



## Notes on the taxonomic position of *Longibulbophora* Yeates, 1967 and *Brachynemella* Cobb, 1933 (Nematoda: Diphtherophoridae)

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### Abstract

The present work is a compilation of the systematics of the two rare genera of Diphtherophoridae including *Longibulbophora* (with two species; *L. ammophilae* and *L. rotundicauda*) and *Brachynemella* (with one species; *B. obtusa*). Morphometric data, descriptions of females and males, relationships with close species, and distribution (associated plants and locality) of the three species are discussed. Furthermore, photomicrographs are provided for type specimens of *L. ammophilae*. The family Diphtherophoridae should be reassigned to include only two genera, namely *Diphtherophora* and *Tyloaimophorus*. The position of amphid apertures and the presence of prerectum exclude the genus *Brachynemella* from the family Diphtherophoridae. The genus *Longibulbophora* is no longer justified as a valid taxon, as *L. rotundicauda*, the type species, should be considered as *species et genera indeterminate*, and the second species, *L. ammophilae*, is proposed here to be a member of the genus *Diphtherophora*.

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### Introduction

The family Diphtherophoridae Micoletzky, 1922 currently contains three valid genera with 52 described species (Ghaderi & Asghari, 2019; Ghaderi et al., 2020): *Diphtherophora* de Man, 1880 (37 valid species), *Longibulbophora* Yeates, 1967 (two valid species), and *Tyloaimophorus* de Man, 1880 (13 valid species). Andrásy (2009) mentioned that these nematodes are soil inhabitants that occur mostly in the vicinity of plant

roots. However, some species may also occur in aquatic habitats as well (Andrásy, 2009).

Members of the family Diphtherophoridae have a characteristic buccal cavity (stoma) which is a complex structure bearing a complicated system of plates and rods which serve as a dome-shaped guiding apparatus for the massive scleroized spear. While stylet knobs in *Tyloaimophorus* are similar in size and shape, those in the genera *Diphtherophora* and *Longibulbophora* show an assymetrical appearance by distinctly larger dorsal

knobs (Andrássy, 2009; Ghaderi & Asghari, 2019). The genus *Longibulbophora* is very similar to *Diphtherophora* but it has been differentiated by having a long pharyngeal basal bulb. Yeates (1967) designated the species *Tyloaimophorus rotundicauda* Paesler, 1955 as type species of a new genus, *Longibulbophora* and described another new species (*L. ammophilae*). Brzeski (1994) considered both species as *species et genera indeterminate*. Andrássy (2009) discussed that these two species may not be congeneric with each other. The other genus, *Brachynemella* (Cobb, 1893) Cobb, 1933 was insufficiently described based on two specimens without illustrations; thus, it was considered as *genus inquirendum* by Andrássy (2009). The present study was conducted to examine the correct taxonomic positions of these rarely occurred nematodes by documenting literature information as well as detailed observations on the deposited type specimens in the international collections.

## Methods

To provide a systematic view on the two rare genera in the family Diphtherophoridae, namely *Longibulbophora* and *Brachynemella*, descriptions, morphological and morphometric characters of the known species were studied and documented here. For each species, taxonomic information including morphometric data, amended description of females and males, taxonomic remarks on the species and its comparison with closely related species, as well as worldwide distribution of the species are provided. Morphological observations were made based on the specimens of *L. ammophilae* deposited in the Rothamsted Nematode Collection, FERA, UK. Photomicrographs were prepared on different body regions particularly those parts with taxonomic importance showing diagnostic characteristics (Fig. 1). Certain diagnostic characters were measured from the drawings in the original descriptions of the species which are given in parentheses. The measurement indices and ratios used in the study are based on Ghaderi & Asghari (2019).

## Results

### Taxonomy

Phylum Nematoda Potts, 1932

Class Enoplea Inglis, 1983

Subclass Enoplia Pearse, 1942

Order Triplonchida Cobb, 1920

Suborder Diphtherophorina Coomans & Loof, 1970

Superfamily Diphtherophoroidea Micoletzky, 1922

Family Diphtherophoridae Micoletzky, 1922

### ***Longibulbophora ammophilae* Yeates, 1967**

(Fig. 1)

*Measurements.* After Yeates (1967)

Holotype female: L = 0.78 mm; a = 14; b = 6.0; spear = 18 µm; c = 34; V = 45.

12 paratype females: L = 0.69-0.85 mm; a = 12-16; b = 4.3-7.7; body width = (55) µm; spear = 17-18 µm; pharynx = (170) µm; tail = (30) µm; c = 32-42; c' = (0.8); V = 42-49.

*Description* (amended from the original description)

*Female.* Body stout and cylindrical, tapering slightly at anterior end. Cuticle with fine transverse striation. Lip region offset from the body contour, but no papillae visible in lateral view (Fig. 1B, C). In *en face* view, two circles of papillae can be observed: the inner circle contains six very small papillae, and the outer with 10 tiny papillae or possibly depressions; around the oral aperture six tiny liplets appear to be present, one in each quadrant and two dorsally. Amphidial aperture oval in shape, as wide as one-third of the head width at base (Fig. 1B). Spear with well-developed basal knobs; with a protuberant dorsal and a simple ventral sector; arch-like nature of dorsal protuberance only apparent in fixed specimens, not visible in live individuals. Spear guiding structure well marked and dorylaimoid in live specimens, but in fixed material three cuticular plates apparently, constitute a guiding structure. Anterior part of pharynx slender, encircled by nerve ring and with a small gland posterior to it. Posterior part occupying two-third of total pharynx length, in the form of a distinct botuliform (sausage shaped) bulb with three distinct gland nuclei. Secretory-excretory pore at about the level of the beginning of basal bulb. Intestine granular in appearance. Ovaries paired, reflexed, the posterior tract usually more developed than the anterior one. Sperm cells with oval-shaped nuclei and distributed throughout the uteri (Fig. 1D). Vulva pore-like; vagina one-third to one-fourth of the vulval body width. Rectum usually obscure, but a half of the anal body diameter in some fixed specimens. An egg in fixed specimen 79 × 36 µm, with a finely sculptured surface. Tail short, with rounded terminus, slightly digitate (Fig. 1E, F). Juveniles similar to females, but possessing a secondary spear tip, pyriform pharyngeal basal bulb, and a rectum as long as anal body diameter.

*Male.* Not found.

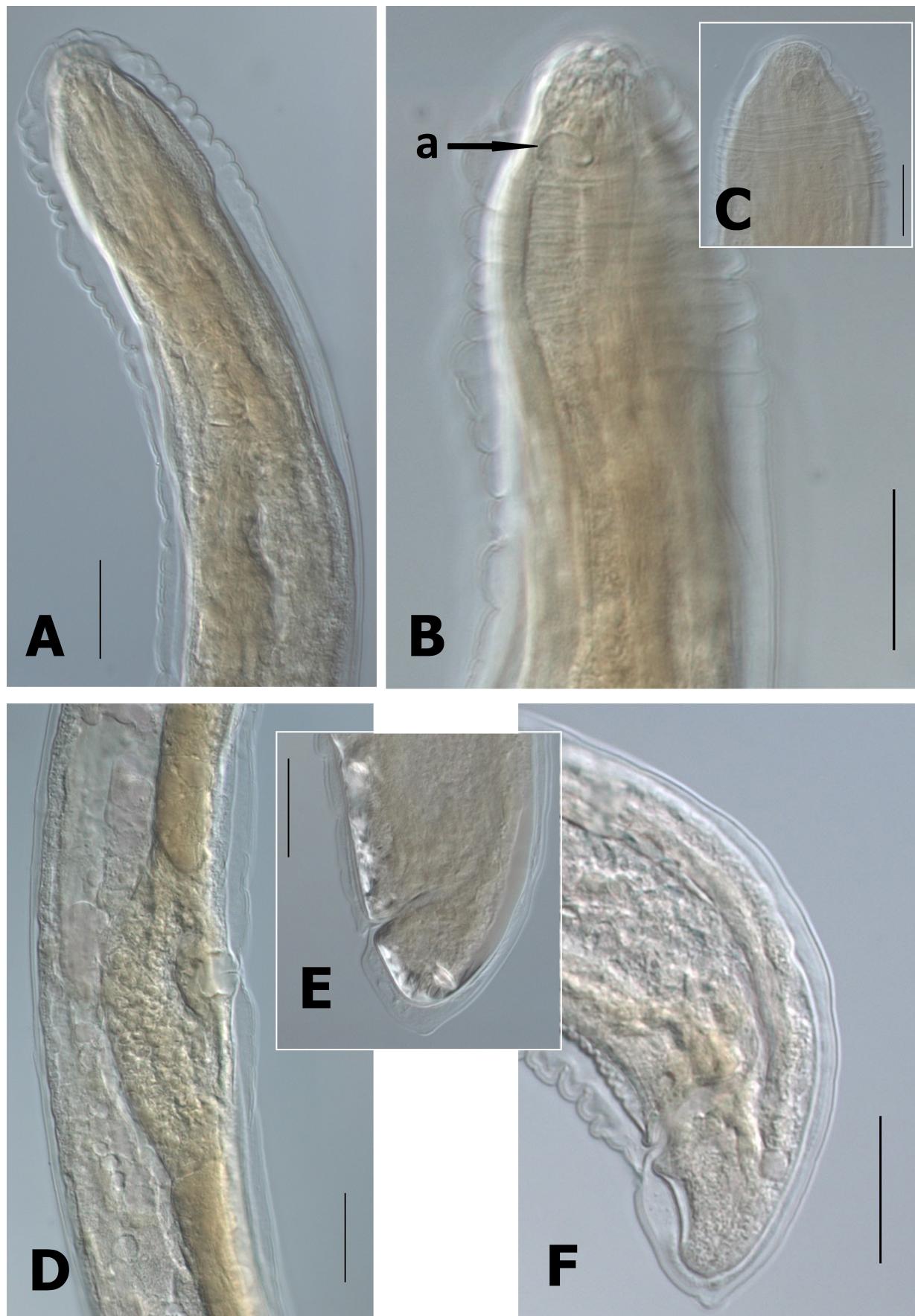
*Diagnosis and relationships.* *L. ammophilae* has been differentiated from *L. rotundicauda* by its longer body (0.69-0.85 vs 0.37-0.43 mm) and having a differentiation between dorsal and ventral sectors of the spear (in *L. rotundicauda*, dorsal and ventral sectors are similar).

*Distribution.* Described from the rhizosphere of a grass, *Ammophila arenaria* L., in Himatangi Beach, Manawatu, New Zealand (Yeates, 1967).

### ***Longibulbophora rotundicauda* (Paesler, 1955) Yeates, 1967**

*Measurements.* After Paesler (1955)

? paratype females: L = 0.37-0.43 mm; a = 21-24; b = 4.2-4.4; body width = (17-20) µm; spear = (16) µm; phar-



**Fig. 1.** Photomicrographs of the type specimens of the females of *Longibulbophora ammophilae* deposited in the Rothamsted Nematode Collection, FERA, UK. A: Anterior end and pharyngeal region; B, C: Lip region; D: Vulva and part of reproductive system; E, F: Posterior end. (Scale-bars = 20  $\mu$ m). [a = amphidial aperture].

ynx = (88-98)  $\mu\text{m}$ ; tail = (15-18)  $\mu\text{m}$ ; c = 24; c' = (1.3-1.5); V = 52-54.

1 paratype male: L = 0.38 mm; a = 22; b = 4.5; tail = (18)  $\mu\text{m}$ ; c = 21; c' = ?; spicules = (15)  $\mu\text{m}$ .

**Description** (amended from the original description and Yeates, 1967)

**Female.** Body slender, cylindrical. Lip region equipped with two circles of papillae, internal papillae small, but external ones setiform. Spear length about one-fifth or one-sixth of pharynx; with symmetrical basal knobs. Nerve ring surrounding the base of the slender part of pharynx. Expanded part of pharynx composed of two offset parts, the posterior with a sclerotized chamber inside. Reproductive system covered with dark granules of the intestine. Tail cylindrical with a rounded terminus.

**Male.** Capsule musculature weakly sclerotized. One supplement located anterior to the cloaca. Spicules large, slightly curved ventrally. Tail similar to that of female.

**Diagnosis and relationships.** *L. rotundicauda* can be compared with *L. ammophilae*; see the discussion of that species. In justifying his separation of *L. rotundicauda* from *Tyloolaimophorus typicus* de Man, 1880, Paesler (1955) stated that these two species share their characteristics for vulva position and the shape of pharynx as a pseudo-bulb can be observed before the wide part of pharynx. However, the original description of *T. typicus* and several other studies (Thorne, 1939; Goodey, 1951; Brzeski, 1994) referred to a pyriform basal bulb in this species without any abnormal section. The length of spicules was surprisingly given as 66  $\mu\text{m}$  (one-sixth to one-seventh of body length) in Ivanova (1977), but it appears as about 15  $\mu\text{m}$  when it compared with tail length in the given drawing.

**Distribution.** Described from the rhizosphere of walnut trees in Germany (Paesler, 1955).

#### ***Brachynemella obtusa* (Cobb, 1893) Cobb, 1933**

= *Brachynema obtusa* Cobb, 1893

**Measurements.** Not given in the related descriptions.

**Description** (amended from Thorne, 1939)

**Female.** Cuticle apparently without striation. Head rounded. Spear resembling that of *Tylenchus*. Six short setae on head margin. Apparently six lips with six small papillae around mouth aperture. Circular amphids one spear length below the base of the spear. Dorsal swelling of stout spear larger and longer than ventral one. Three oxbow-shaped guiding to spear each one third the spear length. Muscular swelling surrounding spear three times as long, and one half as wide, as the head. Anterior part of pharynx one-fourth the neck width, the basal enlarged portion one fourth as long, and two-thirds as wide as the neck. Intestine coarsely granular, two thirds the body width. Rectum length one- and one-half times the anal body diameter. Prerectum twice as long as the rectum. Lateral cords one fourth

as wide as the body. Ventral secretory-excretory pore halfway between nerve ring and cardia. Vulva probably near the middle of body and ovaries double and symmetrical. Tail conoid-hemispherical with rounded terminus.

**Male.** Unknown.

**Diagnosis and relationships.** There is no adequate information on the diagnostic characters to make a comparison between this species and other species of Diphtherophoridae. However, two morphological features of this species, the position of amphid apertures posterior to spear knobs and having a prerectum, are incongruent with those of the family Diphtherophoridae (Andrássy, 2009).

**Distribution.** Recovered from virgin soil from hills opposite Harwood, New South Wales, Australia (Cobb, 1893).

#### **Notes on the taxonomic position of the species**

***Brachynemella obtusa*.** The genus *Brachynemella* (Cobb, 1893) Cobb, 1933 is an obscure genus of doubtful position which was published based on two specimens. Thorne (1939) noticed that the description of the type species contains all known diagnostic information. However, the description is lacking measurements and illustrations. Andrássy (2009) considered *Brachynemella* as genus *inquirendum*, and we accept his opinion here.

***Longibulbophora rotundicauda*.** Goodey (1963) noted that the structure of the posterior part of the pharynx and the form of spicules cast doubt on the correct position of *Tyloolaimophorus rotundicauda* Paesler, 1955, and maybe this species should be placed in a new genus. Yeates (1967) designated the species *T. rotundicauda* as type species of his new genus, *Longibulbophora*. Brzeski (1994) noted that the description of *L. rotundicauda* does not clarify the structure of the anterior end; for this reason, he considered them as *species et genera indeterminate*. However, Andrássy (2009) pointed out that Paesler's species may not be congeneric with *L. ammophilae*. In the illustrations of the original description (Paesler, 1955), the pharynx seems to be composed of two offset sections: an anterior pyriform and a posterior cylindrical. Andrássy remarked that the pharynx most likely terminated in the usual pyriform bulb, but the posterior section belongs in reality to the intestine. He concluded if this is true, then *Longibulbophora* should be considered a synonym of *Diphtherophora*, and the species of Yeates would be provided with a new genus name.

In our opinion, *L. rotundicauda* differs also from all species in Diphtherophoridae by having distinct outer labial sensilla in the form of setae. Moreover, the structure of pharynx is rather unusual; thus, we accept Brzeski's opinion about the unknown taxonomic position of this species.

***Longibulbophora ammophilae*.** This species could only be distinguished from *Diphtherophora* species by its

expanded part of pharynx as cylindrical form, occupying nearly half of pharynx length, as stated in Andrásy (2009). However, certain *Diphtherophora* species have also a cylindrical expanded part of pharynx with more or less comparable length, as can be seen in *D. caudata* Ivanova, 1977, *D. crustosa* Eroshenko & Tepljakov, 1977, *D. kazhachstani* Razjivin, 1971, *D. kirjanovae* Ivanova, 1958, *D. malkovi* Nedelchev & Choleva, 1989, *D. minuta* Ivanova, 1958, *D. papillata* Eroshenko & Tepljakov, 1977, and *D. vanoyei* De Coninck, 1931.

Observations on type specimens of *L. ammophilae* deposited in the Rothamsted Nematode Collection, FERA, UK revealed some information on the shape of cuticle, lip region, amphid structure and position, and tail characteristics; however, the specimens were in an undesirable condition and the detailed structure of spear and pharynx cannot be described (Fig. 1). Drawings and description of the type population (Yeates, 1967) fit well with characteristics of the genus *Diphtherophora*. As there is no stable character allowing separation of *L. ammophilae* from *Diphtherophora* species, we propose that this species to be transferred to the genus *Diphtherophora* and named as *D. ammophilae* (Yeates, 1967) comb. n. Based on the identification keys for *Diphtherophora* species (Ghaderi *et al.*, 2017; Ghaderi & Asghari, 2019), this species differs from all species with transversed cuticle by having a larger body size (690-850 vs 400-590 µm).

## Discussion

In the present study, the taxonomic position of certain rare members of the family Diphtherophoridae was discussed based on documenting literature data and morphological observations on type specimens. The family Diphtherophoridae should be reassigned to include only two genera, namely *Diphtherophora* and *Tylo-laimophorus*. The position of amphid apertures and the presence of prerectum exclude the genus *Bracynemella* from the family Diphtherophoridae, but its destination could not be determined by present information, so in accordance with Andrásy's opinion, it should be considered as *genus inquirendum*. On the other hand, the genus *Longibulbophora* is no longer justified as a valid taxon, as its type species should be considered as *species et genera indeterminate*, and the second species is proposed here to be a member of the genus *Diphtherophora*.

## Taxonomy

Phylum Nematoda Potts, 1932

Class Enoplea Inglis, 1983

Subclass Enoplia Pearse, 1942

Order Triplonchida Cobb, 1920

Suborder Diphtherophorina Coomans & Loof, 1970

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### *Longibulbophora rotundicauda* (Paesler, 1955) Yeates, 1967

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*Male.* Capsule musculature weakly sclerotized. One supplement located anterior to the cloaca. Spicules large, slightly curved ventrally. Tail similar to that of female.

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### ***Brachynemella obtusa* (Cobb, 1893) Cobb, 1933**

**Diagnosis and relationships.** There is no adequate information on the diagnostic characters to make a comparison between this species and other species of Diphtherophoridae. However, two morphological features of this species, the position of amphid apertures posterior to spear knobs and having a prerectum, are incongruent with those of the family Diphtherophoridae (Andrassy, 2009).

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**Male.** Unknown.

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12 paratype females: L = 0.69-0.85 mm; a = 12-16; b = 4.3-7.7; body width = (55)  $\mu\text{m}$ ; spear = 17-18  $\mu\text{m}$ ; pharynx = (170)  $\mu\text{m}$ ; tail = (30)  $\mu\text{m}$ ; c = 32-42; c' = (0.8); V = 42-49.

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**Male.** Not found.

#### **Notes on the taxonomic position of the species**

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nearly half of pharynx length, as stated in Andrásy (2009). However, certain *Diphtherophora* species have also a cylindrical expanded part of pharynx with more or less comparable length, as can be seen in *D. caudata* Ivanova, 1977, *D. crustosa* Eroshenko & Tepljakov, 1977, *D. kazachstani* Razjivin, 1971, *D. kirjanovae* Ivanova, 1958, *D. malkovi* Nedelchev & Choleva, 1989, *D. minuta* Ivanova, 1958, *D. papillata* Eroshenko & Tepljakov, 1977, and *D. vanoyei* De Coninck, 1931.

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## Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors wish to thank Dr Thomas Prior (FERA, UK) for kindly providing light photographs of *Longibulbophora ammophilae* paratypes deposited in the Rothamsted Nematode Collection, FERA, UK.

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