



Marine free-living nematodes in semiarid inland waters

Nematoda marinho de vida livre em águas continentais no semiárido

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Abstract: Aim: This study reports the occurrence of five Nematoda genera previously considered as exclusively marine in inland waters. **Methods:** Sediment samples were taken, weekly, during nine weeks, at a small spring at the Horto Florestal Olho D'Água da Bica, Cuité, PB, ca. 130 km away from the shoreline, between March and May 2016 and fixed in 4% formalin. Nematodes were sorted by manual elutriation through sieves with 0.5 and 0.045 mm and slides were prepared. **Results:** The sediment was classified as coarse sand, with organic matter content ranging between 2.73 and 13.32%, temperature between 27-28°C and constant salinity of 6. From 315 nematodes were sampled among which 18 were from five genera previously thought to be exclusively marine were found. *Bolbolaimus* was the most common, found in 11.1% of the samples, followed by *Gomphionema* (3.7%), *Rynchonema* (7.4%), *Prorynchonema* (7.4%) e *Sabatieria* (3.7%), representing 5.7% from total of nematofauna. These genera are widely distributed worldwide, but had never been recorded from inland environments. **Conclusions:** This study contributes with the distributional knowledge of free-living nematodes. From literature data, these genera are not restricted by low salinity, and can be found in estuarine and/or brackish water environments and body water with direct connection to the sea. In any case, the presence of marine genera in this region which is >130 km away from the nearest shoreline is noteworthy, highlighting the importance of studying nematodes from these poorly known semiarid water bodies. These new occurrences raise questionings about the dispersal mechanisms that certified the colonization of these nematodes in the semiarid.

Keywords: meiofauna; nematofauna; distribution, taxonomy and ecology.

Resumo: Objetivo: Esse trabalho relata a ocorrência de gêneros de Nematoda, que eram previamente considerados restritos a ambientes marinhos, em águas continentais. **Métodos:** Amostras de sedimentos foram coletadas semanalmente, durante nove semanas, entre março e maio de 2016 no Horto Florestal Olho D'Água da Bica, Cuité, PB, cerca de 130 km da região costeira mais próxima e fixados em formol 4%. Os Nematoda foram separados por elutriação manual através de peneira de malha de 0,5 e 0,045 mm e lâminas foram confeccionadas. **Resultados:** O sedimento foi classificado como areia grossa, com teor de matéria orgânica variando entre 2,73 e 13,32%, temperatura entre 27-28°C e salinidade constante de 6. Dentre 315 nematoides coletados, 18 são de cinco gêneros marinhos. *Bolbolaimus* foi o mais frequente, presente em 11,1% das amostras, seguido de *Gomphionema* (3,7%), *Rynchonema* (7,4%), *Prorynchonema* (7,4%) e *Sabatieria* (3,7%), representando 5,7% do total da nematofauna. Esses gêneros têm ampla distribuição no mundo, mas não possuem registros prévios



em ambientes continentais, sendo a primeira ocorrência nesse estudo. **Conclusões:** Este trabalho contribuiu com o conhecimento da distribuição de Nematoda de vida livre. De acordo com os dados de literatura, esses gêneros não possuem restrições a baixas salinidades, sendo encontrados em regiões estuarinas e/ou águas salobras e também em corpos de água doce com conexão direta com o mar. Ainda assim, é notável a presença de gêneros marinhos há ~130 km de distância da costa, enfatizando a importância de estudos em diferentes corpos d'água no semiárido, permitindo questionar sobre quais mecanismos de dispersão permitiram esses indivíduos colonizar essa região.

Palavras-chave: meiofauna; nematofauna; distribuição; taxonomia e ecologia.

Free-living Nematoda are abundant in marine, freshwater and terrestrial environments (Bongers & Bongers, 1998; Bhadury et al. 2006; Leduc & Rowden, 2018). There are around 30,000 free-living nematode species currently described, with appraisals suggesting from 1 to 100 million species to be described (Lambshhead, 2003; Blaxter, 2011; Zhang, 2013; Smythe et al., 2019). Until the beginning of the last decade ~2270 genera from 256 families had been described worldwide (Hodda, 2011), with 78 genera and ~220 species recorded from Brazil (Venekey, 2017). Most of the studies dealing on aquatic free-living nematodes were performed on marine environments and inland ecosystems have been mostly neglected (Traunspurger et al., 2020). Although the number of studies on continental nematodes is growing, they are concentrated on the northern hemisphere, mostly in Europe (Abebe et al., 2008; Traunspurger et al., 2020). Studies dealing on inland water nematodes from Brazil are still very sparse, mostly available in grey literature with a few published papers (e.g., Lucena et al., 2015; Barros et al., 2020; Pinto et al., 2021). Currently, there are 132 genera of freshwater nematodes record on Brazil (Pinto et al., 2021), but this number is certainly underestimated considering both the high nematode diversity (e.g., Zhang, 2013) and the lack of studies. In the current study we report five nematode genera from inland waters which were previously known as exclusive from marine environments. These findings highlight the need of increasing the knowledge of the diversity of free-living nematodes from inland water bodies worldwide and particularly at Brazil.

The material was sampled between March and May 2016 in a permanent small spring at Horto Florestal Olho D'Água da Bica, at Cuité County (6°29'06"S; 36°09'24"W), Paraíba State, in the Brazilian semiarid, located ca. 130 km from the coastline. The reservoir has an area ~230 m², with 2 m of maximum depth, and is preserved by the Campina Grande Federal University (Universidade Federal de Campina Grande, UFCG).

Weekly samplings were performed between 24 March and 12 May 2016 in the margin of the pond.

At each of the nine weeks three replicate sediment samples were taken with a 7 cm² corer 10 cm deep. The material sampled was immediately fixed in 4% formalin. In addition, sediment samples were taken and frozen to perform standard granulometric and organic matter analyses in the laboratory (Suguio, 1973; Walkley & Black, 1934). Water temperature and salinity were measured using a digital (Alfakit, AT-160, Florianópolis, Brazil) and manual field refractometer, respectively. In the laboratory, the samples were manually elutriated in sieves with meshes of 0.5 and 0.045 mm. The material retained in the finer sieve was placed in Dollfus plates and taken to the compound microscope where all nematodes were sorted and permanent slides were made (following Cobb, 1917). The slides were taken to the optical microscope and the genera were identified following mostly Platt & Warwick (1983), Warwick et al. (1998) and Zullini (2010). Voucher slides with the genera reported here were deposited in the Coleção de Invertebrados Paulo Young (CIPY), from Paraíba Federal University (Universidade Federal da Paraíba – UFPB) – CIPY-Nema 5-9.

The organic matter content ranged from 2.73 and 13.32%, and sediment size from 0.8 to 0.97 mm being characterized as coarse sand (Suguio, 1973; Walkley & Black, 1934). Temperature was nearly constant, between 27-28°C and salinity had constant values of 6. From 315 nematodes sampled, 18 belong to five genera previously considered as exclusively marine (Table 1), and will be detailed in this study.

1. *Bolbolaimus* Cobb, 1920

Bolbolaimus has a strongly annulated cuticle, sometimes ornamented with dots. Head not set-off. Cephalic setae close to the front end. Amphideal fovea unispiral or cryptospiral. Buccal cavity strongly sclerotized, with large dorsal tooth, denticles may be present. Pharynx with anterior peribuccal bulb and posterior oval bulb. Copulatory apparatus strongly sclerotized and with gubernacular apophyses directed dorsally or dorsocaudally (Tchesunov, 2014b; Figure 1a).

Table 1. Free-living Nematoda genera reported in this study, their frequency of occurrence (FO; %), number of individuals sampled (Ind.) and environmental parameters of the samples containing them. MO = organic matter content (%); G = granulometry (mm); T = temperature (°C). Environmental parameters are presented as mean \pm standard deviation whenever appropriate; D.N= deposit number.

	<i>Bolbolaimus</i>	<i>Gomphonema</i>	<i>Rynchonema</i>	<i>Prorhynconema</i>	<i>Sabatieria</i>
FO	11.1	3.7	7.4	7.4	3.7
Ind.	4	4	2	5	3
MO	7.70 \pm 5.20	2.72	7.96 \pm 5.36	2 \pm 0.6	2.72
G	0.64 \pm 0.23	0.8	2 \pm 0.71	0.82 \pm 0.09	0.8
T	27.75 \pm 0.25	28	27.25 \pm 0.25	27.25 \pm 0.25	28
D.N	6	7	8	9	5

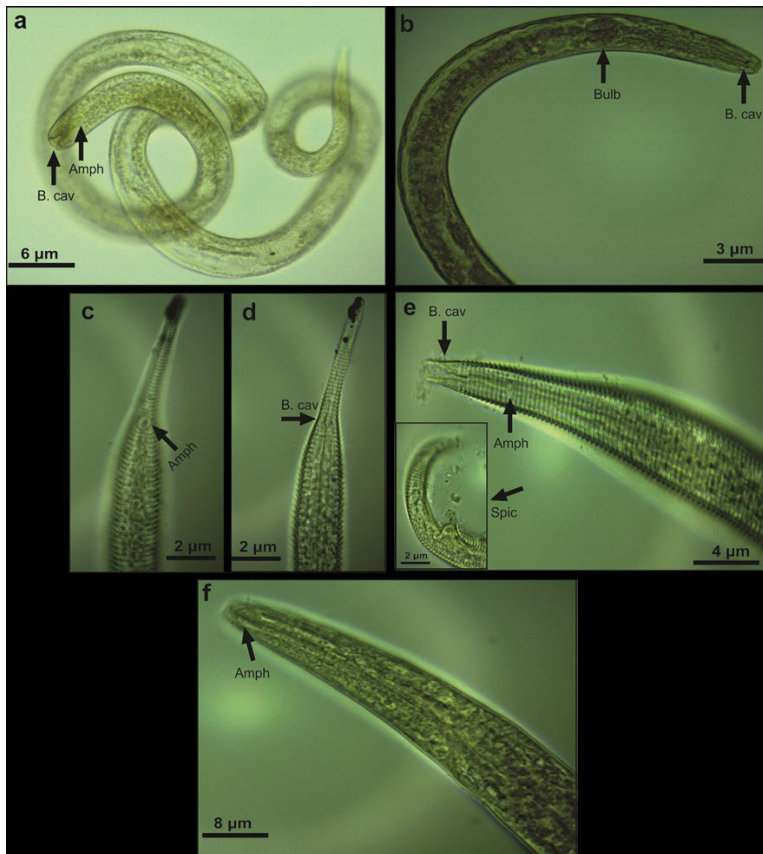


Figure 1. Free-Living Nematode genera in the Semi-Arid region. a= *Bolbolaimus*, b= *Gomphonema*, c-d=*Rynchonema*, e=*Prorhynconema*, f= *Sabatieria*. Amp= Amphidius; B. cav= Buccal Cavity; Bulb= Shape of pharyngeal bulb; Spic= Spicule.

This genus has been recorded between 66°N and 26°S (Muthumbi & Vincx, 1999; Kovalyev & Tchesunov, 2005; Hua et al., 2016; Leduc, 2016; Long et al., 2017). It currently has 11 valid species, all of them from marine environments (Bezerra et al., 2020), although it may also be found in estuarine waters in salinity between 7.0-7.5 (Long et al., 2017). This is the first record of *Bolbolaimus* from inland waters.

2. *Gomphonema* Wieser & Hopper, 1966

Gomphonema presents buccal cavity heavily cuticularized, consisting of an anterior concave

chamber and a cylindroconoid posterior portion, and armed with a massive dorsal tooth not entering the cheilostoma; subventral teeth absent. Pharyngeal bulb large, “barrel-shaped”, occupying approximately one-third of the total pharyngeal length (Tchesunov, 2014a; Figure 1b).

The family Ethmolaimidae is divided into two subfamilies Ethmolaiminae and Neotonchinae this latter encompassing *Gomphonema* (Bezerra et al., 2013). This genus has a wide distribution, between 50°N and 90°S (Boucher & Goubault, 1990; Ndaro & Ólafsson, 1999; Netto & Gallucci, 2003; Fisher & Sheaves, 2003; Ingole & Singh, 2010;

Bezerra et al., 2013; Pastor de Ward et al., 2015; Venekey & Melo, 2016; Thai et al., 2017). There are records of the subfamily Ethmolaiminae in estuarine and inland waters, while the subfamily Neothonchinae was previously recorded only in marine waters (Bezerra et al., 2013).

Gomphionema is typically found on marine (Semprucci et al., 2013), and brackish waters from estuaries and mangroves in salinity as low as 7 (Venekey & Melo, 2016; Thai et al., 2017). This genus currently has five species described and still is poorly studied (Bezerra et al., 2020). Both the genus *Gomphionema* and the subfamily Neothonchinae were not previously found in inland waters with this study being the first such record.

3. *Rhynchonema* Cobb, 1920

Rhynchonema presents cuticle coarsely striated. Amphids placed over or very close to the end of the buccal tube. Buccal cavity in two parts; short anterior chamber, at the level of cephalic setae, narrow tubular part extending along the cervical region. Male with two testes. Spicules short and arcuate. Tail conical (Fonseca & Bezerra, 2014; Figure 1c-d).

Rhynchonema has been found between 18-36°N and 0-40°S (Jesús-Navarrete, 2003; Leduc et al., 2012; Sandulli et al., 2015; Bezerra et al., 2014; Tu & Gagarin, 2017), and was reported from deep seawaters between 750 and 6300 m depth (Vanhove et al., 2004). *Rhynchonema* was also reported from estuarine brackish waters with low salinity between 4.5-6.0 (Tu & Gagarin, 2017).

4. *Prorhynchonema* Gourbault, 1982

Prorhynchonema possess buccal cavity in two parts; anterior short, at level of cephalic setae, posterior tubular, restricted to the anterior part of the cervical region. Amphids circular, placed well posterior to the end of buccal tube. Spicules short and arcuate. Tail conical (Gourbault, 1982; Figure 1e).

Prorhynchonema has been recorded at 16°N (Gourbault, 1982) and 0-80°S (De Broyer et al., 2007; Venekey et al., 2010) and was reported from deep waters between 750 and 6300 m depth (Vanhove et al., 2004). *Prorhynchonema* in estuarine waters with salinity as low as 10 (Tilbert, 2017). None of these genera had been previously recorded in inland waters with this study representing the first record.

5. *Sabatieria* Rouville, 1903

The three individuals belonging to the genus *Sabatieria* were identified by the cuticle striated or

with transverse punctuation, lateral differentiation as larger regular or irregular punctuations may occur. Anterior sensilla arranged in three crowns. Cephalic setae longer than the outer labial. Anterior portion of buccal cavity globular to cup-shaped, posterior portion narrow as a collapsed tube, weakly sclerotized; sometimes with small projections of the wall at the border between the two portions. Male excretory system with two additional uninucleate subventral gland cells far behind the cardia. Spicules usually enlarged proximally, apophyses usually directed dorsocaudally or caudally (Figure 1f). All these characteristics are consistent with those described in the review of Platt (1985).

This genus is widely distributed worldwide, between 50°N and 50°S (Chen & Vincx, 1999; Pastor De Ward, 2003; Botelho et al., 2007; Gagarin & Nguyen, 2008; Ansari et al., 2012; Leduc et al., 2012). It has been commonly found on marine muddy sediments where it is frequently abundant (Yang et al., 2019). *Sabatieria* is commonly amongst the dominant nematode genera on marine environments such as deep water (Ott et al., 1991), oceanic islands, estuaries, sand beaches and continental shelf (Vanreusel et al., 2010; Venekey et al., 2010). This genus has been found with salinity down to 1 (Adão et al., 2009), and recently recorded inside the São Francisco River (NE Brazil) relatively close (<20 km) and with direct connection to the sea (Pinto et al., 2021). The current record emphasizes its presence on freshwater >100 km away from the coast in a body of water completely independent from the sea.

6. Final remarks

Apart from the five genera reported here, *Pseudosteneria* and *Odontophoroides* also were previously thought to be exclusive from marine environments but have been reported in the Brazilian semiarid (Lucena et al., 2015). It is noteworthy the presence of many marine genera in closed body of waters of this region which is >130 km away from the nearest shoreline. All these genera are frequently found in estuarine brackish waters with low salinity (Adão et al., 2009; Long et al., 2017; Tu & Gagarin, 2017). Consequently, the saline condition in many of the semiarid water bodies and inland freshwater closer seawater (6 in this study; 19 in Lucena et al., 2015; and <0.5 in Pinto et al., 2021) may not be a restrictive factor to the survival of these nematode genera. These observations raise questionings about the dispersal mechanisms of these tiny organisms and highlight the importance of studying

nematodes from different semiarid water bodies. Further taxonomic studies with identification down to the species level will be important to test for endemism of these Nematoda from the Brazilian semiarid water bodies.

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