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Research article

## A review on and identification tabular key for the species of the genera *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus* (Nematoda: Telotylenchinae)

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### Abstract

The genus *Tylenchorhynchus* is one of the largest and most problematic taxa of plant-parasitic nematodes. The separation or combining of certain genera into a large genus as *Tylenchorhynchus sensu lato* has long been the subject of debate. Mostly due to incomplete descriptions of valid species and the limited number of diagnostic characters, species identification has also been a challenging task in the genus, even in *Tylenchorhynchus sensu stricto* with fewer species. In this study, a tabular identification key is provided for 136 valid species of *Tylenchorhynchus* and the closely related genera *Bitylenchus* and *Sauertylenchus*. Species are coded based on the status of nine diagnostic characters *viz.* the number of transverse annuli on lip region, lip region demarcation, stylet length, presence of post-rectal sac, number of cuticular annuli on the ventral side of tail, tail shape, value of  $c'$  ratio, tail terminus striation, and gubernaculum structure, as well as special characters for certain species. As a result, sixteen groups were identified that show the same status for all these characters. Further discussions are provided on the detailed characterization of the closely related species in each group, if needed.

**Keywords:** Diagnostic characters, identification, plant-parasitic nematodes, taxonomy

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## بازبینی و کلید شناسایی جدولی برای گونه‌های سه جنس *Sauertylenchus*, *Bitylenchus* و *Tylenchorhynchus* (Nematoda: Telotylenchinae)

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### خلاصه

جنس *Tylenchorhynchus* یکی از بزرگ‌ترین و مسأله‌دارترین گروه از نماتدهای انگل گیاهی است. تفکیک یا تجمیع جنس‌های معین در داخل جنس بزرگ *Tylenchorhynchus sensu lato* یک موضوع بحث‌برانگیز است. اغلب به دلیل توصیف‌های ناقص گونه‌های قابل قبول و محدود بودن ویژگی‌های ریخت‌شناختی متمایزکننده، حتی در *Tylenchorhynchus sensu stricto* با داشتن تعداد گونه بسیار کمتر، شناسایی گونه‌ها کاری دشوار است. در این مطالعه مروری، یک کلید شناسایی جدولی برای ۱۳۶ گونه معتبر از *Tylenchorhynchus* و دو جنس نزدیک به آن، *Bitylenchus* و *Sauertylenchus* فراهم شده است. گونه‌ها بر اساس نه ویژگی تمایزی شامل تعداد حلقه‌های سر، نحوه اتصال سر به بدن، طول استایلت، وجود کیسه عقبی راست‌روده، تعداد حلقه‌های کوتیکولی در سطح شکمی دم، شکل دم، شاخص 'c'، شیاردار بودن انتهای دم و ساختار گوبرناکولوم، کدگذاری شدند. در نتیجه، ۱۶ گروه شناسایی گردید که تمامی ویژگی‌ها برای گونه‌های داخل هر گروه دارای وضعیت‌های مشابه بودند. در صورت نیاز، بحث بیشتری روی ویژگی‌های جزئی برای گونه‌های نزدیک به هم در هر گروه ارائه شده است.

**واژه‌های کلیدی:** آرایه‌بندی، شناسایی، نماتدهای انگل گیاهی، ویژگی‌های متمایزکننده

## Introduction

The genus *Tylenchorhynchus* Cobb, 1913 with more than 130 species, is known as “stunt nematodes” and is one of the largest and most problematic taxa of plant-parasitic nematodes. About 20 species of them form the *Bitylenchus* Filipjev, 1934 subgroup that is sometimes regarded as a valid genus (Hunt *et al.* 2013; Decraemer & Hunt 2013). Fortuner & Luc (1987) proposed the concept of ‘large genus’ for *Tylenchorhynchus*, in which *Bitylenchus*, *Neodolichorhynchus* Jairajpuri & Hunt, 1984, *Telotylenchus* Siddiqi, 1960 and several other genera were considered as synonyms of *Tylenchorhynchus*. Nematologists, however, did not universally agree on this concept, hence they attempted at splitting this large genus (Geraert 2011). Species with five and three incisures in their lateral fields were considered as *Quinisulcius* Siddiqi, 1971 and *Trichotylenchus* Whitehead, 1960, respectively. The presence of longitudinal ridges and an overlapping pharyngeal lobe were used as the main characteristic of *Neodolichorhynchus* and *Telotylenchus*, respectively. Species with an abnormally thickened tail end were characterized as *Paratrophurus* Arias, 1970. Some species with special asymmetrical stylet were considered as *Histotylenchus* Siddiqi, 1971, and a single species bearing the largest stylet was transferred to *Sauertylenchus* Sher, 1974. Moreover, *Bitylenchus* was distinguished from *Tylenchorhynchus* by having lateral fields with areolated outer bands, a large post-rectal intestinal sac containing intestinal granules and fasciculi, relatively more thickened cuticle at the female tail terminus, and gubernaculum lacking a crest (Gomez-Barcina *et al.* 1992).

Several studies have contributed to the taxonomy of the genera *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus* based on morphological or molecular approaches during recent years (Carta *et al.* 2010; Ganguly *et al.* 2013; Handoo *et al.* 2014; Ghaderi *et al.* 2014a, 2014b, 2017, 2018a; Alvani *et al.* 2017; Zhang *et al.* 2018; Hosseinvand *et al.* 2020; Mwamula *et al.* 2020; Xu *et al.* 2020). Molecular studies have confirmed that *Bitylenchus* and *Sauertylenchus* could be considered as separate genera from *Tylenchorhynchus* (Handoo *et al.* 2014; Hosseinvand *et al.* 2020).

Hosseinvand *et al.* (2020) provided a dichotomous species identification key for the genera *Bitylenchus*,

*Sauertylenchus* and *Tylenchorhynchus* without diagnostic tabular key. The present study has provided important diagnostic characters for each species included in these genera, aiming to provide a tabular key that could be used as a supplement to the previously published key (Hosseinvand *et al.* 2020) for more accurate identification of the species.

## Materials and methods

For having a systematic view on the species treated under *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus*, morphological and morphometric characters of all known species from their original descriptions or the reference book "Dolichodoridae of the world" (Geraert 2011) were compared with each other, and all data documented. All species were coded based on the status of the nine important diagnostic characters *viz.* the number of transverse annuli on lip region, lip region demarcation, stylet length, presence of post-rectal sac, number of cuticular annuli on the ventral side of tail, tail shape, the value of *c'* ratio, tail terminus striation, and gubernaculum structure as well as special characters for certain species. After sorting and arranging the data, a tabular key was provided for species identification. Sixteen groups were formed based on having the same status in their all nine diagnostic characters. Each group was named after the oldest described species in that group.

## Results and discussion

A complete list of the valid species of *Tylenchorhynchus* with their synonyms as well as *species inquirendae* and *nomina nuda* species are given in Geraert (2011) and Hosseinvand *et al.* (2020). Certain species are added to the species list in Geraert (2011) in recent years including *T. bambusi* Singh, Lal, Rathour & Ganguly, 2010, *B. capsicumi* Akhter & Zarina, 2014, *B. hispaniensis* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014, *T. iranensis* Azimi, Mahdikhani-Moghadam, Rouhani & Rajabi Memari, 2016, *T. mediterraneus* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014, *T. casigo* (Castillo, Siddiqi & Gomez-Barcina, 1989) Sturhan, 2014, and *B. parvulus* Hosseinvand, Eskandari, Ganjkhani, Ghaderi, Castillo and Palomares-Rius,

2020 (Singh *et al.* 2010; Akhter & Zarina 2014; Handoo *et al.* 2014; Sturhan 2014; Azimi *et al.* 2016; Hosseinvand *et al.* 2020).

Morphological (Barcina *et al.* 1992), molecular (Handoo *et al.* 2014; Ghaderi *et al.* 2014b; Hosseinvand *et al.* 2020), and numerical analyses (Ghaderi *et al.* 2017) support *Bitylenchus* and *Sauertylenchus* as valid genera separable from *Tylenchorhynchus*, but there is no adequate information on the important diagnostic characters such as lateral field areolation, lip region structure, presence of post-rectal sac, or the shape of gubernaculum on many of described species in the literature. Moreover, certain features, particularly gubernaculum shape, were sometimes not described in a detailed or even accurate manner.

All known species of these three genera are treated in a single tabular key here. For the taxonomic status of the species, we followed Hosseinvand *et al.* (2020). Although *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus* are treated as the three separate valid genera in literature, there are problems in the identification of the species (Hosseinvand *et al.* 2020). In the tabular key, 16 groups bearing more than one species were identified that showed the same states for all these characters (Table 1). Further discussions were made on the detailed characterization of the closely related species in each group, as well.

### Genus *Bitylenchus* Filipjev, 1934

#### Diagnosis (After Siddiqi 2000):

Telotylenchinae. Body small to medium sized. Cuticle with fine and distinct annuli. Lateral field with three band or four incisures, outer bands areolated; longitudinal incisures excluding lateral field in pharynx region in some species, Cephalic region finely annulated, usually offset by a constriction or depression, squarish-shaped, with 6-10 annuli, framework lightly sclerotized. Stylet less than 30  $\mu\text{m}$ , conus about half of total stylet length, basal knobs rounded often sloping backwards. Median bulb well developed, not offset from precorpus by a constriction. Basal bulb offset from intestine. Vulva a transvers slit, in some species cuticle irregularly undulations (wrinkling) at anterior or/and posterior of vulva. Spermatheca rounded, axial. Ovaries paired. Postanal intestinal sac present, filling one-quarter or more of tail cavity. Female tail cylindrical, subcylindrical or subclavate, tail tip rounded and usually

striated. Spicules usually with velum, tip notched. Bursa enveloped to tail tip. Gubernaculum large, boat-shaped in lateral view, lacking crest.

#### Type species

*Bitylenchus dubius* (Bütschli, 1873) Filipjev, 1934

#### Other species

*B. botrys* Siddiqi, 1985

*B. brevilineatus* (Williams, 1960) Jairajpuri, 1982

*B. bryobius* (Sturhan, 1966) Jairajpuri, 1982

*B. canalis* (Thorne & Malek, 1968) Jairajpuri, 1982

*B. capsicum* Zarina, Akhtar, Khan & Zaki, 2014

*B. colombianus* Siddiqi, 1985

*B. cuticaudatus* (Ray & Das, 1983) Siddiqi, 1986

*B. depressus* Jairajpuri, 1982

*B. erevanicus* (Karapetjan, 1979) n. comb.\*

*B. equatorialis* Talavera & Siddiqi, 1995

*B. goffarti* (Sturhan, 1966) Jairajpuri, 1982

*B. hispaniensis* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014

*B. huesingi* (Paetzold, 1958) Jairajpuri, 1982

*B. iphilus* Minagawa, 1995

*B. kidwaini* (Rashid & Heyns, 1990) Siddiqi, 2000

*B. mediocris* Talavera & Siddiqi, 1995

*B. natalensis* (Kleynhans, 1984) Siddiqi, 1986

*B. paracanal* (Khan, 1991) n. comb.\*\*

*B. parvus* (Allen, 1955) Jairajpuri, 1982

*B. parvulus* Hosseinvand, Eskandari, Ganjkanloo, Ghaderi, Castillo & Palomares Rius, 2020

*B. quaidi* (Golden, Maqbool & Handoo, 1987) Siddiqi, 2000

*B. queirozi* (Monteiro & Lordello, 1976) Jairajpuri, 1982

*B. serranus* Gomez-Barcina, Siddiqi & Castillo, 1992

*B. singularis* Siddiqi & Sharma, 1994

*B. swarupi* (Singh & Khera, 1978) Jairajpuri, 1982

*B. teeni* (Hashim, 1984) Siddiqi, 1986

*B. tobari* (Sauer & Annells, 1981) Siddiqi, 1986

*B. usmanensis* (Khurma & Mahajan, 1988) Siddiqi, 2000

*B. ventrosignatus* (Tobar Jiménez, 1969) Jairajpuri, 1982

*B. vulgaris* (Upadhyay, Swarup & Sethi, 1972) Jairajpuri, 1982

*B. zambiensis* (Venditti & Noel, 1995) Siddiqi, 2000

\* This species with areolated outer bands, distinctly annulated tail tip is very similar to *B. huesingi* except in having a finger-shaped outgrowth (Geraert 2011). Also Siddiqi (2000) pointed out this species probably is a *Bitylenchus*.

\*\* This species with simple gubernaculum and distinctly fasciculi posterior to anus (in line drawing) fitted to the genus *Bitylenchus*.

### Genus *Sauertylenchus* Sher, 1974

#### Diagnosis (After Siddiqi 2000):

Telotylenchinae. Body medium-sized, 1.4–2 mm. Annuli distinct. Lateral fields each with four incisures, outer bands incompletely areolated. Deirids absent. Cephalic region offset, annulated, divided into six sectors anteriorly, labial disc round, distinct, slightly prominent; framework weakly sclerotized. Stylet long (33–40 µm), very slender, conus appearing solid anteriorly, 50% or more of total stylet length; knobs small and rounded. Median bulb oval. Basal bulb saccate, rounded posteriorly; cardia large. Intestine with fasciculi extending into tail cavity. Female tail subcylindrical, over two anal body widths long. Male tail enveloped by a simple crenate bursa. Spicules with large distal flanges. Gubernaculum large, with titillae, recurved distally. Hypoptygma absent.

#### Type species

*Sauertylenchus labiodiscus* Sher, 1974

#### Other species

*S. maximus* (Allen, 1955) Siddiqi, 2000

= *S. ibericus* (Mahajan & Nombela, 1987) Siddiqi, 2000

*S. pamiricus* (Ivanova, 1989) Hosseinvand *et al.*, 2020

*S. pratensis* (Gomez-Barcina, Siddiqi & Castillo, 1992) Siddiqi, 2000

*S. velatus* (Sauer & Annells, 1981) Siddiqi, 2000

### Genus *Tylenchorhynchus* Cobb, 1913

#### Diagnosis (After Siddiqi 2000):

Telotylenchinae. Body about 1 mm or less long. Cuticle prominently annulated, may be marked by longitudinal striae, longitudinal ridges or lamellae outside lateral fields absent; not abnormally thickened on tail. Lateral field with three band or four incisures, generally not areolated behind oesophageal region. Cephalic region offset from body or continuous, annulated, or rarely smooth, without longitudinal

indentations on annuli; labial disc indistinct, in SEM *en face* squarish, flattened, fused with first lip annulus; framework light to moderately sclerotized. Stylet well developed, generally 15–25 µm long; conus anteriorly solid-appearing; knobs prominent. Median bulb round or oval, with distinct refractive thickenings, usually marked off from precorpus and isthmus. Basal bulb offset from intestine, or with base slightly extending over intestine. Cardia prominent. Vulva near middle of body, lips not modified. Spermathecae round, axial. Ovaries paired, outstretched. Female tail usually conoid with blunt tip as in type species, but may be subcylindroid, cylindroid or subclavate; terminus smooth, rarely striated or narrowing to a point. Males generally present. Tail enveloped by a large simple bursa. Spicules distally flanged, terminus narrow, indented or pointed. Gubernaculum well developed, generally rod-like and with a velum, protrusible.

#### Type species

*Tylenchorhynchus cylindricus* Cobb, 1913

#### Other species

*Tylenchorhynchus aduncus* de Guiran, 1967

*T. aerolatus* Tobar Jiménez, 1970

*T. agri* Ferris, 1963

*T. alami* Shaw & Khan, 1996

*T. amgi* Kumar, 1981

*T. ancorastyletus* Ivanova, 1983

*T. annulatus* (Cassidy, 1930) Golden, 1971

*T. antarcticus* Wouts & Sher, 1981

*T. areoterminalis* Siddiqi, 2008

*T. aspericutis* Knobloch, 1975

*T. badliensis* Saha & Khan, 1982

*T. bambusi* Singh, Lal, Rathour & Ganguly, 2010

*T. bicaudatus* Khakimov, 1973

*T. bohrrensis* Gupta & Uma, 1980

*T. brassicae* Siddiqi, 1961

*T. casigo* (Castillo, Siddiqi & Gomez-Barcina, 1989) Sturhan, 2014

*T. chirchikensis* Mavlyanov, 1978

*T. clarus* Allen, 1955

*T. clavus* Khan, 1990

*T. claytoni* Steiner, 1937

*T. coffeae* Siddiqi & Basir, 1959

*T. contractus* Loof, 1964

*T. crassicaudatus* Williams, 1960

*T. crotoni* Pathak & Siddiqi, 1997

- T. cynodoni* Kumar, 1981  
*T. dactylurus* Das, 1960  
*T. delhiensis* Chawla, Bhamburkar, Khan & Prasad, 1968  
*T. digitatus* Das, 1960  
*T. ebriensis* Seinhorst, 1963  
*T. elamini* Elbadri, Moon, Lee & Choo, 2010  
*T. elegans* Siddiqi, 1961  
*T. eremicolus* Allen, 1955  
*T. eroshenkoi* Siddiqi, 1986  
*T. ewingi* Hopper, 1959  
*T. fatimae* Khan, Saeed & Akhter, 2004  
*T. georgiensis* Eliashvili, 1971  
*T. gossypii* Nasira & Maqbool, 1996  
*T. graciliformis* Siddiqi & Siddiqui, 1983  
*T. haki* Fotedar & Mahajan, 1971  
*T. handooi* Khan, 2004  
*T. hordei* Khan, 1972  
*T. iarius* Saha, Gaur & Lal, 1998  
*T. idahoensis* Siddiqi, 2008  
*T. iranensis* Azimi, Mahdikhani-Moghadam, Rouhani & Rajabi Memari, 2016  
*T. irregularis* Wu, 1969  
*T. ismaili* Azmi & Ahmad, 1991  
*T. kamlae* Shaw & Khan, 1996  
*T. kashmirensis* Mahajan, 1974  
*T. kegasawai* Minagawa, 1995  
*T. kegenicus* Litvinova, 1946  
*T. latus* Allen, 1955  
*T. leucaenus* Azmi, 1991  
*T. leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982  
*T. lucknowensis* Singh & Jain, 1983  
*T. malinus* Lin, 1992  
*T. mangiferae* Luqman & Khan, 1986  
*T. manubriatus* Litvinova, 1946  
*T. mashhoodi* Siddiqi & Basir, 1959  
*T. mediterraneus* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014  
*T. mexicanus* Knobloch & Laughlin, 1973  
*T. microcephalus* Siddiqi & Patel, 1990  
*T. microconus* Siddiqi, Mukherjee & Dasgupta, 1982  
*T. minutus* Karapetjan, 1979  
*T. musae* Kumar, 1981  
*T. neoclavicaudatus* Mathur, Sanwal & Lal, 1979  
*T. nordiensis* Khan & Nanjappa, 1974  
*T. nudus* Allen, 1955  
*T. ooti* Siddiqi, 2008  
*T. oryzae* Kaul & Waliullah, 1995  
*T. paracanalisis* Khan, 1991  
*T. parasudanensis* (Elbadri, Moon, Lee & Choo, 2010) Geraert, 2011  
*T. paratriversus* Brzeski, 1992  
*T. paulettae* Bloemers & Wanless, 1998  
*T. persicus* Sultan, Singh & Sakhuja, 1991  
*T. projectus* Khan, 1990  
*T. qasimii* Rarnzan, Handoo & Fayyaz, 2008  
*T. pruni* Gupta & Uma, 1981  
*T. robustus* Thorne & Malek, 1968  
*T. rudis* Siddiqi, 2008  
*T. sanwali* Kumar, 1982  
*T. shimizui* Talavera, Watanabe & Mizukubo, 2002  
*T. shivanandi* Shaw & Khan, 1992  
*T. siccus* Nobbs, 1990  
*T. silvaticus* Ferris, 1963  
*T. spinaceai* Singh, 1976  
*T. striatus* Allen, 1955  
*T. subcylindricus* Singh & Jain, 1982  
*T. sudanensis* (Decker, Yassin & El-Amin, 1975) Castillo, Siddiqi & Gomez-Barcina, 1989  
*T. tarjani* Andrásy, 1969  
*T. tenuicauda* Wouts & Sher, 1981  
*T. teres* (Khan & Darekar, 1979) Siddiqi, 1986  
*T. thermophilus* Golden, Baldwin & Mundo-Ocampo, 1995  
*T. tritici* Golden, Maqbool & Handoo, 1987  
*T. tuberosus* Zarina & Maqbool, 1994  
*T. variannus* Mavlyanov, 1978  
*T. variannulatus* Siddiqi, 2008  
*T. varicaudatus* Singh, 1971  
*T. vishwanathensis* Pathak & Siddiqui, 1997  
*T. zae* Sethi & Swarup, 1968
- Species inquirendae**
- Tylenchorhynchus alatus* (Cobb, 1930) Filipjev, 1936  
*T. brachycephalus* Litvinova, 1946  
*T. browni* (Kreis, 1929) Filipjev & Schuurmans Stekhoven, 1941  
*T. bucharicus* (Tulaganov, 1949) Tulaganov, 1954  
*T. caromatae* (Tulaganov, 1949) Tulaganov, 1954  
*T. gadeai* (Arias Delgado, Jiménez Millán & López Pedregal, 1965) Braun & Loof, 1966  
*T. galeatus* Litvinova, 1946

- T. paucus* Kirjanova, 1951  
*T. sexamamillatus* (Kirjanova, 1938) Kirjanova, 1961  
*T. styriacus* Micoletzky, 1922  
*T. symmetricus* (Cobb, 1914) Filipjev, 1936

#### Nomina nuda

- T. ascicaudatus* Chang, 1991  
*Tylenchorhynchus caricae* Kapoor, 1983  
*T. fugianensis* Chang, 1990  
*T. imitans* Kapoor, 1983  
*T. phallocercus* Chang, 1991  
*T. robustus* var. *pseudorobustus brasiliensis* Rahm, 1928  
*T. stabilis* Kapoor, 1983  
*T. sulciceps* Kapoor, 1983  
*T. valerianae* Kapoor, 1983  
*T. triversus* Siddiqi, 2000

#### Tabular key to the species of *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus* (mainly based on females)

##### A- The number of transverse annuli on lip region (excluding labial disc)

- 1: without annuli; smooth (Fig. 1A)
- 2: usually 2 or 3 annuli, rarely 4 annuli (Fig. 1B)
- 3: usually 4 or 5 annuli, rarely 6 annuli (Fig. 1C)
- 4: 6-10 mostly fine annuli (Fig. 1D)

##### B- Stylet length

- 1: average shorter than 18  $\mu\text{m}$
- 2: average 18-22  $\mu\text{m}$
- 3: average more than 22  $\mu\text{m}$

##### C- Post-rectal sac

- 1: absent or very slightly overlaps rectum (Fig. 1F)
- 2: present (Fig. 1G, H)

##### D- Cuticular annuli on the ventral side of tail

- 1: average less than 25
- 2: average 25-35
- 3: average more than 35

##### E- Lip region demarcation

- 1: continuous to slightly offset (Fig. 1A-D)
- 2: distinctly offset (Fig. 1E)

##### F- Tail shape

- 1: conical to sub-cylindrical (Fig. 1F, G)
- 2: cylindrical or clavate (Fig. 1H, I)

##### G- Value of $c'$ index

- 1: average below 2
- 2: average 2 to nearly 3.5
- 3: average nearly 4 or more

##### H- Tail terminus striation commonly seen in the population

- 1: smooth (Fig. 1F-H)
- 2: striated (Fig. 1I)

##### I- Gubernaculum in males

- 1: with crest, an expansion in distal half (Fig. 1J, K)
- 2: lacking crest (Fig. 1L-N)
- 3: unknown

##### J- Special characters

- 1: body nearly 1 mm or larger
- 2: longitudinal striae are present on whole body or on anterior end
- 3: cuticle near vulva depressed or irregular
- 4: vulva with very distinct outer epiptygma
- 5: female tail with a distinct hyaline region (more than 10  $\mu\text{m}$  thick)
- 6: female tail with very narrow terminus
- 7: female tail with terminal outgrowth or bursa-like structure
- 8: spicules in males nearly 25  $\mu\text{m}$  or longer

#### Remarks on the groups with similar species

##### Group 1

*Tylenchorhynchus tuberosus* group (two species: *T. parasudanensis* and *T. tuberosus*). In *T. parasudanensis* lip region is hemispherical, basal bulb saccate, and stylet knobs posteriorly or laterally directed, but in *T. tuberosus* lip region conoid truncate, basal bulb pyriform, and stylet knobs anteriorly directed.

##### Group 2

*Tylenchorhynchus microcephalus* group (two species: *T. microcephalus* and *T. ooti*). Stylet of *T. ooti* is slightly longer than that of *T. microcephalus* (21-22 vs. 18-21  $\mu\text{m}$ ). No sperm was detected in the spermatheca of *T. microcephalus*, while the male was not found in *T. ooti*. Siddiqi (2008) noted that *T. microcephalus* has a characteristic dorsal shallow depression in the female tail.

**Table 1.** Diagnostic tabular key for the species identification of the genera *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus*.

	Species	Characters										
		A	B	C	D	E	F	G	H	I	J	
1	<i>T. cynodonti</i>	1	1	1	1	1	2	3	1	2	5	
	<i>T. sudanensis</i>	1	1	1	3	1	1	2	1	3	-	
	<i>T. tuberosus</i>	1	2	1	1	1	2	2	1	3	5	
	<i>T. parasudanensis</i>	1	2	1	1	1	2	2	1	3	5	
	<i>T. leviterminalis</i> *	1	2	1	1	1	2	3	1	1	5	
2	<i>T. oryzae</i>	1	2	1	1	2	1	2	1	1	-	
	<i>T. microcephalus</i>	1	2	1	2	1	2	3	1	1	-	
	<i>T. ooti</i>	1	2	1	2	1	2	3	1	3	-	
	<i>T. amgi</i>	1	2	2	1	1	1	3	1	3	-	
3	<i>T. robustus</i> *	1	3	1	3	1	2	2	1	3	1	
	<i>T. bohrrensis</i>	2	1	1	1	1	1	2	1	1	-	
	<i>T. elamini</i>	2	1	1	1	1	1	2	1	3	-	
	<i>T. elegans</i> *	2	1	1	1	1	1	2	1	1	-	
	<i>T. haki</i>	2	1	1	1	1	1	2	1	3	-	
	<i>T. iarius</i>	2	1	1	1	1	1	2	1	3	-	
	<i>T. mashhoodi</i> *	2	1	1	1	1	1	2	1	1	-	
	<i>T. qasimii</i>	2	1	1	1	1	1	2	1	1	-	
	<i>T. rudis</i>	2	1	1	1	1	1	2	1	1	-	
	<i>T. tritici</i> *	2	1	1	1	1	1	2	1	1	-	
	<i>T. varicaudatus</i>	2	1	1	1	1	1	2	1	3	-	
4	<i>T. gossypii</i>	2	1	1	1	1	2	2	1	1	-	
	<i>T. lucknowensis</i>	2	1	1	1	1	2	2	1	1	-	
5	<i>T. aspericutis</i>	2	1	1	1	2	1	2	1	1	-	
	<i>T. microconus</i>	2	1	1	1	2	1	2	1	3	-	
	<i>T. handooi</i>	2	1	1	2	1	1	3	1	3	-	
	<i>T. delhiensis</i>	2	1	1	2	1	1	3	2	3	-	
	<i>T. karnalensis</i>	2	1	1	2	1	2	2	1	3	-	
	<i>T. microcephaloides</i>	2	1	2	1	1	2	2	2	3	5, 8	
	6	<i>T. ancorastyletus</i>	2	2	1	1	1	1	2	1	1	-
		<i>T. annulatus</i> *	2	2	1	1	1	1	2	1	3	-
		<i>T. badliensis</i>	2	2	1	1	1	1	2	1	1	-
		<i>T. coffeae</i>	2	2	1	1	1	1	2	1	1	-
<i>T. mexicanus</i>		2	2	1	1	1	1	2	1	1	-	
<i>T. musae</i>		2	2	1	1	1	1	2	1	3	8	
<i>T. claytoni</i> *		2	2	1	1	1	1	2	2	1	2	
<i>T. crassicaudatus</i>		2	2	1	1	1	2	3	1	1	5	



Table 1. Continued

	<i>T. kegasawai</i>	2	2	1	1	1	2	3	1	1	-
	<i>T. thermophilus</i>	2	2	1	2	1	1	2	1	3	-
	<i>T. paratriversus</i>	2	2	1	2	1	1	2	2	1	6, 8
	<i>T. clavicaudatus</i>	2	2	1	2	1	2	3	2	1	5
	<i>T. sanwali</i>	2	2	1	2	2	1	2	2	3	-
7	<i>T. agri</i> *	2	2	2	1	1	1	2	1	1	-
	<i>T. alami</i>	2	2	2	1	1	1	2	1	1	-
	<i>T. ewingi</i>	2	2	2	1	1	1	2	1	1	-
8	<i>T. nudus</i> *	2	2	2	1	1	2	2	1	1	-
	<i>T. bambusi</i>	2	2	2	1	1	2	2	1	2	-
	<i>T. kashmirensis</i>	2	2	2	1	2	1	1	2	1	-
	<i>B. equatorialis</i>	2	2	2	2	1	2	3	2	2	5
	<i>T. neoclavicaudatus</i>	2	2	2	3	1	2	2	1	2	5
	<i>T. clavus</i>	2	3	1	1	1	2	2	1	1	5
	<i>T. silvaticus</i>	2	3	1	1	1	2	2	1	1	1, 8
	<i>T. botrys</i>	2	3	2	3	2	2	3	2	2	5
	<i>T. contractus</i>	3	1	1	1	1	1	2	1	2	3
9	<i>T. aerolatus</i>	3	1	1	1	1	1	2	1	1	-
	<i>T. brassicae</i> *	3	1	1	1	1	1	2	1	1	-
	<i>T. clarus</i> *	3	1	1	1	1	1	2	1	3	-
	<i>T. nordiensis</i>	3	1	1	1	1	1	2	1	1	-
	<i>T. shivanandi</i> *	3	1	1	1	1	1	2	1	3	-
	<i>T. spinaceae</i> *	3	1	1	1	1	1	2	1	1	-
	<i>T. variannus</i>	3	1	1	1	1	1	2	1	3	-
	<i>T. striatus</i>	3	1	1	1	1	2	2	1	1	-
10	<i>T. crotoni</i>	3	1	1	1	2	1	2	2	3	-
	<i>T. leucaenus</i>	3	1	1	1	2	1	2	2	3	-
	<i>T. variannulatus</i>	3	1	1	2	2	1	2	1	1	8
	<i>T. minutus</i>	3	1	1	3	1	1	2	1	3	-
	<i>B. usmanensis</i> *	3	1	1	3	2	1	2	1	2	3
11	<i>T. fatimae</i>	3	1	1	3	2	1	2	1	1	-
	<i>B. quaidi</i>	3	1	1	3	2	1	2	1	1	-
	<i>T. mangiferae</i>	3	1	1	3	2	1	2	2	1	-
	<i>B. zambiensis</i>	3	1	2	2	1	1	2	1	1	-
	<i>B. queirozi</i>	3	1	2	2	1	1	2	2	2	4
	<i>B. ventrosignatus</i> *	3	1	2	2	2	1	2	1	2	3
	<i>T. subcylindricus</i>	3	1	2	2	2	1	2	1	2	-
	<i>B. swarupi</i>	3	1	2	2	2	1	2	2	2	-
	<i>B. cuticaudatus</i>	3	1	2	2	2	2	2	2	2	-
	<i>B. paracanalisis</i>	3	1	2	3	2	1	2	1	3	8

Table 1. Continued

	<i>B. capsicum</i>	3	1	2	3	1	2	2	1	2	-
12	<i>T. chirchikensis</i>	3	2	1	1	1	1	2	1	1	-
	<i>T. projectus</i>	3	2	1	1	1	1	2	1	1	-
	<i>T. zaeae*</i>	3	2	1	1	1	1	2	1	1	-
	<i>T. paulettae</i>	3	2	1	1	1	1	2	1	1	6
	<i>T. irregularis</i>	3	2	1	1	1	1	2	2	1	8
13	<i>T. dactylurus</i>	3	2	1	1	1	2	2	1	2	-
	<i>T. georgiensis*</i>	3	2	1	1	1	2	2	1	3	-
	<i>T. idahoensis</i>	3	2	1	1	1	2	2	1	3	-
	<i>T. aduncus*</i>	3	2	1	1	2	1	2	1	1	6
	<i>T. graciliformis*</i>	3	2	1	1	2	1	2	1	1	-
	<i>T. ismaili</i>	3	2	1	1	2	1	2	2	3	-
	<i>T. ebriensis</i>	3	2	1	2	1	1	2	1	1	-
	<i>T. eremicolus</i>	3	2	1	2	1	1	2	2	1	-
	<i>T. digitatus</i>	3	2	1	2	1	2	2	1	3	-
	<i>B. erevanicus</i>	3	2	1	3	1	2	2	2	3	7
	<i>T. areoterminalis</i>	3	2	1	3	1	2	3	2	3	5
	<i>T. hordei*</i>	3	2	1	3	2	2	2	1	2	-
	<i>T. kamlae</i>	3	2	2	1	1	1	3	1	3	-
	<i>B. colombianus</i>	3	2	2	2	1	1	2	2	1	-
	<i>B. malinus</i>	3	2	2	2	1	2	2	2	2	5
	<i>B. iphilus*</i>	3	2	2	2	1	2	3	2	1	-
	<i>B. pratensis</i>	3	2	2	2	2	2	2	2	2	1,8
	<i>B. bryobius</i>	3	2	2	3	1	1	2	2	2	1,8
	<i>T. bursifer</i>	3	3	1	1	1	1	2	1	1	7
	<i>S. velatus</i>	3	3	1	1	1	1	2	2	3	4,8
	<i>T. tarjani</i>	3	3	1	1	1	2	2	1	1	8
	<i>T. cylindricus*</i>	3	3	1	1	2	1	2	1	1	-
	<i>T. bicaudatus</i>	3	3	1	2	2	2	2	1	3	-
	<i>T. antarcticus</i>	3	3	1	3	1	1	2	2	1	6
	<i>T. latus*</i>	4	1	1	1	2	1	2	1	3	-
	<i>T. persicus</i>	4	1	1	2	2	1	2	1	3	-
	<i>B. goffarti*</i>	4	1	2	2	2	1	2	1	2	3
<i>B. brevilineatus*</i>	4	1	2	2	2	1	2	1	2	2,3	
	<i>B. mediocris</i>	4	1	2	3	1	1	2	1	2	-
14	<i>B. huesingi</i>	4	1	2	3	1	2	2	2	2	8
	<i>B. parvus*</i>	4	1	2	3	1	2	2	2	2	8
	<i>B. parvulus*</i>	4	1	2	3	1	2	2	2	2	3,8
	<i>B. teeni*</i>	4	1	2	3	1	2	2	2	2	8
	<i>B. hispaniensis</i>	4	1	2	3	1	2	2	2	2	8

Table 1. Continued

	<i>T. iranensis</i> *	4	1	2	3	1	2	2	2	1	8
	<i>B. vulgaris</i> *	4	1	2	3	2	1	2	1	2	-
	<i>B. singularis</i>	4	1	2	3	2	2	2	1	3	-
	<i>B. depressus</i>	4	1	2	3	2	2	2	2	1	3
	<i>T. shimizui</i>	4	2	1	1	1	1	2	1	3	-
	<i>T. casigo</i>	4	2	1	2	1	2	2	2	2	5,8
	<i>T. teres</i>	4	2	1	2	2	2	3	1	1	1
	<i>T. mediterraneus</i>	4	2	1	3	1	1	3	2	1	1,8
	<i>T. manubriatus</i> *	4	2	1	3	1	2	2	1	3	-
	<i>B. tobari</i>	4	2	1	3	2	1	3	1	2	3
	<i>B. kidwaii</i>	4	2	2	2	1	2	1	2	2	1,8
15	<i>B. canalis</i>	4	2	2	3	1	2	2	2	1	1,8
	<i>B. serranus</i> *	4	2	2	3	1	2	2	2	2	8
	<i>B. natalensis</i>	4	2	2	3	1	2	2	2	3	-
	<i>B. dubius</i> *	4	2	2	3	2	2	2	2	2	3,8
	<i>T. eroshenkoi</i>	4	3	1	1	1	2	1	1	3	-
	<i>T. siccus</i>	4	3	1	1	2	1	2	2	1	4,8
	<i>T. kegenicus</i>	4	3	1	3	1	2	3	1	1	1,8
	<i>T. tenuicauda</i>	4	3	1	3	2	1	3	2	3	6,8
16	<i>S. maximus pakistanensis</i>	4	3	2	3	1	2	2	2	3	1
	<i>S. maximus</i> *	4	3	2	3	1	2	2	2	2	1,8
	<i>S. pamiricus</i>	4	3	2	3	2	2	2	2	3	1
	<i>S. labiodiscus</i>	4	3	2	3	2	2	2	2	3	1,8

\*: Reported from Iran (according to Ghaderi *et al.*, 2018b; Ghaderi, 2020 and Hosseinvand *et al.*, 2020).

### Group 3

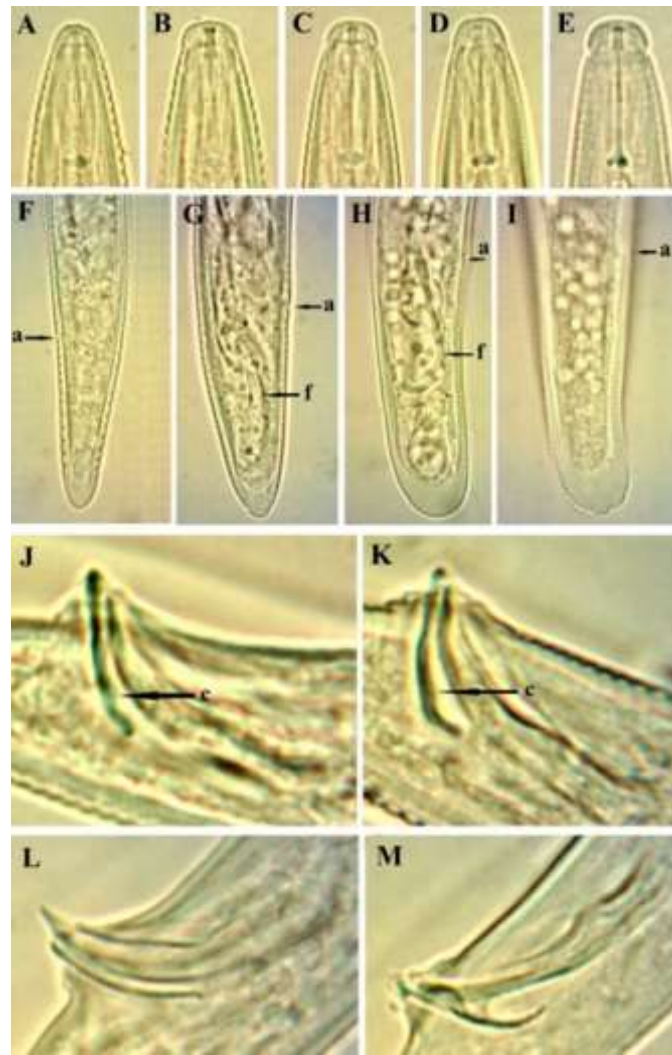
*Tylenchorhynchus mashhoodi* group (10 species: *T. bohrrensis*, *T. elamini*, *T. elegans*, *T. haki*, *T. iarius*, *T. mashhoodi*, *T. qasimii*, *T. rudis*, *T. tritici* and *T. varicaudatus*). *Tylenchorhynchus tritici* has a slightly shorter stylet compared to other nine species (12.5-14.5 vs. 14.0-19.0  $\mu$ m). Males have not been recovered for three species; however, spermatheca described as having sperm in *T. elamini*, no sperm for *T. varicaudatus*, and unknown (not mentioned nor illustrated) for *T. haki*. However, Siddiqi (2008) discussed that *T. rudis* can be differentiated from *T. mashhoodi* by smaller body and stylet, presence of intestinal fasciculi, and different structure and length of spicules.

### Group 4

*Tylenchorhynchus lucknowensis* group (two species: *T. gossypii* and *T. lucknowensis*). The only observed difference is the slightly more number of tail annuli in *T. lucknowensis* (19-23 vs. 15-17 in *T. gossypii*) and lateral fields marked by peculiar oblique striae.

### Group 5

*Tylenchorhynchus aspericutis* group (two species: *T. aspericutis* and *T. microconus*). The value of *c* ratio of *T. microconus* is slightly higher (17-24 vs. 14-17) and cuticular annuli at mid-body are coarser (1.6-1.8 vs. 2.7  $\mu$ m). The males of *T. microconus* have not been found in the original description.



**Fig. 1.** Some important diagnostic characters of the genera *Bitylenchus*, *Sauertylechus*, and *Tylenchorhynchus*. The number of transverse annuli on lip region: vary from without (A) to more than six fine annuli (D); Lip region demarcation: continuous or slightly offset (A-D) to distinctly offset (E); Tail shape: conical to subcylindrical (F, G) to cylindrical (H, I); Tail terminus striation: smooth (F-H) or striated (I); Post-rectal sac: absent (F) or present (G, H); Gubernaculum: having (J, K) or lacking (L, M) crest. (a = anus; f = intestinal fasciculi; c = gubernaculum crest).

### Group 6

*Tylenchorhynchus annulatus* group consists of five species (*T. anchorastyletus*, *T. annulatus*, *T. badliensis*, *T. coffeae* and *T. mexicanus*). Male has never been found for *T. annulatus*, even in populations with very high number of females. However, males are common in the other four species. *Tylenchorhynchus mexicanus* has a shorter tail (average of  $c'$  nearly 2 vs. 2.5 in *T. anchorastyletus* and more than 3 in *T. coffeae* and *T. badliensis*) and slightly longer stylet than *T. anchorastyletus*, *T. badliensis*, and *T. coffeae* (19-23 vs. 17-20  $\mu\text{m}$ ). *Tylenchorhynchus anchorastyletus* can be differentiated by the refractive cephalic framework. *Tylenchorhynchus badliensis* is very similar to *T. coffeae* with minor differences in the number of lip

region annuli (3 vs. 2) as well as in the body (0.62-0.71 vs. 0.57-0.63 mm) and spicules lengths (20-22 vs. 23-24  $\mu\text{m}$ ).

### Group 7

*Tylenchorhynchus ewingi* group (three species: *T. agri*, *T. alami* and *T. ewingi*). *Tylenchorhynchus alami* is distinguishable from the other species by longer tail ( $c' = 2.8-4.1$  vs. 2.1-2.6 in *T. agri* and *T. ewingi*), smaller post-rectal sac (filling less than 20 % vs. about 45-70 % of the tail length) and slightly narrower tail terminus in females. Likewise, tails of females in *T. agri* and *T. ewingi* have similar shape with a comparable number of cuticular annuli (16-26 and 15, respectively). However, the length of spicules could be considered as distinguishing character (21-25 vs. 18  $\mu\text{m}$ , respectively).

### Group 8

*Tylenchorhynchus nudus* group (two species: *T. bambusi* and *T. nudus*). Both species differ only in terms of tail shape (cylindrical to slightly clavate vs. distinctly clavate).

### Group 9

*Tylenchorhynchus clarus* group (seven species: *T. aerolatus*, *T. brassicae*, *T. clarus*, *T. nordiensis*, *T. shivanandi*, *T. spinaceae*, and *T. variannus*). Spermatheca is non-functional and males are absent or very rare in *T. clarus* and *T. variannus*, but spermatheca is functional in the other five species and males commonly occur except in *T. shivanandi*. Ghaderi and Karegar (2016) synonymized *T. variannus* Mavlyanov, 1978 with *T. clarus*, since they found individuals in a *T. clarus* population from Iran with combined annuli on the ventral side of the tail to form larger-sized but fewer annuli. Among the other five species, *T. nordiensis* has typically smaller stylet (11-13 vs. 15-19  $\mu\text{m}$ ), and *T. aerolatus* has a tail with broadly rounded terminus (narrower in the other species), areolated lateral fields (vs. non-areolated), and unique shape of gubernaculum. *Tylenchorhynchus shivanandi*, *T. brassicae* and *T. spinaceae* are very similar together; however, *T. shivanandi* has a slightly different by tail terminus (flattened vs. conoid) and somewhat longer tail ( $c' = 3.1-4.0$  vs.  $1.7-3.7$ ) than *T. brassicae*, and *T. spinaceae* only has larger spicules (21-26 vs. 18-21  $\mu\text{m}$ ) than *T. brassicae*. Two species from the *T. zae* group, namely *T. chirchikensis* and *T. zae* significantly resemble and have comparable but slightly longer stylets than those found in the *T. clarus* group.

### Group 10

*Tylenchorhynchus leucaenus* group (two species: *T. crotoni* and *T. leucaenus*). Pharyngeal median bulb is slightly anterior in *T. leucaenus* (MB = 47 vs. 53 % in *T. crotoni*). Spermatheca filled with sperm in *T. crotoni*, but the males have not been recovered. Males of *T. leucaenus* have the smallest spicules in the genus (13.5  $\mu\text{m}$ ). Only minor differences in the stylet and tail length are observed between *T. ismaili* and *T. leucaenus*.

### Group 11

*Bitylenchus quaidi* group (two species: *T. fatimae* and *B. quaidi*). Besides of diagnostic characters, both species have also similar ranges of morphometric

characters, but in *T. quaidi*, the lip region is sunken and dome-shaped (vs. hemispherical), the lateral fields are areolated (vs. non-areolated), and the vulva is not covered with a lateral membrane.

### Group 12

*Tylenchorhynchus zae* group (three species: *T. chirchikensis*, *T. projectus* and *T. zae*). These three species are very similar with slight differences in their body lengths (530-640, 620-700 and 700-780  $\mu\text{m}$  in *T. zae*, *T. chirchikensis* and *T. projectus*, respectively).

### Group 13

*Tylenchorhynchus dactylurus* group (three species: *T. dactylurus*, *T. gerogiensis* and *T. idahoensis*). *Tylenchorhynchus gerogiensis* can be distinguished from the other members of group 13 by a shorter tail ( $c' = 2.5$  vs. more than 3) with fewer annuli (8-10 vs. 21-28) and slightly posterior position of the vulva (57-61 vs. 51-55). *Tylenchorhynchus idahoensis* has slightly longer stylet (20-23 vs. 19-21  $\mu\text{m}$ ) and body (730-830 vs. 640-710  $\mu\text{m}$ ) length than *T. dactylurus*. Males have not been mentioned, and spermatheca has been empty in the latter species.

### Group 14

*Bitylenchus parvus* group (five species: *B. huesingi*, *B. parvus*, *B. parvulus*, *B. teeni*, and *B. hispaniensis*). *Bitylenchus parvulus* differs by tail tip, vulva region and molecular phylogenetic position in the 18S and 28S rRNA phylogenetic trees (Hosseinvand *et al.* 2020). The characterization of this group remains challenging since they commonly occur in the collected soil samples, and classifying their populations into one of the above species is usually a confusing task for researchers. A detailed morphological and molecular work is needed to address the taxonomic problems in the group. The other very similar species, *B. dubius*, has a different position in the present grouping due to having a distinctly offset lip region.

### Group 15

*Bitylenchus canalis* group (three species: *B. canalis*, *B. serranus*, and *B. natalensis*). *Bitylenchus natalensis* has a slightly longer tail ( $c' = 3-4$  vs. 2-3) from other species of this group, and *B. serranus* has a shorter post-rectal sac filling up to one-fourth of the tail length (vs. almost total length in the other two species).

### Group 16

*Sauertylenchus maximus* group (two species: *S. maximus* and *S. maximus pakistanensis*). These two species are very similar concerning the morphological and morphometric characters. *Sauertylenchus ibericus* is very close to *S. maximus* and differs only by tail terminus annuli (as wide as vs smaller than other tail annuli). Considering morphological and molecular evidence, Hosseinvand *et al.* (2020) synonymized *S. ibericus* with *S. maximus*.

### Concluding remarks

Several morphological and molecular studies (Handoo *et al.* 2014; Hosseinvand *et al.* 2020) well support the genera *Bitylenchus*, *Sauertylenchus*, and *Tylenchorhynchus* as three valid separate genera. In the present study and the previously published work (Hosseinvand *et al.* 2020), we tried to classify all the described species into one of the genera based on morphological diagnostic characters. However, as discussed by Hosseinvand *et al.* (2020), original descriptions of several species have not provided detailed morphological information, particularly on the structure of gubernaculum, post-rectal sac, lip region, or lateral fields. In the other words, the exact taxonomic position of certain species is still questionable. Therefore, certain species from one genus may come

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close to certain species from the other genus, as can be seen for *B. quaidi* and *T. fatimae*.

Although some works have focused on the taxonomy of these closely related genera using morphological or molecular approaches, more detailed studies are needed to clarify the accurate taxonomic position of certain problematic members in these genera. Taking together, it is frequently clear that detailed morphological studies on certain characters particularly gubernaculum structure, SEM observations on the lip region face view, as well as molecular data are essential in advancing these genera taxonomy. Conducting integrative taxonomic works by using those approaches could resolve some problems in the taxonomy of the stunt nematodes, hopefully.

### Conflict of interest

None conflict of interest between authors.

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