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Zygonemella: the forgotten genus of the family Xyalidae (Nematoda)

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Species descriptions of the family Xyalidae, as well as of most marine nematodes, were in general largely made in the past century (e.g. Allgén, 1927; Cobb, 1920; Gerlach, 1957; Lorenzen, 1977). Many of these descriptions were based on one or two specimens or even on juveniles with relatively few features of taxonomic value. Lack of types and inaccuracy in sampling localities are other problems associated with taxonomy of marine nematodes. These issues, together with the fact that in the past researchers had slower exchange of information and reduced access to some journals, led to the multiplication of synonyms. To propose a new species of nematode, particularly within a genus with a convoluted historical background, requires caution and critical taxonomical review prior to the description (Adams, 2001; Fonseca & Decraemer, 2008).

Nematodes species delimitation based solely on morphological characters is problematic for three reasons: a) high phenotypic plasticity among populations (Sommer and Ogawa, 2011), which reduces the number of diagnostic characters; b) poor taxonomical descriptions (Nadler, 2002); and c) existence of cryptic species (Derycke *et al.*, 2005; Fonseca *et al*., 2008). These problems often lead to greater taxonomic uncertainty within very speciose genera and may bias diversity studies.

This problem is particularly evident in the species-rich and often ecologically dominant Xyalidae Chitwood, 1951. This family contains 44 genera, with some genera, such as *Daptonema* Cobb, 1920 and *Theristus* Bastian, 1865, having more than one hundred nominal species (Fonseca & Bezerra, 2012). Most species descriptions are limited to a few and poorly described diagnostic morphological characters. Moreover, identification keys and systematic revisions (e.g. Lorenzen, 1977; Fonseca & Bezerra, 2012) need to be revised and updated.

Daptonema matrona Neres, Fonseca-Genevois, Torres, Cavalcanti, Castro, Da Silva, Rieger, & Decraemer 2010 was recently described from Pina Basin, an estuarine area located on the coast of the state of Pernambuco (Brazil). The main diagnostic characters given by the authors were: 1) reduced cephalic setae in relation to the head diameter, 2) straight shape of the spicules, 3) amphidial fovea slightly oval, situated less than one head diameter from the anterior end and 4) viviparous reproduction. To accommodate this new species, the authors amended the diagnosis of the genus *Daptonema* proposed by Lorenzen (1977). According to Lorenzen, *Daptonema* species had L-shaped spicules and four cephalic setae longer than 5µm in length. The spicules of *D. matrona* are straight, and their cephalic setae are smaller than 5µm (Neres *et al.*, 2010).

However, the four characters listed by Neres *et al.* (2010) are also the diagnostic characters of the genus *Zygonemella* erected by Cobb (1920) from material sampled in Punta Arenas, Pacific coast of Costa Rica. Gerlach (1957) identified some specimens of *Z. striata* from the mangrove of Cananéia, south coast of São Paulo, Brazil. In his work, Gerlach pointed out that although the specimen from Costa Rica had a larger b-ratio (rate of body length divided by the pharynx length) and tail length than specimens from Cananéia, he considered these differences insufficient to erect a new species. Further revisions recognized the validity of *Zygonemella* as a distinct genus from *Daptonema* (Lorenzen, 1977; Gerlach and Riemann, 1974; Nicholas & Trueman, 2002; Fonseca & Bezerra, 2012). When comparing the descriptions of *Z. striata* and *D. matrona*, the only two morphological differences reported are the presence of a gubernaculum and the presence of five ejaculatory glands in the specimens examined by Neres *et al.* (2010). The gubernaculum was neither mentioned by Cobb or Gerlach, and the male described by Cobb had 10 ejaculatory glands.

To determine if *D. matrona* should be synonymized with *Z. striata*, we sampled specimens from the coast of São Paulo (mangroves at Ubatumirim, Guaratuba, Juréia-Itatins and Cananéia) including the sampling localities previously sampled by Gerlach (1957) at Cananéia and compared with the published data by Neres *et al.* (2010) from Pernambuco.

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The material was fixed with DESS (Yoder *et al*., 2006) and stored at room temperature. One specimen from Ubatumirim mangrove was picked for molecular analyses. DNA extraction was done with Worm Lysis Buffer according to Derycke *et al.* (2005) and the primer set G18S-18P was used to amplify the small ribosomal subunit (18S rDNA) according to Fonseca & Fehrlauer-Ale (2012). Complementary strands were combined, edited and compiled using Geneious Pro v5.6.5 created by Biomatters (Available from http://www.geneious.com/).

When compared with the sequence from Pernambuco obtained by Neres *et al.* (2010), our sequence (GenBank KC920423) showed only four nucleotide bases of difference, representing 99.8% similarity between the two sequences. This congruence supports the idea that specimens from Pernambuco and São Paulo belongs to the same species (Bhaduri *et al*., 2006).

For morphological comparisons, 24 specimens from São Paulo coast were measured (Table 1 and 2). Specimens of *Z. striata* from all four localities (Costa Rica, Cobb 1920; Cananéia, Gerlach 1957; Pernambuco, Neres *et al.* 2010; São Paulo present study) were analyzed by means of cluster analysis calculated on a Euclidean matrix using the group linkage algorithm and associated SIMPROF procedure (Clarke & Gorley, 2006). Prior to the analysis characters were standardized and highly correlated characters (r>0.8) were excluded.

TABLE 1. Females body measurements of *Zygonemella striata* [means and (range) in µm]. Measurements available to only one location were excluded from the table. n, number of specimens measured; a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length; c′, tail length divided by anal body diameter; L, body length; mbd, maximum body diameter; ph, pharynx length; ph bd, pharynx base diameter; t, tail length ; abd, anal body diameter; b.cav, buccal cavity length ; hd, head diameter; n. ring, position of nerve ring from anterior body end; n. ringbd, body diameter in nerve ring region; Amph%, percentage of diameter amphidial fovea in relation to corresponding body diameter; amphd pos, distance of amphidial fovea from anterior end; els, external labial setae length; cs, cephalic setae length; ts, caudal setae length; V%, position of the vulva as percentage of body length from anterior end; V, position of vulva from anterior body end; vbd, body diameter in vulva.

	Pernambuco (Neres *et al*. 2010)	São Paulo (present study)	Cananéia-São Paulo (Gerlach, 1957)
n	10	14	1
L	1290.6 (1122–1524)	898.5 (728.5–1097.4)	1480
ph	201.4 (178.5–222)	157.2 (133.4–215.6)	250
mbd	88.4 (73.2–106.2)	61.3 (38.7–86.3)	125
t	218.8 (199.5–253.5)	170 (126.5–254.8)	280
a	14.7 (12–16.7)	14.9 (12.5–18.8)	12
b	6.4 (6–6.9)	5.8 (5–6.6)	5.9
c	5.89 (5.5–6.3)	5.4 (4.3–6.1)	5.3
c`	3.9 (3.6–4.4)	5 (3.8–6)	2.9
V%	74.2 (72.5–75.6)	71 (64–76)	70
els	3.4 (3.0–3.6)	3.7 (2–5.6)	3.5
cs	2 (1.8–2.4)	2 (1.13–2.6)	-
ph bd	75.4 (58.5–88.8)	54.4 (37.9–79.3)	108
b.cav	12.5 (9–14.4)	10.1 (7–15.7)	-
hd	31.8 (30–36)	17.6 (13.4–21)	38
n.ring	98.5 (74.4–130.2)	-	141
n.ringbd	63.4 (51–74.4)	-	95
Amph%	11.2 (9.1–13.8)	14 (7.4–19)	-
amph pos	14.3 (11.4–18)	13.3 (10–20)	5
abd	55.7 (48–65.4)	34.8 (25.6–50.6)	95
V	957.4 (840–1128)	645.5 (545.5–754.45)	1040
vbd	75.2 (60–89.4)	-	109
ts	8.2 (6.6–10.8)	6.7 (3.9–9.6)	-

TABLE 2. Male's body measurements of *Zygonemella striata* [means and (range) in µm]. Measurements available to only one location were excluded from the table. n, number of specimens measured; a, body length divided by maximum body diameter; b, body length divided by pharynx length; c, body length divided by tail length; c′, tail length divided by anal body diameter; L, body length; mbd, maximum body diameter; ph, pharynx length; ph bd, pharynx base diameter; t, tail length; abd, anal body diameter; b.cav, buccal cavity length ; hd, head diameter; n. ring, position of nerve ring from anterior body end; n. ringbd, body diameter in nerve ring region; Amph%, percentage of diameter amphidial fovea in relation to corresponding body diameter; amphd pos, distance of amphidial fovea from anterior end; els, external labial setae length; cs, cephalic setae length; ts, caudal setae length; spic, spicule length (along the spicule); gub, gubernaculum length.

	Pernambuco (Neres *et al.*, 2010)	São Paulo (present study)	Cananéia-São Paulo (Gerlach, 1957)	Costa Rica (Cobb, 1920)
n	10	10	1	1
L	1209.9 (1041-1356)	844.9 (762.2-1002.4)	960	1200
ph	195.1 (171-213)	139.8 (103-165.6)	200	136
mbd	64.4 (56.4-72.6)	46.4 (33-63.2)	65	53
t	192 (157.5-207)	132.1 (117-160.6)	160	352
a	18.8 (15.9-22.1)	18.6 (14.8-24.5)	15	22.6
b	6.2 (6-6.6)	6.1 (5.3-7.8)	4.8	7.4
c	6.5 (6-6.8)	6.4 (5.8-7.6)	6	3.4
c`	4.4 (3.9-4.8)	4.4 (3.7-5)	3.6	9.8
els	3.1 (3-3.6)	4.1 (3.2-5.2)	3.5	-
cs	1.8	2.7 (1.7-5.5)	-	-
ph bd	58.9 (53.4-64.8)	41.6 (32.7-59.2)	61	46
b.cav	11.8 (10.2-13.8)	8.4 (7.2-9.8)	-	-
hd	24.7 (21.6-27)	16.8 (14.3-19)	21	31
n.ring	92.9 (72-102.6)	71.7 (60.2-81.7)	107	73
n.ringbd	49.4 (43.2-56.4)	40 (33.2-47.8)	51	41
Amph%	20 (17.8-26)	20 (16-26)	-	-
amph pos	12.8 (9.6-15.6)	10.6 (10-10.9)	6.5	-
abd	44.1 (40.5-48)	30.4 (26-37.8)	45	36
spic	30.3 (28.2-34.8)	23 (20.8-37.8)	30	-
gub	6.6 (5.4-7.8)	5.5 (4.4-7.2)	-	-
sipc/abd	0.7	0.76	0.67	-
ts	8.6 (6.6-10.8)	6.2 (4.6-8)	-	-

Specimens from the four localities are morphologically similar (Fig. 1) with considerable overlap between morphometric characters (Table 1, 2). The only exceptions are: (1) the females from Pernambuco are longer than specimens from the present study (Table 1); (2) the males from Pernambuco are slightly longer (365 µm on average) and with longer pharynx (55 µm on average) than males from the present study (Table 2). When the specimens from Pernambuco are compared with those described by Gerlach, also from Cananéia, the only character that is different is the total body length of the males (a difference of 249 µm), the others are within the range reported by Neres *et al.* (2010). Apart from body length, which can vary within a population according to food availability (Herman and Vranken, 1988), the measurements do not give enough support to distinguish the specimens from the four localities.

Morphometric analysis of the females confirmed no clear clustering of the specimens from the different locations (Figure 2a). Female specimens from Pernambuco are clustered with the specimens from São Paulo (present study). Analysis using the males also showed no separation between locations (Figure 2b). Multivariate analysis indicated that although these specimens are several hundreds of kilometers apart from each other, they are morphologically similar. Molecular and morphometric data suggest that these specimens are probably from one single species broadly spread along the Brazilian coast up to Costa Rica. So far, this species has been reported from estuarine areas with mangrove

forest. It is important to note, however, that our conclusions are based on evidence from the ribosomal gene 18S-DNA. This is a conservative gene unsuitable for detecting population level structuring in nematodes (Bhaduri *et al*., 2006). Additional information could be gained if a more variable molecular marker, such as mitochondrial COI, was used. At the moment, we cannot exclude the possibility that these populations are in fact a complex of cryptic species without morphological differences (Derycke *et al.*, 2008; Fonseca *et al*., 2008).

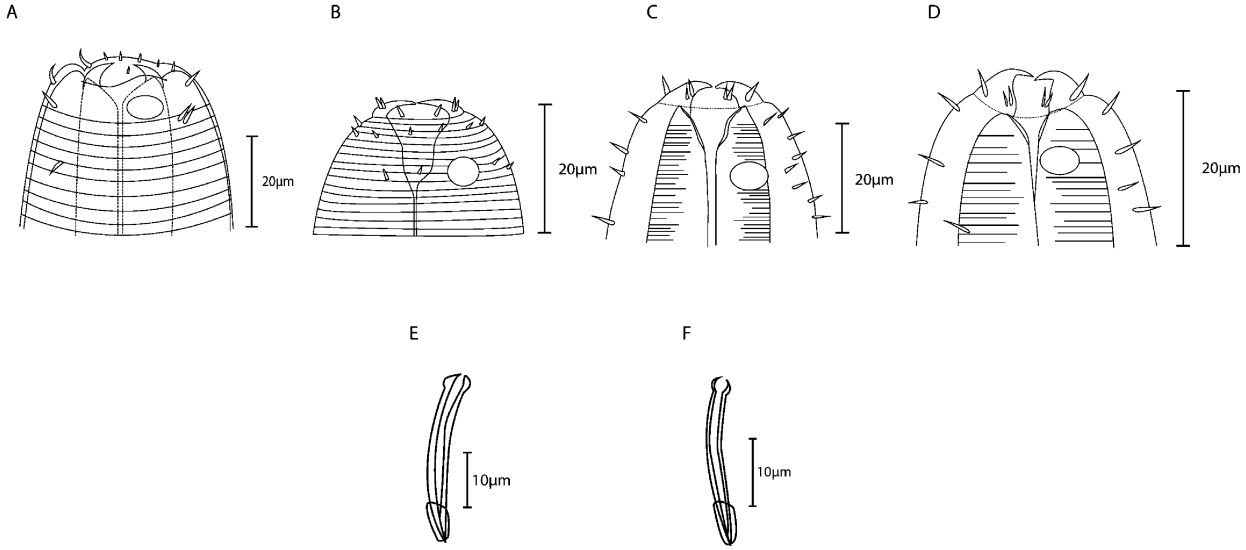

FIGURE 1. Anterior end of the males from the four localities where *Zygonemalla striata* has been reported. A) Costa Rica (adapted from Cobb, 1920); B) Cananéia – São Paulo (adapted from Gerlach,1957); C) Pernambuco (adapted from Neres *et al*., 2010); D) São Paulo (present study). Depicted spicules and gubernaculum from Pernambuco (E; adapted from Neres *et al*., 2010) and São Paulo (F; present study).

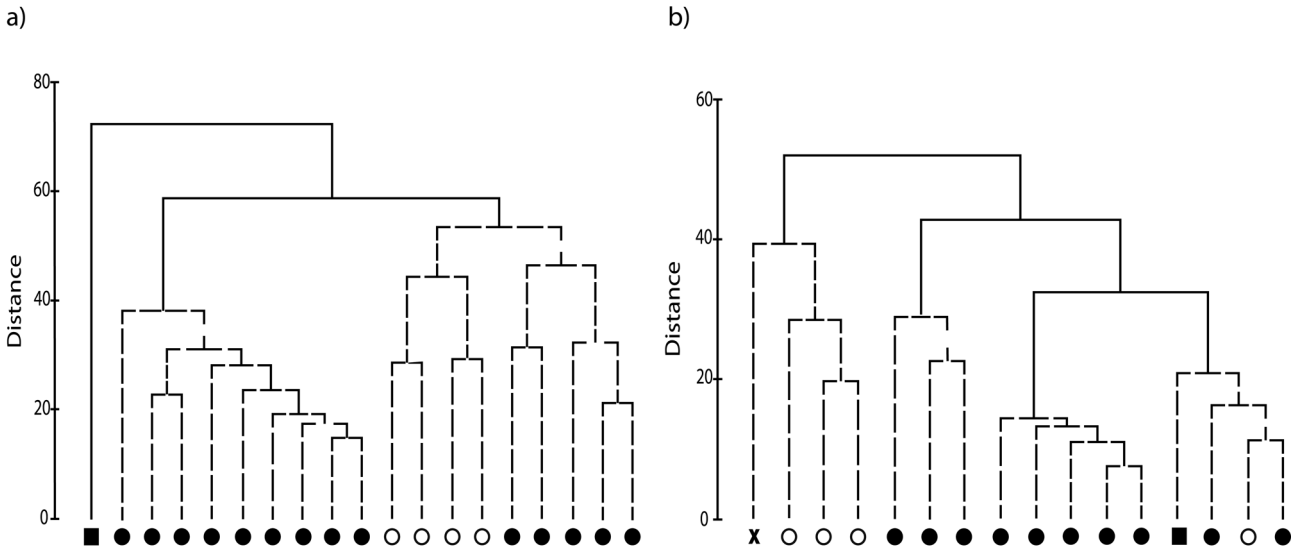

FIGURE 2. Dendogram based on the morphometrics of *Zygonemella striata* specimens from the four locations. Dashed lines represent specimens with no significant difference ($p > 0.5$; SIMPROF). a) females; b) males. ●: São Paulo (present study); ○: Pernambuco (Neres *et al.*, 2010); ■: Cananéia - São Paulo (Gerlach, 1957); **X**: Costa Rica (Cobb, 1920).

In addition to the overlapping of characters between populations, another feature in common among specimens from São Paulo and the Pernambuco is the presence of a small gubernaculum without apophysis at the tip of the spicules (Figure 1E, F). Such a character is difficult to observe and might have been overlooked by Cobb (1920) and Gerlach (1957). Moreover, the material deposited in the National Museum of Rio de Janeiro by Neres *et al.* (2010) shows the presence of ten ejaculatory glands, five on each side of the intestine, in agreement with the description made by Cobb and the specimens analyzed in the present study. There is so far no strong evidence to separate *D. matrona* from *Z. striata*,

and they should be considered synonyms. This study reinforces the importance of critically reviewing the literature before describing new species, especially when the family in question has several species with poorly defined morphological characters.

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