TAXONOMY AND RELATIONSHIPS OF A NEW AND THE FIRST CONTINENTAL SPECIES OF *TRISSONCHULUS* COBB, 1920 ALONG WITH TWO SPECIES OF *IRONUS* (NEMATODA: IRONIDAE) COLLECTED FROM COAL MINES

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Summary. The morphological and taxonomic account of a new and the first terrestrial species of the largely marine genus *Trissonchulus* Cobb, 1920 is given, along with descriptions of two known species of the sister taxon *Ironus* Bastian, 1865, reported for the first time from India. *Trissonchulus baldwini* sp. n. is characterised by the lip region markedly offset from adjoining body by a deep groove; lips strongly amalgamated forming a band or collarette; inner margins of lips serrated; small, setose and backwardly directed cephalic sensilla; narrow funnel-shaped amphids; inconspicuous excretory pore; larger stoma with two dorsal and two ventral outwardly curved, reversible teeth at anterior margins; expanded part 65-70% of the corresponding pharyngeal length; anterior vulva (38-39%); reduced anterior genital branch; long filiform tail and absence of males. The species *Trissonchulus lichenii* Nasira *et* Turpeenniemi, 2002 has been placed under genus *Syringolaimus* de Man, 1888 as *S. lichenii* on the basis of affinities with the latter. The population of *Ironus dentifurcatus* Argo *et* Heyns, 1972 shows slight variations from the original population in having narrower amphids, fang-like projections of the dorsal tooth, conspicuous crystalloids and the presence of caudal pores, while the population of *I. terranovus* Ebsary, 1985 shows variations from the original specimens in having a smaller body, dome-shaped head and a pair of caudal pores. On morphological grounds, among the congeners *T. baldwini* sp. n. has been found to show a close relationship with *T. acutus* and *T. benepapillosus* while showing many affinities to *I. terranovus* and *I. dentifurcatus* on account of morphometric characteristics.

Keywords: *Ironus dentifurcatus*, *I. terranovus*, morphology, morphometrics, new record, taxonomy, *Trissonchulus baldwini*.

The family Ironidae de Man, 1876 is a predominantly aquatic group of nematodes, with species found in sea, continental waters and occasionally on land. The group is comprised of large-sized enoplid predators that are *k*-strategists in the environment. The representative species tend to have long life cycles (Yeates, 1967) and life spans and are very sensitive to slight changes in the environment (Bongers, 1990). de Man (1876) placed *Ironus* Bastian, 1865 under Ironidae as its type genus. In view of their similarities, including the position of replacement teeth in juveniles, Filipjev (1918) placed *Ironus* along with *Thalassironus* de Man, 1889, *Dolicholaimus* de Man, 1888, *Aulolaimus* de Man, 1880, *Cephalonema* Cobb, 1893, *Nanonema* Cobb, 1905 and *Syringolaimus* de Man, 1888 in the subfamily Dorylaiminae de Man, 1876. Later, Filipjev (1934) shifted *Ironus*, *Ironella* Cobb, 1920, *Thalassironus*, *Dolicholaimus*, *Trissonchulus* Cobb, 1920 and *Syringolaimus* to subfamily Ironinae de Man, 1976. Thorne (1939) considered Ironidae to be only distantly related to others of the group and recommended its exclusion from Dorylaimoidea Thorne, 1934. This view was further supported by Coomans and Van der Heiden (1978), who considered the synapomorphies of Ironids and Dorylaimids to be due to parallel evolution. Chitwood and Chitwood (1937) placed Ironidae under Enoploidea Dujardin, 1845 but later (1950) considered it under Tripyloidea de Man, 1876. Chitwood (1951) shifted *Syringolaimus* from Ironidae to Leptolaimidae Örley, 1880, considering its bulbar pharyngeal base and cylindroid stoma similar to that found in Rhabdolaiminae Chitwood, 1951. Riemann (1970), Gerlach and Riemann (1973), and Andrássy (1976) approved the placement but several workers, including Schuurmans Stekhoven (1935), Schneider (1939), Goodey (1951), Wieser (1953), Platonova and Mokievsky (1994) and Lorenzen (1994), considered *Syringolaimus* best placed in Ironidae. Andrássy (1976) considered Ironoidea de Man, 1876 to come under suborder Tripylina Andrássy, 1974 and included the two families Cryptonchidae Chitwood, 1937 and Ironidae under it. He included the subfamily Ironinae under Ironidae, with genera *Ironus*, *Dolicholaimus* and *Pheronous* Inglis, 1966 placed in it. The other subfamily, Thalassironinae Andrássy, 1976, included the genera *Conilia* Gerlach, 1956, *Ironella*, *Parironus* Micoletzky, 1930 and *Thalassironus*. Lorenzen (1994) considered the superfamily Ironoidea de Man, 1876 under Enoplina Chitwood et Chitwood, 1937 while approving the same two subfamilies under Ironidae. However, he placed only *Ironus* in Ironinae, considering the genera *Conilia*, *Dolicholaimus*, *Ironella*, *Parironus*, *Pheronous*, *Syringolaimus*, *Thalassironus* and *Trissonchulus* to be under Thalassironinae. There have been several revisions of the group from time to time by Andrássy (1968), Ebsary (1985), Tsalolikhin (1987), Gagarin (1993), Lorenzen (1994) and Platonova and Mokievsky (1994). Smol and

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[*] The species is named in honour of Prof. James G. Baldwin a renowned Nematologist.

Coomans (2006) considered Ironina Siddiqi, 1983 as a sub-order of Enoplida and, following the scheme of Lorenzen (1994), regarded *Ironus* as the only representative of Ironinae and placed the remaining genera under Thalassironinae. It is, therefore, a fact that the taxonomy of the family has been subject to alterations due to insufficient descriptions and inadequate weighting and comparison of morphological characters (Chitwood, 1960; Van der Heiden, 1974). The members are unique in possessing three claw-like teeth in the anterior part of the stoma. The morphology of stomal teeth is one of the important taxonomic features to distinguish between the genera and species. Van der Heiden (1974) studied in detail the anterior feeding apparatus in selected members of Ironidae. *Ironus* is a fairly cosmopolitan genus, although most species have been reported from the African continent. Though there are morphological similarities between *Ironus* and the genus *Trissonchulus*, their distribution patterns show marked differences. *Ironus* is largely a continental genus with species mostly found in terrestrial habitats and in inland waters, whereas *Trissonchulus* is mostly represented by marine species.

During a survey of nematodes from the Sonbhadra coal mining area we discovered *Trissonchulus baldwini* sp. n., the first terrestrial representative of the genus. Here we describe this new species along with two known species of *Ironus* reported for the first time from India. The relationships of the species have also been worked out.

MATERIALS AND METHODS

Soil samples were collected from the Sonbhadra coal mining area and nematodes were extracted by Cobb's (1918) sieving and decanting and modified Baermann's funnel techniques. For light microscopy, nematodes were fixed in 4% formaldehyde, dehydrated (Seinhorst, 1959) and later mounted on slides using the wax ring technique. The nematodes were measured with an ocular micrometer and drawn using a drawing tube. LM photographs were taken by an Olympus DP-11 digital camera mounted on an Olympus BX-51 DIC Microscope.

Cluster analysis. Sixteen morphological characters were taken to compare *T. baldwini* sp. n. with other species of *Trissonchulus*, while fourteen morphological characters were selected for the subsequent analyses using *T. benepapillosus* Schulz, 1935 (a good representative of *Trissonchulus*) as an outgroup to appraise the status of *T. baldwini* sp. n. with respect to *Ironus* and *Trissonchulus*. All the characters were important with good taxonomic value. The characters were ranked on the basis of commonality principle. Character state '0" represented the most commonly occurring trait whilst a gradual increase in value represented increasing deviation. Data matrices (Appendix 1-4) were prepared and cluster analysis was made. The data matrices were analyzed using Statistica-99 software. The relationships were interpreted from the dendrograms obtained.

DESCRIPTION

*TRISSONCHULUS BALDWINI** sp. n.
(Figs 1 and 2; Table I)

Measurements. See Table I.

Female. Body large-sized, strongly arcuate upon fixation, tapering acutely at posterior extremity. Cuticle smooth without transverse or longitudinal striations. Body pores usually obscure. Crystalloids of variable shapes and sizes ranging from rectangular to spheroid types. Lip region markedly offset by a distinct groove, with three labial flaps. Lips with inner margins serrated, strongly amalgamated to form a band or collarette. Lip sensilla in two circlets of 6 + (6 + 4). Inner labials papilliform, faintly visible; outer labials, papilliform; cephalic sensilla, at level of outer labials, 3-4 µm long, unjointed and backwardly directed. Amphids pocket-like, located at lip-body junction, about $1/4^{th}$ - $1/5^{th}$ corresponding body diameter. Oral aperture surrounded by three liplets with serrated margins. Excretory pore faintly visible, located in lip region, 5-8 µm anterior to lip-body junction. Renette cell obscure. Stoma divided into small, anterior cheilostom and long oesophastom surrounded by pharyngeal tissue or a vestibule and a cylindroid part. Three stomal walls conspicuously sclerotized with outwardly curved, reversible teeth at anterior margins. Stoma widened anteriorly at level of teeth, posterior part cylindroid with subventral walls thicker and striated. Dorsal stomal wall bearing two narrower teeth; subventral tooth on each subventral wall relatively stouter but equal in length to dorsal teeth. Pharynx cylindroid, widening at *ca* 30-35% of its length. Nerve ring prominent, at *ca* 50-65% of pharyngeal length. Body at pharyngeal end *ca* 2-2.5 times labial diameter. Cardia hemispheroid, about one-third of corresponding body diameter. Intestine isocytous with polygonal cells. Pre-rectum indistinct. Reproductive system having anterior genital branch longer and weaker with reduced/rudimentary ovary. Both ovaries located on right side of intestine. Oviduct narrow, spermatheca not differentiated. Uterus long, muscular, without any sperms. Vagina well developed, *ca* 0.6-0.7 times corresponding body diameter with conspicuous muscles. Vulva a transverse slit. Tail long, filiform with an anterior conoid part that gradually tapers to a whip-like terminus. Two pairs of ventral body pores present in conoid part of tail, located latero-dorsally at 18-22 µm and 22-28 µm from anal level or 5-8 µm apart. Caudal glands rudimentary, spinneret absent.

Male. Not found

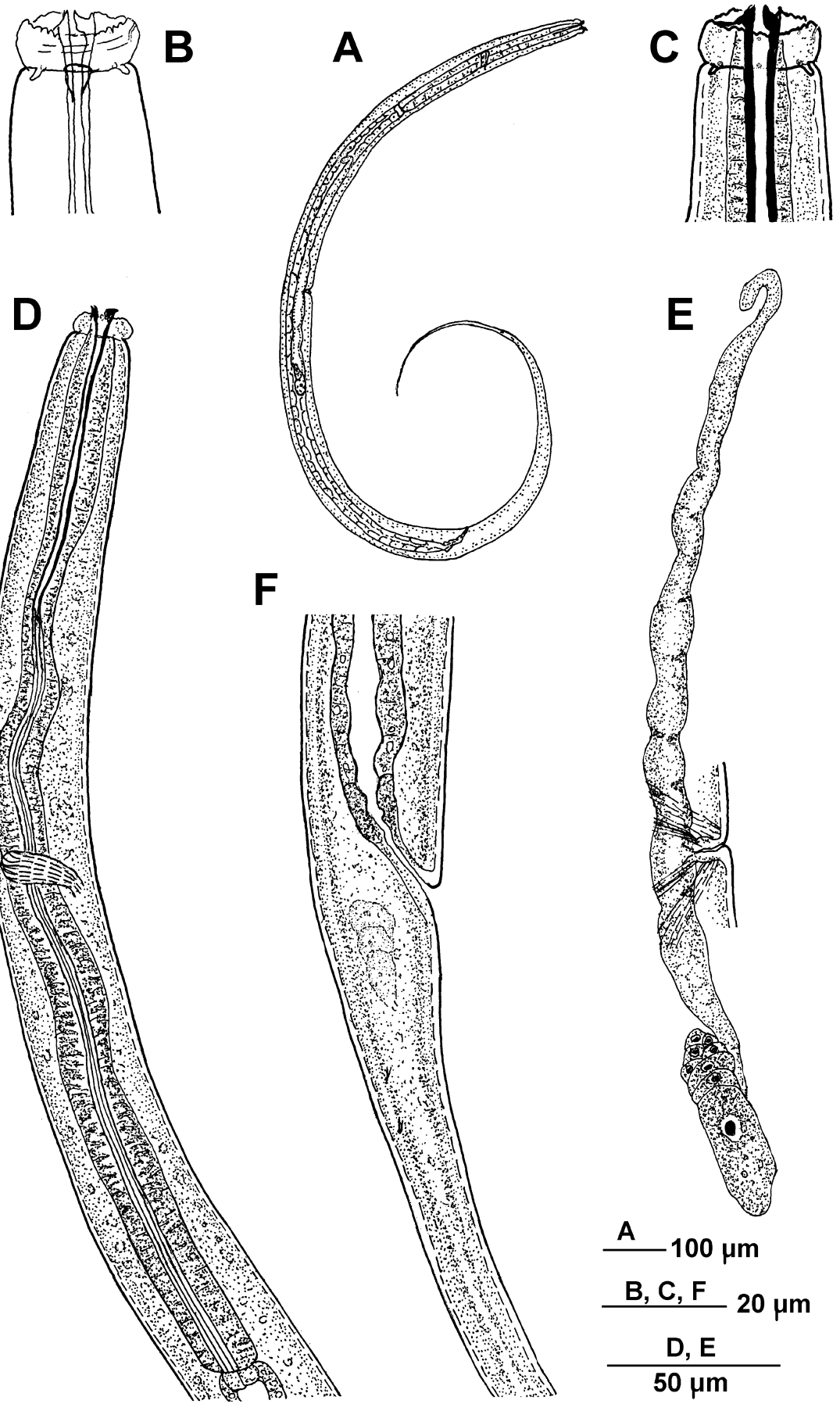

Fig. 1. *Trissonchulus baldwini* sp. n. A: Entire female; B, C: Female anterior end; D: Pharyngeal region; E: Female reproductive system; F: Female caudal region.

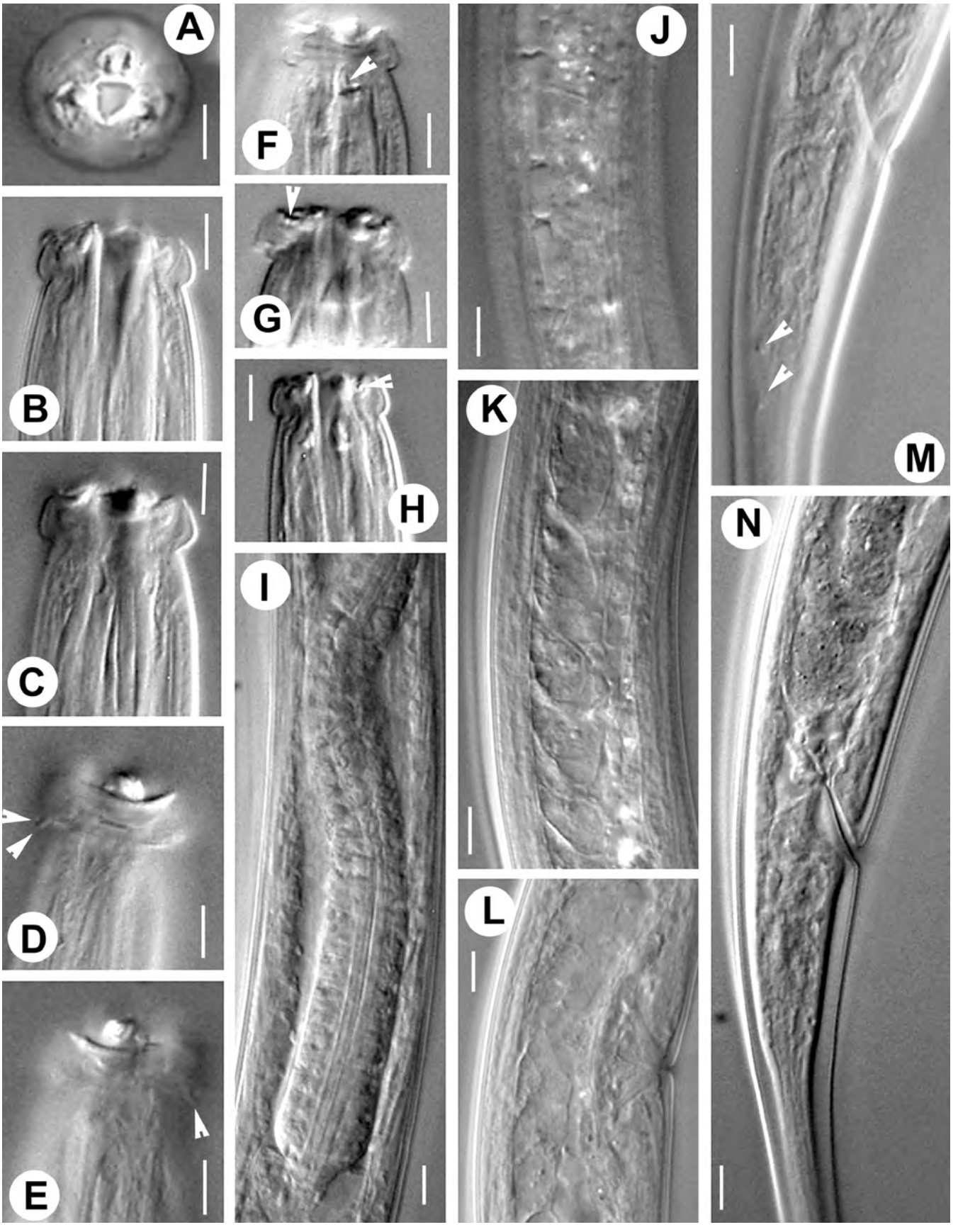

Fig. 2. *Trissonchulus baldwini* sp. n. A: *En face* view; B-E: Female anterior end (arrows indicate D, E- lip sensilla); F-H: Anterior end of juvenile (arrows indicate F- replacement tooth, G- serrated inner margin of lips, H- inner labial sensillum); I: Female posterior pharyngeal end; J: Mid-body showing crystalloids; K: Intestinal cells in mid body region; L: Vulval region; M: Anal region (arrows indicate caudal pores); N: Female caudal region. (Scale bars = 5 μm)

Table I. Morphometric data of *Trissonchulus baldwini* sp. n. Measurements are in µm and in the form: mean ± standard deviation (range).

Character	Female	
	Holotype	Paratypes
No.		5
L	1045	1100 ± 43.6 (1045-1575)
a	33.7	34.1± 0.5 (33.7-34.6)
b	4	4.0 ± 0.1 (4.0-4.2)
c	1.9	2.0 ± 0.1 (1.9-3.2)
c'	31.8	29.2 ± 2.6 (26.8-32.6)
V	38	38.8 ± 0.3 (38-39)
G_1	12.8	13.8 ± 1.4 (12.4-15.1)
G_2	12.2	12.5 ± 0.4 (11.2-13.3)
Body diam.	31	32 ± 4.0 (31-38)
Lip diam.	17	17± 0.1 (17-18)
Lip height	3	2.5 ± 0.6 (2-4)
Stoma length	65	65 ± 0 (65-65)
Pharynx length	260	262 ± 4.8 (260-270)
Nerve ring position	120	125 ± 4.9 (120-130)
Vulva-anus distance	445	450 ± 5.1 (445-458)
Rectum length	23	20.5 ± 2.4 (18-24)
Anal body diam.	17.5	18 ± 0.63 (17-19)
Tail length	540	510 ± 30 (480-540)

Fourth stage female juvenile. Similar to female in morphology. Replacement teeth (smaller dorsals and larger subventrals) present in the corresponding pharyngeal walls.

Type habitat and locality. Soil (sandy clay) samples collected from coal mining area of Sonbhadra, Uttar Pradesh, India at coordinates 24.783°N 82.833°E.

Type material. Holotype female, five paratype females and two fourth stage juveniles on slide *Trissonchulus baldwini* sp. n. no ST/1-4 deposited in the Nematode Collection of the Department of Zoology, Aligarh Muslim University, Aligarh, India. One paratype female slide no. ST/5 deposited at the Laboratory of Nematology, Wageningen University and Research Centre (WUR), 6700 ES Wageningen, The Netherlands.

Diagnosis and relationships. Trissonchulus baldwini sp. n. is characterised by markedly offset lip region demarcated from adjoining body by a deep groove; lips strongly amalgamated and forming a band or collarette with inner margins serrated; papilloid inner labial and outer labial sensilla; setose and backwardly directed cephalic sensilla at the level of outer labials; narrow amphids; anteriorly placed excretory pore; larger stoma with two dorsal and two sub-ventral outwardly curved, reversible teeth at anterior margins, expanded part 65-70% of the corresponding pharyngeal length; anterior vulva (38-39%); reduced anterior genital branch; long filiform tail and apparent absence of males.

The new species most closely resembles *T. benepapillosus* Schulz, 1935 but differs in having smaller 'a' value (34.2 *vs* 46.7), smaller 'b' value (4.0 *vs* 9.4), reduced (*vs* well developed) anterior gonad, anteriorly located vulva (38.8% *vs* 52-54%), longer (510 µm *vs* 75 µm) and narrower (*vs* broader tail in *T. benepapillosus* Schulz, 1935).

The new species differs from *T. dubius* Orselli *et* Vinciguerra, 1997 in having smaller body (1 mm *vs* 2 mm), smaller 'a' value (34.2 *vs* 44), smaller 'b' value (4.0 *vs* 7.2), reduced (*vs* well developed) anterior gonad, anteriorly located vulva (38.8% *vs* 57.4%), longer (510 µm *vs* 57.5-65.0 µm) and filiform tail (*vs* bluntly conoid in *T. dubius* Orselli *et* Vinciguerra, 1997). However, the illustration Fig. 2F *apud* Orselli *et* Vinciguerra, 1997 does not correspond with the range of values given in the text.

Trissonchulus baldwini sp. n. further differs from *T. provulvatus* Orselli *et* Vinciguerra, 1997 in having smaller 'a' value (34.2 *vs* 58), smaller 'b' value (4.0 *vs* 8.5), larger stoma (65.0 µm *vs* 50.6 µm), long reduced (*vs* well developed) anterior gonad, longer (510 µm *vs* 57.5 µm) and narrower tail (*vs* broader tail in *T. provulvatus* Orselli *et* Vinciguerra, 1997).

Remarks. The genus *Trissonchulus* is, hereby, reported for the first time from India. The new species *T. baldwini* is unique in having a terrestrial origin in contrast to other species of the genus. The long whip-like tail, the amalgamated lips with serrated margins forming a band or collarette and demarcated from the adjoining body by a deep groove or suture, a pre-equatorial vulva and reduced anterior genital branch are other distinct features. There is a great possibility of an amictic mode

of reproduction as sperms could not be found in the female genital tracts and males were absent from the population.

IRONUS TERRANOVUS **Ebsary, 1985**
(Figs 3 and 4; Table II)

Measurements. See Table II.

Female. Body large-sized, strongly curved ventrally when fixed, more tapering at posterior extremity. Cuticle smooth without transverse or longitudinal striations. Hypodermal glands absent, body pores confined to tail region. Lip region slightly flattened to dome-shaped, offset; lips rounded. Amphids narrow, located 6-8 µm from the anterior end with sensillar pouches barely visible. Amphidial aperture about one-fourth of corresponding labial diameter. Lip sensilla in two circlets of 6 + (6 + 4). Inner labial sensilla papilliform; outer labials slightly raised, hardly 2 µm long. Cephalic sensilla setose about 3-4 µm long or one-sixth of labial diameter, located 5-6 µm from anterior end. Crystalloids thin, rod-like or spherical, sparse, variable in shape and size, scattered. Stoma comprised of triangular, serrated, heavily sclerotized anterior vestibule partly surrounded by pharyngeal tissue and posterior cylindroid part wholly surrounded by pharyngeal tissue. An obtuse, bifurcate dorsal tooth and two stouter sub-ventral teeth attached to sclerotized stomal walls narrowing at basal end of vestibule. Dorsal tooth bifurcated along its length with each half appearing as a separate tooth. Sub-ventral stomal walls conspicuous and twice as thick as dorsal wall. A small denticle present at the base of each sub-ventral tooth. Pharynx cylindroid with narrow anterior and relatively wider posterior regions. Nerve ring prominent at *ca* 50-75% of pharyngeal length. Body at pharyngeal end *ca* 2-2.5 times labial diameter. Excretory pore located in lip region leading to a faint excretory duct. Renette cell not visible. Cardia short, hemispheroid, *ca* one-fourth of corresponding body diameter. A small pre-rectum observed in few specimens. Rectum *ca* 0.8-1.0 anal body diameters long. Reproductive system didelphic, amphidelphic. Ovaries reflexed, relatively slender, on right side of intestine; germinal zone markedly narrower than growth zone with oocytes arranged in one or two rows. Oviduct narrow, uterus long with thick, muscular walls. Vulva a transverse slit, vagina *ca* 10-11 µm long, almost at right angles to longitudinal body axis, provided with strong and conspicuous muscle bands. Tail divided into short conoid anterior part and a long, filiform posterior part. Body pores present in tail region as dorso-lateral (anterior) and ventro-lateral (posterior) pairs.

Males. Not found.

Habitat and locality. Soil (sandy loam) samples having *I. terranovus* population collected from Naqvi Park, Uttar Pradesh, India at coordinates 27.544°N, 78.427°E.

Table II. Morphometric data of *Ironus terranovus* Ebsary, 1985 and *Ironus dentifurcatus* Argo *et* Heyns, 1972. Measurements are in µm and in the form: mean ± standard deviation (range).

Character	*I. terranovus* Ebsary, 1985	*I. dentifurcatus* Argo *et* Heyns, 1972
	Female	Female
No.	19	7
L	1578.9 ± 110.8 (1387-1816)	1440.8 ± 104.9 (1268-1568)
a	50.7 ± 2.9 (44.3-58.4)	29.3 ± 0.5 (29-30)
b	5.9 ± 0.4 (5.7-8.9)	5.9 ± 0.4 (5.0-6.2)
c	3.9 ± 0.4 (3.2-4.9)	3.8 ± 0.9 (3.1-5.6)
c'	21.6 ± 3.4 (16.3-28.1)	25.8 ± 6.9 (14.0-36.4)
V	41.3 ± 2.2 (37.2-44.8)	45.3 ± 6.1 (38.9-46.4)
G_1	6.8 ±0.9 (6.3-7.8)	9.6 ± 0.9 (8.2-11.1)
G_2	8.5 ± 0.6 (7.3-9.1)	10.8 ± 0.8 (9-12)
Body diam.	31.2 ± 2.2 (28-37)	29.3 ± 0.5 (29-30)
Lip diam.	13.8 ± 0.8 (13-16)	14.3 ± 0.8 (13-15)
Lip height	6.7 ± 1.2 (5-10)	7 ± 0.6 (6-8)
Stoma length	91.2 ± 4.5 (77-100)	91.5 ± 4.9 (85-100)
Pharynx length	255.2 ± 21.7 (220-296)	244 ± 12.5 (218-253)
Nerve ring position	123.4± 5.9 (110-130)	118.3 ± 3.7 (110-120)
Vulva-anus distance	521.8 ± 54.4 (452-640)	443.3 ± 13.8 (424-463)
Rectum length	21 ± 1.2 (20-23)	18.6 ± 3.7 (15-24)
Anal body diam.	18.8 ± 0.9 (18-21)	14 ± 1.16 (13-16)
Tail length	404.7 ± 60.9 (310-532)	403.6 ± 91.8 (225-430)

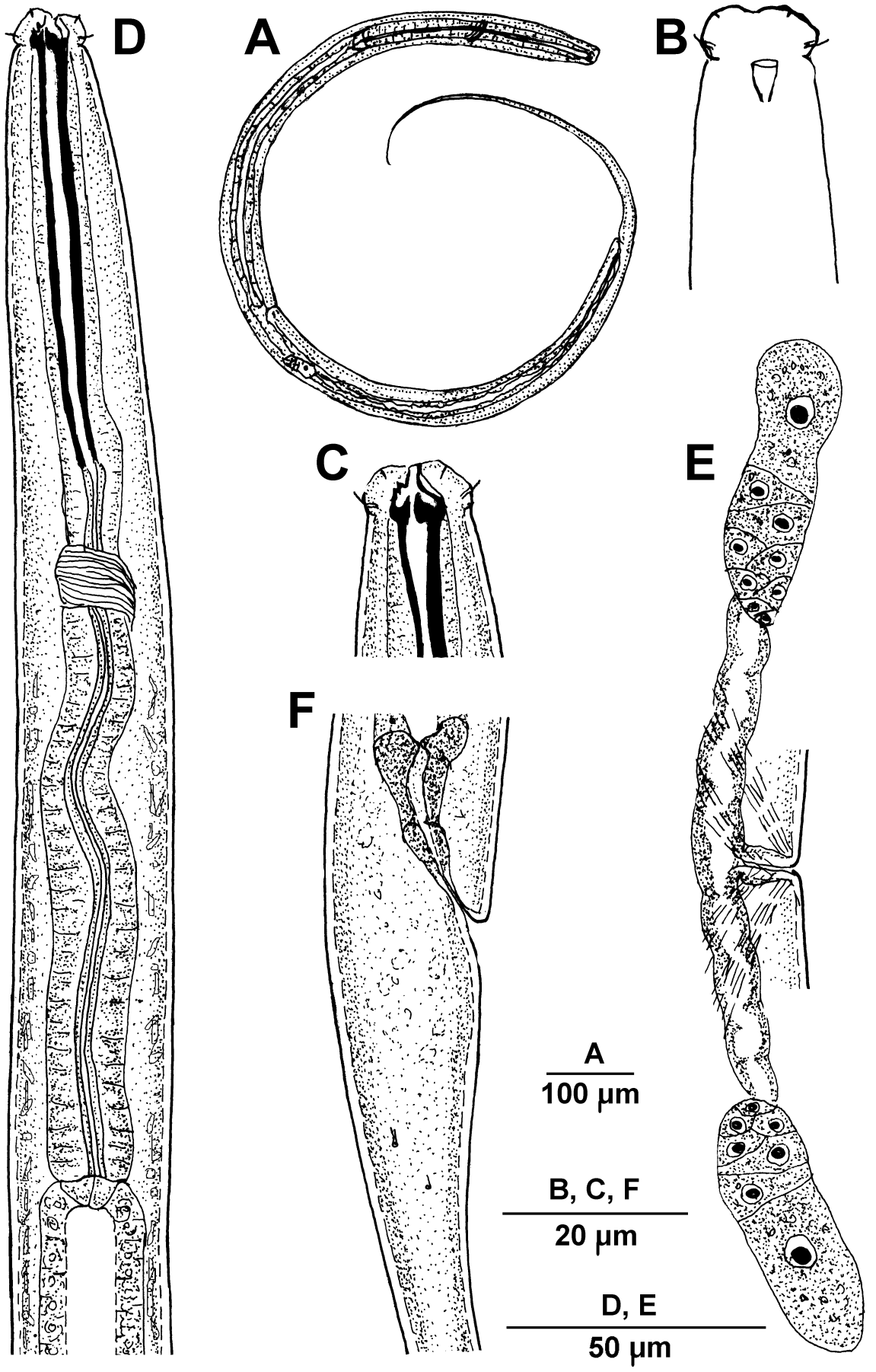

Fig. 3. *Ironus terranovus* Ebsary, 1985. A: Entire female; B, C: Female anterior end; D: Pharyngeal region; E: Female reproductive system; F: Female caudal region.

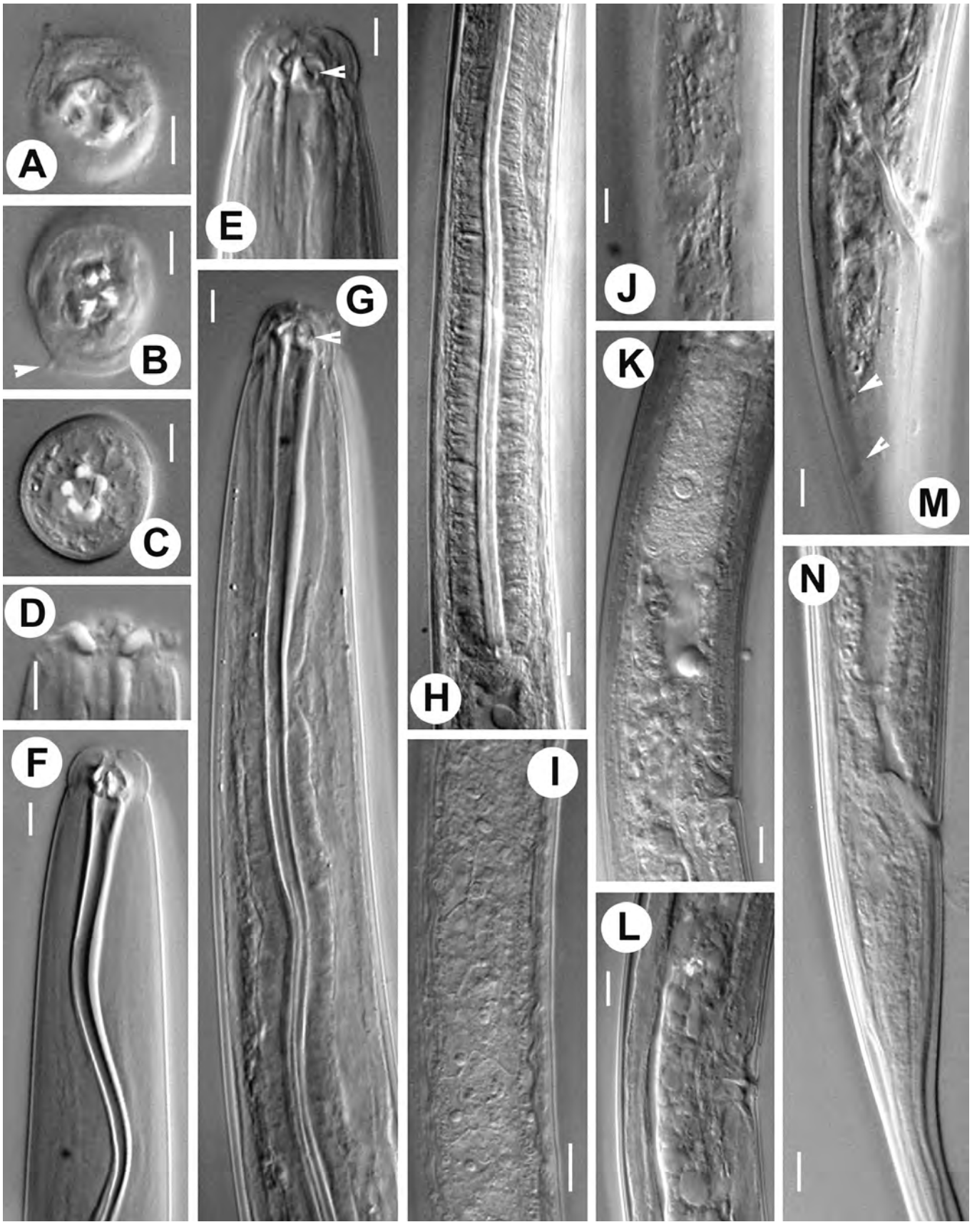

Fig. 4. *Ironus terranovus* Ebsary, 1985. A-C: *En face* view at different levels (arrow indicates cephalic and outer labial sensilla); D: Lip region; E: Female anterior end (arrow indicates denticle at base of subventral tooth); F, G: Anterior region of pharynx (G- arrow indicates denticle at base of sub-ventral tooth); H: Posterior region of pharynx; I: Intestinal cells; J: Mid-body showing crystalloids; K: Part of female genital tract (anterior); L: Vulval region; M: Anal region (arrows indicate caudal pores); N: Female caudal region. (Scale bars = 5 µm).

Voucher material. Nineteen females and two fourth stage juveniles on slide *Ironus terranovus* slide no. NOA/1-6 deposited in the Nematode Collection of the Department of Zoology, Aligarh Muslim University, Aligarh, India. Two females NOA/7 deposited at the Laboratory of Nematology, Wageningen University and Research Centre (WUR), 6700 ES Wageningen, The Netherlands.

Remarks. The present specimens form the basis of the first report of *I. terranovus* Ebsary, 1985 from India. The specimens agree well in most morphometric and morphological details with *I. terranovus* Ebsary, 1985 but differ in having smaller body, dome-shaped head and a pair of caudal pores. In view of poor descriptions and illustrations of the three Indian species of this genus viz., *I. indicus* Sharma *et* Saxena, 1980, *I. iarius* Saha, Lal *et* Singh, 2004 and *I. kaneri* Rathour, Sharma *et* Ganguly, 2004, and the unavailability of their type specimens, adequate comparisons could not be made with the present specimens. Hence, the above species are considered as *species inquirendae*.

IRONUS DENTIFURCATUS **Argo *et* Heyns, 1972**
(Figs 5 and 6; Table II)

Measurements. See Table II.

Female. Body large-sized, strongly curved ventrally when relaxed, finely tapering towards the posterior extremity. Cuticle smooth with fine longitudinal striations. Body pores largely absent except in tail region. Lip region offset, 13-15 µm wide, lips rounded. Amphidial fovea stirrup-shaped, 6-7 µm from anterior body end with indiscernible sensillar pouches. Amphidial aperture 4-5 µm across at posterior border of lip region. Lip sensilla in two circlets of 6 + (6 + 4). Inner labials papilloid, outer labials slightly raised. Cephalic sensilla four, 3-4 µm long. Crystalloids numerous, conspicuous, 4-9 µm long, mostly rectangular in shape aggregated in mid-body region, hiding most of internal details. Stoma sclerotised, divided into anterior vestibule partly surrounded by pharyngeal tissue and posterior cylindroid part wholly surrounded by pharyngeal tissue. Anterior vestibular part shows a dorsal tooth and two sub-ventral teeth borne on corresponding sclerotized stomal walls. Dorsal tooth bifurcated, each finely pointed prong appearing fang-like, arising from a common flattened base. Sub-ventral teeth massive and conspicuously curved. A small denticle present at the base of each sub-ventral tooth. The sub-ventral walls prominent, striated, of almost twice as thick as dorsal wall. Pharynx cylindroid with narrow anterior and relatively wider posterior regions. Nerve ring prominent at *ca* 45-50% of pharyngeal length. Body at pharyngeal end *ca* 2-2.5 times labial diameter. Cardia conoid, about one-third the corresponding body width. Intestine with prominent polygonal cells. Pre-rectum indistinct. Rectum *ca* 1.1-1.3 times anal body diameter long. Reproductive system didelphic, amphidelphic, short and compact. Ovaries broad, reflexed, connected to narrow oviducts. Uteri large with thick muscular walls, without any sperm. Vulva a transverse slit, vagina conspicuous, 10-11 µm long provided with strong muscles. Tail divided into short conoid anterior part and a long, filiform posterior part. Two pairs of body pores at base of conical part of tail, dorso-lateral in position.

Male. Not found.

Fourth stage female juveniles. Similar to females in morphology. Replacement teeth (smaller dorsal and larger sub-ventrals) present in the corresponding pharyngeal walls.

Habitat and locality. Soil (sandy clay) samples collected from coal mine area of Sonbhadra, Uttar Pradesh, India at coordinates 24.783°N, 82.833°E.

Voucher material. Seven females and three fourth stage juveniles on slide *Ironus dentifurcatus* no. SI/1-4 deposited in the Nematode collection of the Department of Zoology, Aligarh Muslim University, Aligarh, India. One female on slide no. SI/5 deposited at the Laboratory of Nematology, Wageningen University and Research Centre (WUR), 6700 ES Wageningen, The Netherlands.

Remarks. This is the first report of *I. dentifurcatus* Argo *et* Heyns, 1972 from India. The present specimens agree well in their morphometrics and morphology with *I. dentifurcatus* Argo *et* Heyns, 1972 but differ in some minor details, such as narrower amphids, fang-like arms of dorsal tooth, conspicuous crystalloids and presence of caudal pores.

DISCUSSION

The genus *Ironus* is unique in having predominantly a continental limnetic distribution, with the exception of a few marine or brackish water species, and therefore, occupies the separate subfamily Ironinae. However, it is a heterogeneous taxon with much variation within the genus. Usually, most of the species lack detailed description and are differentiated on the basis of superficial features (Van der Heiden, 1974). In general, the species are large-sized with attenuated posterior ends. The stoma as a rule bears one dorsal and two sub-ventral teeth. However, there is remarkable diversity in the structure of dorsal tooth, which may be single or slightly to deeply forked, giving the appearance of two teeth. Nometheless, most earlier descriptions lack morphological details of the dorsal tooth, which looks undivided in lateral view.

The presence of a very anterior pre-equatorial vulva in some species (*I. luci* Andrássy, 1956) is quite contrary

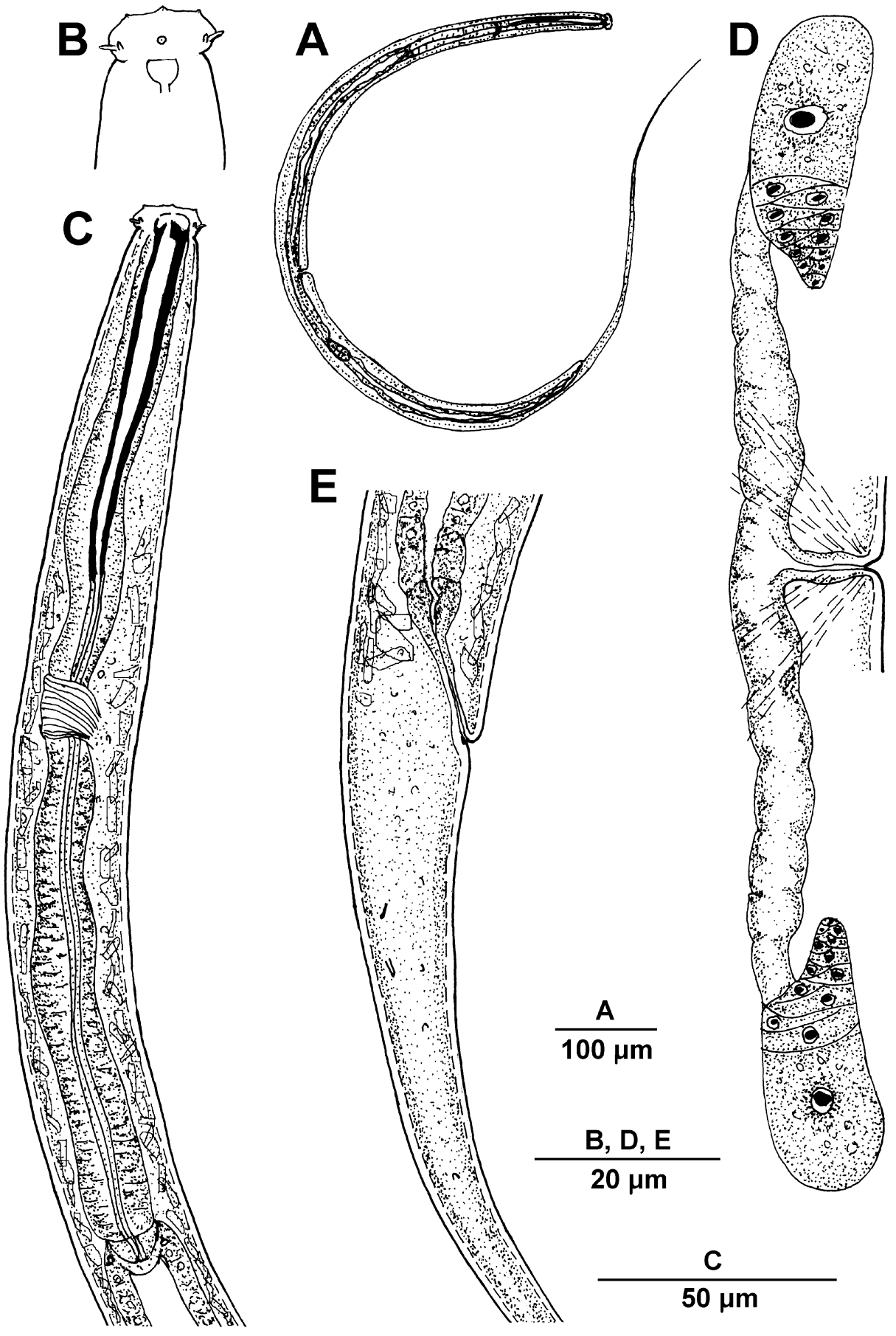

Fig. 5. *Ironus dentifurcatus* Argo *et* Heyns, 1972. A: Entire female; B: Female anterior end; C: Pharyngeal region; D: Female reproductive system; E: Female caudal region.

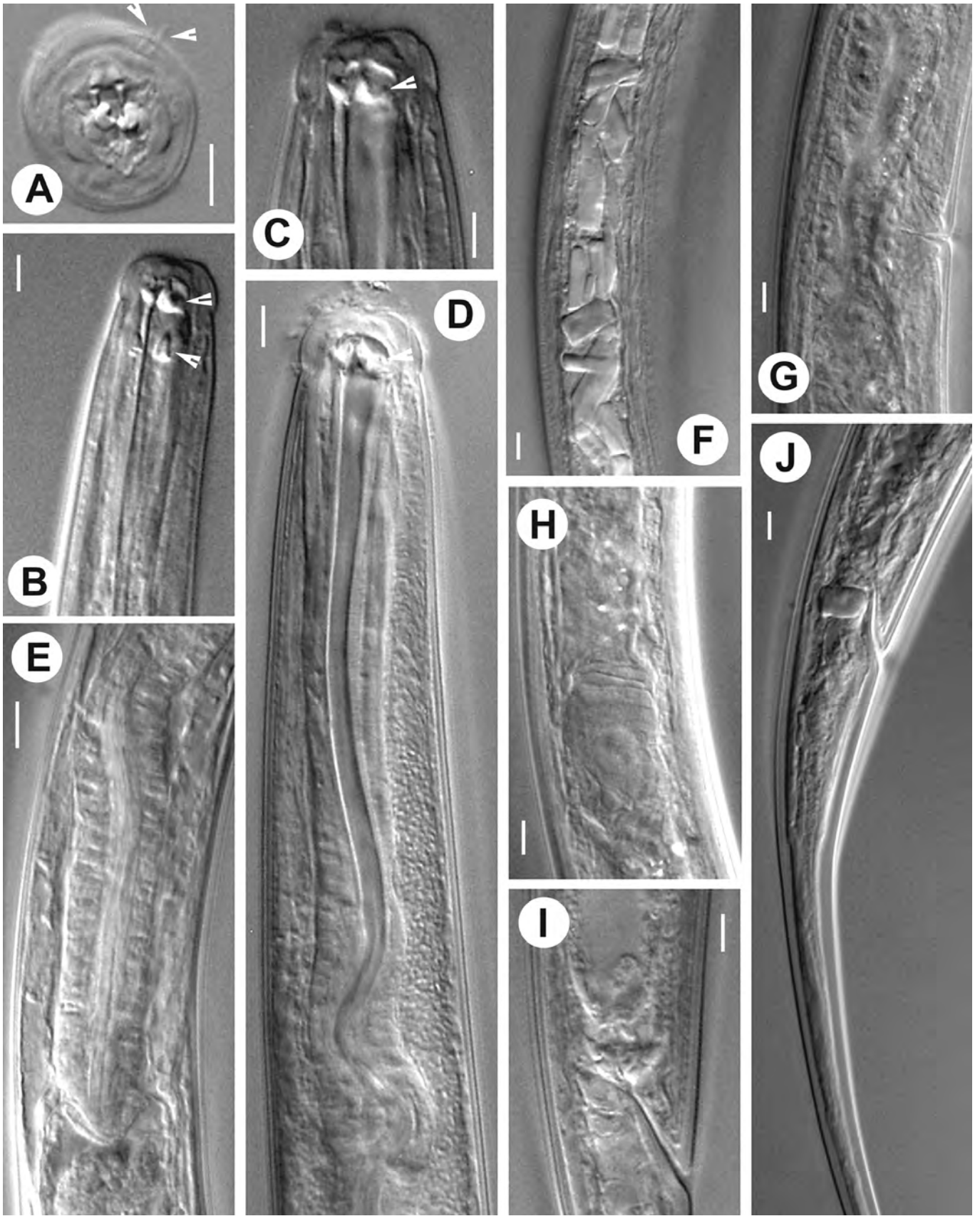

Fig. 6. *Ironus dentifurcatus* Argo *et* Heyns, 1972. A: *En face* view (arrow indicates cephalic and outer labial sensilla); B: Anterior end of juvenile (arrows indicate denticle at base of subventral tooth and the replacement tooth); C: Female anterior end (arrow indicates denticle at base of sub-ventral tooth); D: Female anterior pharyngeal region (arrow indicates denticle at base of sub-ventral tooth); E: Female posterior pharyngeal end; F: Mid-body showing crystalloids; G: Vulval region; H: Part of female genital tract (posterior); I: Anal region; J: Female caudal region. (Scale bars = 5 µm).

to those (*I. macramphis* Schuurman Stekhoven *et* Teunissen, 1938) having a post equatorial vulva. Likewise, the number of genital branches in females can be one (*I. luci* and *I. lautus*) or two as found in most species of the genus. The tail size and shape is also another character which is quite variable among different species. The tail can be elongate hemispheroid (*I. rotundicaudatus* Kreis, 1924) to dorsally convex with a narrow rounded tip. It can also be conoid (*I. sphincterus* Ebsary, 1985), spicate (*I. elegans* Colomba *et* Vinciguerra, 1979) or whip-like (*I. ernsti* Argo *et* Heyns, 1972). Its length can vary from 158 µm (*I. sphincterus*) to 710 µm (*I. ernsti*). In view of the cosmopolitan distribution of the genus, with species occupying different habitats, the diversity in morphological characters is to be expected.

The genera *Dolicholaimus* de Man, 1888 and *Trissonchulus* Cobb, 1920 are very closely related and often are a source of controversy among taxonomists. Schuurmans Stekhoven (1943) considered them to be the same and synonymised them under *Dolicholaimus.* Chitwood (1951, 1960) regarded *Trissonchulus* to be a distinct genus, based on the difference in number of female genital branches, while Wieser (1953) could find no difference between *Dolicholaimus* and *Trissonchulus* other than the shape of the tail. Yeates (1967) also considered *Trissonchulus* a valid genus. Gerlach and Riemann (1974) considered *Dolicholaimus* a valid genus with *D. marioni* as its type and unique species while Andrássy (1976) considered *Dolicholaimus* as the senior synonym of *Trissonchulus.* Later, Platt and Warwick (1983), Platonova and Mokievsky (1994) and Lorenzen (1994) considered them separate genera on account of the dorsal tooth and tail shape. The dorsal tooth and sub-ventral teeth remain single or non-bifid and the tail has an anterior conical and posterior cylindrical segment in *Dolicholaimus* compared to the bifid dorsal tooth (as in *T. janetae*, *T. obtusus*) and largely conical tail in *Trissonchulus*.

The genus *Trissonchulus* shows striking similarities with *Pheronous* Inglis, 1966 in labial arrangement, pharynx structure, position of amphids, presence of two dorsal stomal teeth and fine denticles in vestibular wall. The presence of a spinneret is not a too convincing character on its own to differentiate *Trissonchulus* from the relatively recent genus *Pheronous*. Therefore, the absence of a spinneret in *T. baldwini* does not justify its placement under *Pheronous* (type sp. *Pheronous ogdeni* Inglis, 1966), especially as there are other marked differences *viz.*, smaller amphids, a prominent collarette, longer and setose cephalic sensilla, reproductive system with reduced anterior branch, a filiform tail and weakly developed/rudimentary caudal glands, along with differences in certain allometric ratios ('b', 'c' and 'V'), its seemingly amictic mode of reproduction and terrestrial habitat.

Another closely related genus, *Syringolaimus* de Man, 1888, can be differentiated from the above genera on the basis of striated cuticle, less developed lips and lip sensilla, incompletely or completely divided stomal teeth, pharynx differentiated into basal bulb, long conoid or whip-like tail with well developed caudal glands. The inadequately described species *Trissonchulus lichenii* Nasira *et* Turpeenniemi, 2002, better fits within the genus *Syringolaimus* on account of its continuous/non-offset lip region, pharyngeal bulb and a conical tail with caudal glands and terminal spinneret, as *S. lichenii*. The pharynx with posterior muscular bulb can in particular be considered as an apomorphic character of the genus *Syringolaimus*. The elongated cardia and relatively posterior excretory pore are similar to those found in *S. renaudae* Gourbault *et* Vincx, 1985 and *S. loofi,* respectively.

While examining the descriptions and illustrations of the species of *Trissonchulus* we became aware of much variation in the teeth, a feature commonly reported in *Ironus* also. Another limitation in the descriptions of species of *Trissonchulus* is that most of them lack an *en face* view to clearly show the arrangement of the teeth. There are three undivided teeth in *T. acutus* Gerlach, 1953, *T. reversus* Chitwood, 1951, *T. raskii* Chitwood, 1960, *T. littoralis* Yeates, 1967, *T. quiquepapillatus* Yeates, 1967, *T. dubius* Orselli *et* Vinciguerra, 1997 and *T. provulvatus* Orselli *et* Vinciguerra, 1997. The dorsal tooth is double in *T. benepapillosus* Schulz, 1935, *T. obtusus* Bresslau *et* Schuurmans Stekhoven, 1940 and *T. janetae* Inglis, 1961, while the sub-ventrals are forked or double in *T. oceanus* Cobb, 1920 and *T. nudus* Schuurmans Stekhoven, 1943. Hence the tooth type is a character showing adaptive radiation in these Ironidae and cannot be a reliable character for differentiation at genus level. Likewise, the pharynx seems to be variable in different species of *Trissonchulus*. Though the expanded part never forms a bulb, as in *Syringolaimus*, its lengths are very different in *T. raskii* and *T. littoralis.* The features that can be counted important, giving the genus a separate identity over the closely related *Dolicholaimus,* are the nature of the lips and the lip region (a very consistent and reliable character in some Aporcelaimids). *Trissonchulus* usually shows a truncate or dome-shaped lip region with three labial flaps, but the lips are very strongly amalgamated with their bases considerably wider and separated from the rest of the body by a prominent groove or suture. Further, the inner labial margins are finely serrated or corrugated, as also observed in *T. oceanus*, *T. dubius*, *T. provulvatus* and even in *T. baldwini* sp. n. The inner labial sensilla of *Trissonchulus* are papilliform while the outer circlet and cephalic sensilla are usually raised outward or directed backward. In *Dolicholaimus* the sensilla are papilliform. Another noticeable feature in *Trissonchulus* is a tendency towards reduction of the anterior genital branch, as most species (except for *T. obtusus, T. janetae* and *T. benepapillosus*) show a non-functional, reduced or rudimentary anterior genital branch as also found in *T. baldwini* sp. n. However, it is a fact that detailed information on reproductive system structure is lacking in the

Appendix 1. Characters and character states of *Ironus dentifurcatus*, *I. terranovus*, *Trissonchulus baldwini* sp. n. and the outgroup *T. benepapillosus*.

1. Cuticle: smooth (0), transversely striated (1)
2. Longitudinal striae: absent (0), present (1)
3. Lip region: slightly offset (0),distinctly offset (1)
4. Labial flaps: inconspicuous (0),conspicuous (1)
5. Labial flaps: with smooth (0), serrated(1) margins
6. Labial collar: absent (0), present (1)
7. Cephalic setae: anteriorly (0), backwardly(1) directed
8. Amphid: wide stirrup-shaped (0), narrow funnel-shaped (1)
9. Cheilostom and Oesophastom: differentiated (0), undifferentiated (1)
10. Teeth: Normal (0), reversible (1)
11. Teeth location: subapical (0), apical (1)
12. Buccal denticles: absent (0), present (1)
13. Dorsal tooth: slightly furcated (0), two/deeply furcated (1)
14. Dorsal tooth tip: relatively blunt (0), needle-like (1)
15. Female anterior genital branch: well developed (0), rudimentary (1)
16. Tail: filiform (0), blunt conoid (1)

Appendix 2. Matrix of species and character states of *Ironus dentifurcatus*, *I. terranovus*, *Trissonchulus baldwini* sp. n. *and the outgroup T. benepapillosus*.

Taxon	Characters															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
I. dentifurcatus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I. terranovus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
T. baldwini	0	0	1	1	1	1	1	0	1	1	1	0	1	1	1	0
T. benepapillosus	0	0	1	0	1	0	1	0	1	1	1	1	1	0	0	1

Appendix 3. Characters and character states of species of *Trissonchulus.*

1. Body length: 1-3 mm (0), 3-5 mm (1), 5-7 (2) mm
2. Lip region with wide base (0), slightly wide (1), more or less uniformly wide (2)
3. Lip- body junction: offset (0), continuous (1)
4. Labial flaps: absent (0), present (1)
5. Labial sensilla: papilliform (0), setose (1)
6. Cephalic sensilla: setose (0), papilliform (1)
7. Amphids: 1/3 (0), 1/2 or more (1) of body width
8. Teeth: all single (0), dorsal bifid/two (1), all bifid (2)
9. Inner labial margins: serrated (0), smooth (1)
10. Peribuccal denticles: absent (0), present (1)
11. Pharyngeal tissue surrounding stoma: strongly ellipsoidal (0), weakly or not ellipsoidal (1)
12. Posterior pharyngeal part: gradually expanded (0), elongate bulb (1)
13. Tail: short, blunt conoid to hemispheroid (0), long cylindroid (1)
14. Caudal gland opening in females: ventral (0), dorsal (1), terminal (2), absent (3)
15. Vulva: post-equatorial (0), equatorial (1), pre-equatorial (2)
16. Gonads: paired with anterior ovary reduced (0), ophisthodelphic (1), didelphic (2)

descriptions of most species of the genus. The tail type is considered to be a differentiating feature and is generally conical, but can be short and bluntly rounded (*T. reversus*) or long, gradually tapering and acute (*T. acutus* and *T. baldwini* sp. n.). The tail shape seems to be more consistent in *Dolicholaimus* (*D. minor, D. filipjevi* and *D. marioni*), where the tail possesses an anterior conical and a posterior narrower and cylindroid segment. The position of the caudal glands opening or spinneret in females of most species of *Trissonchulus* is ventral. The spinneret is reported to open dorsally in *T. reversus,* ventrally in *T. acutus* and terminally in *T. benepapillosus*. *Trissonchulus baldwini* sp. n. has rudimentary caudal glands but lacks a spinneret.

Appendix 4. Matrix and character states of species of *Trissonchulus*.

Taxon	Characters															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
T. acutus	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	2
T. baldwini n.sp.	0	0	0	0	0	0	0	1	0	0	0	0	2	3	2	0
T. benepapillosus	0	0	0	0	0	0	0	1	1	1	0	0	1	2	0	0
T. dubius	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
T. janetae	1	1	2	1	0	0	0	1	1	1	0	0	0	1	0	2
T. latus	1	2	2	1	1	0	0	0	1	0	0	0	0	0	0	2
T. littoralis	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0
T. nudus	2	0	0	0	0	1	0	2	1	0	1	0	0	0	2	2
T. obtusus	1	2	1	0	0	1	0	1	1	0	0	1	0	0	1	2
T. oceanus	1	0	0	0	0	1	0	2	0	1	0	0	0	0	2	1
T. provulvatus	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0
T. quiquepapillatus	2	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0
T. raski	1	0	0	0	0	1	0	?	1	0	0	0	0	0	0	1
T. reversus	0	2	2	0	0	1	0	0	1	0	0	0	1	1	?	?

TRISSONCHULUS **Cobb, 1920**

Diagnosis (emended). Genus *Trissonchulus*, represented predominantly by marine species, is characterised by medium to very large-sized individuals with conspicuous crystalloids. Lip region truncate or dome-shaped, separated from adjoining body by a deep groove. Lip region with three labial flaps and lips strongly amalgamated to form a band or collarette, with inner margins corrugated. Lip sensilla in two circlets: six inner sensilla papilliform, six sensilla of outer circlet papilliform or slightly raised, four cephalic sensilla of outer circlet usually setose and directed outward or backward. Stoma provided either with three reversible, undivided teeth or occasionally with one forked dorsal or two dorsals and rarely with biuncinate sub-ventral teeth. Pharynx strongly muscular showing gradual dilation posterior to nerve ring. Female reproductive system didelphic, amphidelphic with reflexed ovaries or pseudomonodelphic with reduced anterior genital branch. Vulva equatorial or slightly post-equatorial, rarely pre-equatorial. Tail variable in shape from short conoid to long cylindroid, in rare instances filiform. Caudal glands usually present, mostly located precaudally, opening through a ventral spinneret in most species, spinneret occasionally dorsal or terminal and rarely absent.

The relationships, based on important morphological characters (Appendix 3), between *T. baldwini* sp. n. and other species of *Trissonchulus* revealed (Fig. 7) a closer affinity of the former with *T. acutus* and *T. benepapillosus*, which group together. Interestingly the labial morphologies of these three species are identical, along with the long tapering tail type which is exceptional in the genus. However, the dorsal tooth in *T. acutus* has been reported to be single, slightly forked in *T. benepapillosus* while completely divided or double in *T. baldwini* sp. n. *Trissonchulus raskii* and *T. quinquepapillatus*, *T. dubius* and *T. littoralis* formed separate groups indicating closer identity among themselves and collectively with *T. provulvatus*. *Trissonchulus oceanus* and *T. nudus* grouped together, supporting Wieser's (1953) decision

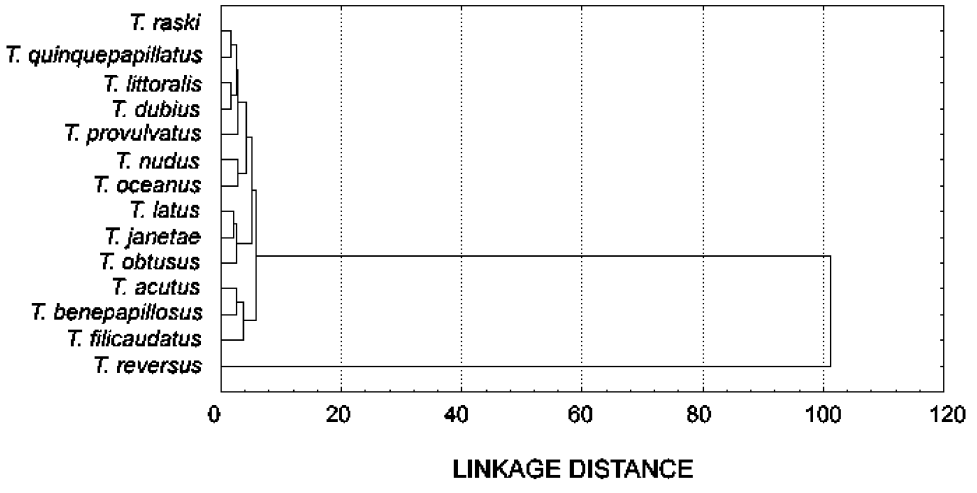

Fig. 7. Dendrogram showing relationship between species of *Trissonchulus* based on morphological characters.

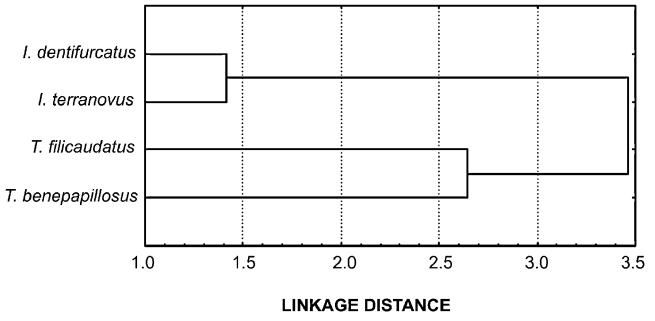

Fig. 8. Dendrogram showing relationship between *I. dentifurcatus, I. terranovus, T. baldwini* sp.n. and *T. benepapillosus* based on morphological characters.

to consider the latter as a junior synonym of the former. *Trissonchulus latus* and *T. janetae*, though clustered together, also show affinities with *T. obtusus. Trissonchulus reversus* appeared on a separate branch showing significant differences from other members of the genus.

The morphometrics of *T. baldwini* sp. n. show similarity with the filiform-tailed species, *I. terranovus* and *I. dentifurcatus*. However, the analysis of morphological characters (Fig. 8) indicates its close relationship with *T. benepapillosus* rather than with *I. dentifurcatus* and *I. terranovus*, which group together. The analysis thus suggests that, as a large-sized terrestrial species of Ironidae, *T. baldwini* sp. n. has most morphometric characteristics similar to *Ironus* species *viz.*, body length, pharyngeal length, length of filiform tail and allometric ratios, but that it is distant from them morphologically due to the apomorphic differences *viz.*, backwardly directed cephalic sensilla, colarette and reduced anterior genital branch.

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