncated head with three annuli, spindle shaped vs rectangular shaped oral disc, I-shaped vs X-shape oral aperture, large and distinct submedian lobes vs small and obscure submedian lobes from the lateral view, shorter stylet (37-49  $\mu\text{m}$ ) vs longer stylet (59-66  $\mu\text{m}$ ), presence vs absence of anastomoses, simple anterior vulval lip without lobe vs elongated and retrorse anterior vulval lip with two spines, 7-9 annuli vs 12-13 annuli from tail tip to the vulva, 5-7 annuli vs 3-5 annuli from tail terminus to anus, larger V value (91-93%) vs smaller V value (88-90%), smaller VL/VB value (0.9-1.2) vs greater VL/VB value (1.3-1.6), and a bilobed vs pointed tail terminus.

*Mesocriconema mamitensis* n. sp. is further similar to *M. paradenoudenii* (Rashid, Geraert, & Sharma 1987) Loof & De Grisse (1989) in body length, presence of few anastomoses, moderately robust stylet and anteriorly directed knobs, simple anterior vulval lip without any lobe or projection, and similar values of R, RV, Rvan, Ran, V and VL/VB. However, *M. mamitensis* n. sp. differs from *M. paradenoudenii* in having a labial plate uninterrupted laterally, trilobed on one side and bilobed on another side vs absence of lobes, shorter stylet (37-49  $\mu\text{m}$ ) vs slightly longer stylet (39-52  $\mu\text{m}$ ), conical vs truncated post vulval region, bilobed tail terminus vs complex tail terminus containing deeply cleft projection within the last annule.

*Mesocriconema mamitensis* n. sp. is also further similar to *M. ornicauda* Vovlas, Inserra & Esser (1991) in having few anastomoses, non-offset lip, I-shaped oral aperture, moderately robust stylet with anteriorly directed basal knobs, and similar values of RV, Ran, Rvan, V and VL/VB. However, it differs from *M. ornicauda* in having a laterally uninterrupted labial plate, trilobed on one side and bilobed on the other side vs trilobed on one side and unilobed on the other side, a greater number of body annuli (117-120) vs lesser number of body annuli (91-111), vulva without any lobes or projection from anterior vulval lips vs anterior vulval lip with two small rounded or sometimes pointed lobes, conical tail with bilobed terminus vs conical tail with irregular cuticle on 3<sup>rd</sup> & 4<sup>th</sup> annulus from the tail terminus, and the absence vs presence of a deep notch at the site of the anus.

Lastly, *M. mamitensis* n. sp. shares more similarities with *M. theobromae* Crozzoli & Lamberti (2001) such as the presence of anastomoses, the head region with two annuli, 1<sup>st</sup> annule smaller than 2<sup>nd</sup> annule, and similar values of Rv, Ran and V. However, it differs from *M. theobromae* in having a smooth posterior margin vs irregular margin of body annuli, labial plate laterally uninterrupted trilobed on one side and bilobed on the other side vs trilobed on both sides, simple and open type vulva without projection vs lobulated anterior vulval lip, bilobed tail terminus vs presence a single projection of tail terminus, greater body length (0.34-0.45 mm) vs shorter body length (0.27-0.32 mm), shorter stylet length (37-49  $\mu\text{m}$ ) vs longer stylet length (47-51  $\mu\text{m}$ ), and more body annuli (117 - 120) vs lesser body annuli (73-74).

Table 2: Comparative account of morphometric data of *Mesocriconema mamitensis* n. sp. with closely related species. All the measurements are in  $\mu\text{m}$  except for the body length which is in mm. The data is presented as min-max values.

Characters	<i>Mesocriconema mamitensis</i> n. sp.	<i>M. curvatum</i> (Raski, 1952) Loof & De grisse, 1967	<i>M. denoudenii</i> (de Grisse, 1967) Loof & de Grisse, 1989	<i>M. parareedi</i> (Ebsary, 1981) Loof & De grisse, 1967	<i>M. paradenoudenii</i> (Rashid, Geraert, & Sharma, 1987) Loof & De Grisse, 1989	<i>M. ornicauda</i> (Vovlas, Inserra & Esser, 1991)	<i>M. theobromae</i> (Crozzoli & Lamberti, 2001)
Body length	0.34-0.45	0.29-0.56	0.39-0.57	0.38-0.48	0.32-0.47	0.37-0.46	0.27-0.32
Stylet length	37-49	47-68	53-59	59-66	39-52	43-50	47-51
Knob width	7.4-8.5	-	-	-	6-7.5	7.5-10.5	7.5-8
R	117-120	74-106	102-127	111-121	102-130	92-111	73-74
Rex	-	20-29	32-37	31-34	28-35	24-33	-
RV	7-9	5-10	8-11	12-13	8-10	9-11	7
Rvan	1-2	0-3	0-2	-	2-4	1-2	1
Ran	5-7	2-6	6-9	3-5	4-7	6-9	5
V	91-93%	92-96%	90-94%	88-90%	90-94%	88-93%	93-94%
VL/VB	0.9-1.2	0.7-1.2	1.0-1.3	1.3-1.6	0.8-1.4	1.1-1.6	-

(Figure 3; Table 1)

**Description:**

Female: Body slightly arcuate upon fixation, both ends taper, posterior end somewhat more tapering than the anterior end. Body annule retrorse except for the first annule, anastomoses present, but few in number. Head conical, not set off, not retrorse; four distinctly separated submedian lobes present in the equally spaced area around the labial region. Lip height and width are 1.7-2.1  $\mu\text{m}$  and 10.5-12.7  $\mu\text{m}$ , respectively. The stylet is robust with moderate length and basal knobs anteriorly directed, 3.7-4.9  $\mu\text{m}$  in height and 7.4-10.1  $\mu\text{m}$  in width. Oesophagus criconematid type and 77.5-88.1  $\mu\text{m}$  long. 26-29 annuli from the anterior end to the base of the oesophagus. The excretory pore is not clear. Reproductive system mono-pro delphic, ovary outstretched, spermatheca rounded. Vulva open type with a simple anterior vulval lip which is forwardly inclined. Tail short and conoid with 1-2 platelets. 5-7 annuli between the anal opening and posterior end of the body and 1-2 annuli between the vulva and anus.

Male: Not found

**Host and Habitat:** *Ananas* sp. (common name: pineapple and local name: La-khuih-thei) from Ailawng, Mamit district, Mizoram with longitude and latitude of 23'693621" E & 92'628979" N, respectively.

**Specimen:** Specimens on the slide MU/MM1 *M. medani* have been deposited at the nematode collection center of the Parasitology Section, Department of Zoology, Manipur University, Canchipur 795003, Manipur, India.

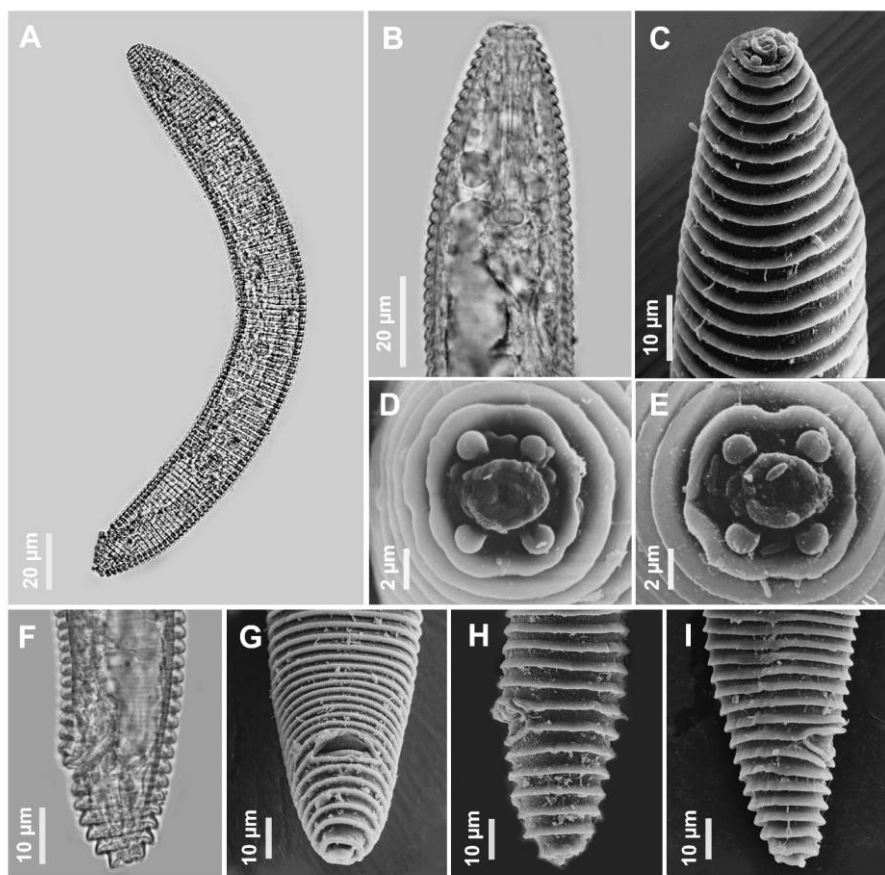




Figure 3. Light and scanning electron microscopy images of *Mesocriconema medani* females. A: Total body, B, C: Anterior regions showing lip region, stylet and knobs, D, E: *En face* view showing labial disc and four submedian lobes, F-I: Posterior region showing open type vulva and conoid tail.

### 3. Results and Discussion:

The diversity of *Mesocriconema* appears to be rich in India as evidenced by a total number of 27 *Mesocriconema* species described from India Geraert (2010). Eighteen *Mesocriconema* species have been originally described from mainland India – i.e., from the states of Uttar Pradesh, Tamil Nadu, Kerala, Himachal Pradesh, Maharashtra, West Bengal and Mysore, and nine species were described from the northeastern part of India. Among the northeastern states, six species were described from the state of Manipur – i.e. *M. lobellum* Pramodini, Mohilal & Gambhir (2007), *M. oblongatum* (Renubala, Dhanachand & Gambhir, 1991) Brzeski, Loof & Choi, (2002), *M. ovospermatum* (Mohilal & Dhanachand, 1998) Brzeski, Loof & Choi, (2002), *M. paraxeste* (Dhanachand & Renubala, 1991) Brzeski, Loof & Choi, (2002), *M. serratum* (Renubala, Dhanachand & Gambhir, 1991) Brzeski, Loof & Choi, (2002) and *M. waitha* Pramodini, Mohilal and Dhanachand (2006) and three species from the state of Assam – i.e. *M. onostre* (Phukan & Sanwal, 1981) Loof & De Grisse (1989), *M. orientale* Rahaman & Ahmed (1996) and *M. medani* (Phukan & Sanwal, 1981) Loof & De Grisse, (1989).

Although the pathogenicity of most of the *Mesocriconema* spp. is unknown, a few studies have reported that some *Mesocriconema* species can cause substantial damage to crops including legumes and a variety of fruit trees. Even though they are weak pathogenic nematodes, there is a report from the USA and a few countries from Africa that the occurrence of *Mesocriconema ornatum* in large percentages or densities in peanut farms caused great damage to peanut Dickson & De Waele (2005). For instance, an unknown *Mesocriconema* sp. caused damage to guava, pomegranate, papaya and passion fruits resulting in the economic loss Castellano *et al.* (2012); *Mesocriconema* spp. have been found to feed on vine roots, thereby reducing water and nutrient uptake and increasing vine stress to eventually cause yield reduction Mckenry & Bettiga (2013). They have also been reported to decrease the number and volume of feeder roots in peach, plum and prune trees thus decreasing the fresh and dry weight of roots and also destroying cortical root tissue causing stunted growth of trees Braun *et.al.* (1975), English *et al.* (1982), Lownsbery *et al.* (1977), Mojtahedi & Lownsbery (1975), Mojtahedi *et.al.* (1975).

### 4. Conclusion

The present new species was identified in this study are of a small population, consisting of predominantly only females in the rhizosphere of lemongrass. This is an economically important plant used in the preparation of tea, in cooking, and other local medicinal practices in this part of India. In the vicinity of the type host, other small plants (*Ageratum* sp. and unknown species of small herbs) were also found growing. However, the real pathogenicity of the newly found species to the host remains un-investigated. On the other hand, *M. medani* was found associated with the rhizosphere of pineapples cultivated by jhuming practice by the locals for commercial purposes. Our morphological data fits very well with the original description of *M. medani*. This species was also found as a small population consisting of only females. This is the first report of *M. medani* from the state of Mizoram, which is a neighbouring state of Assam where the type locality is present.

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### Conflict of interest:

The authors declare no conflict of interest.

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