

## Two new species of *Rhynchonema* Cobb, 1920 from a Brazilian sandy beach

Tania Nara Bezerra · Nic Smol · Magda Vincx

Received: 10 November 2013 / Revised: 26 March 2014 / Accepted: 31 March 2014 / Published online: 11 June 2014  
© Senckenberg Gesellschaft für Naturforschung and Springer-Verlag Berlin Heidelberg 2014

**Abstract** *Rhynchonema* Cobb, 1920 is a genus with worldwide distribution. It is found in diverse coastal environments ranging from brackish water to exposed beaches in intertidal and low subtidal zones, from clay to coarse sand and in sediments under seagrass beds. In this study we describe two new tropical species from Northeast South America: *Rhynchonema cemaie* sp.n. and *R. veronicae* sp. n. from Olinda's Isthmus, Pernambuco, Brazil. These two species are mainly characterized by the shapes of the spicules and gubernaculum, which are described here for the first time. The two species can be differentiated primarily on the basis of the spicules, which in *R. cemaie* are symmetrical, fine and long, while in *R. veronicae* they are asymmetrical, more robust and shorter. We describe for the first time the presence of *Rhynchonema* species on the east side of South America and provide a review of the genus and a polytomous identification key. Our review of the literature and construction of a polytomous key demonstrated that most of the species descriptions are incomplete.

**Keywords** *Rhynchonema* sp.n · Sandy beach · Tropical · Marine nematode · Revision · South America

### Introduction

*Rhynchonema* Cobb, 1920 has an anterior sharply tapered "beak-like head" (Cobb, 1920) with a distinctly annulated

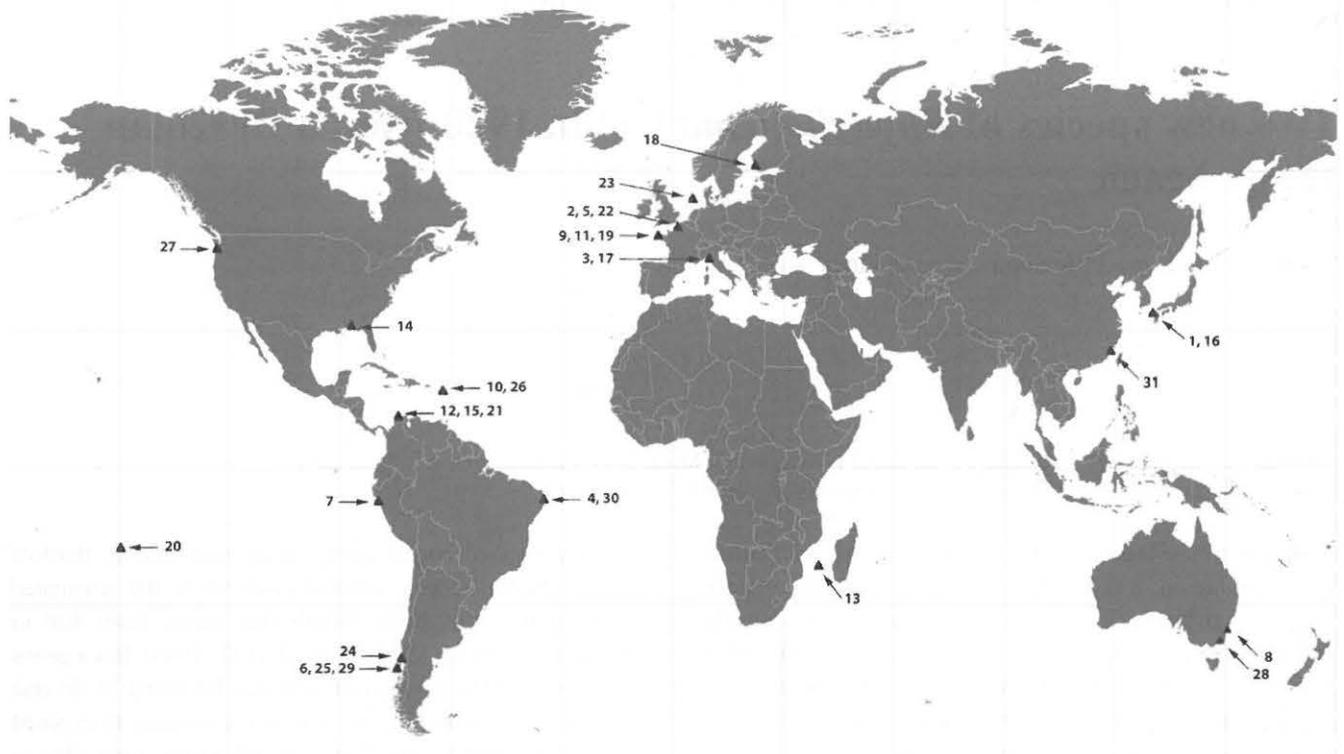
cuticle, elongated buccal cavity, attenuated anterior third of the oesophageal region, amphid posterior to the attenuated section and a total body length that varies from 400 to <900 µm (Lorenzen, 1975; Warwick et al., 1998). It is a genus with a worldwide distribution and can be found in diverse coastal environments ranging from brackish water to exposed beaches in intertidal and low subtidal zones, from clay to coarse sand and in sediments under seagrass beds (Cobb, 1920; Gerlach, 1953a; Gerlach, 1953b; 1955; Wieser, 1956, 1959; Hopper, 1961; Murphy, 1964; Vitiello, 1967; Lorenzen, 1972, 1975; Boucher, 1974; Gourbault, 1982; Aryuthaka, 1989; Nicholas and Stewart, 1995; Huang and Liu, 2002) (Fig. 1).

The genus *Rhynchonema* was erected by Cobb (1920) with the type species *R. cinctum* from Salaverry, Peru. According to Lorenzen (1975) all references that indicate *R. cinctum* for other parts of the world are uncertain. Some limited descriptions or descriptions based only on females led, in the past, taxonomists to mistaking or confusing identifications. In his extensive revision, Lorenzen (1975) states that the synonym by Hopper (1961) for *R. cinctum* sensu Gerlach (1955) and sensu Wieser (1959) (both described with one female) with *R. hirsutum* are both uncertain, since both females may be identical to *R. ornatum*. *R. lyngei* (Allgen 1940) Gerlach, 1953 was originally described as *Leptolaimus lyngei* based on a female specimen from Northern Norway. Gerlach (1953a) described one male and one female under the same species name from Finnish brackish water (5–6 PSU). Lorenzen (1975) considered that he found the same species in the Bay of Kiel, also in brackish water (15–20 PSU) but in the coarse sand of the sub-littoral zone.

Since the publication of Lorenzen's (1975) review, in which he described seven new species, eight additional species have been described, expanding the known distribution of this genus. These eight species are originated from the Antilles (three species; Gourbault, 1982), Japan (two species; Aryuthaka, 1989), Australia (two species; Nicholas and

T. N. Bezerra (✉) · M. Vincx  
Department of Biology, Ghent University, Marine Section,  
Krijgslaan 281/S8, 9000 Ghent, Belgium  
e-mail: tanianara@telenet.be

N. Smol  
Department of Biology, Ghent University, K. L. Ledeganckstraat 35,  
9000 Ghent, Belgium



**Fig. 1** Geographical distribution of *Rhynchonema* Cobb, 1920: 1 *R. amakusanum* Aryuthaka, 1989; 2 *R. ambianorum* Boucher, 1974; 3 *R. brevītuba* Gerlach, 1953; 4 *R. cemaē* sp. n.; 5 *R. ceramotos* Boucher, 1974; 6 *R. chiloense* Lorenzen, 1975; 7 *R. cinctum* Cobb, 1920; 8 *R. collare* Stewart and Nicholas, 1995; 9 *R. deconincki* Vitiello, 1967; 10 *R. dispar* Gourbault, 1982; 11 *R. falciferum* Boucher, 1974; 12 *R. fossum* Lorenzen, 1975; 13 *R. gerlachi* Vitiello, 1967; 14 *R. hirsutum* Hopper, 1961; 15 *R. impar* Lorenzen, 1975; 16 *R. kikuchii* Aryuthaka, 1989; 17 *R.*

*longituba* Gerlach, 1953; 18 *R. lyngei* (Allgén, 1940) Gerlach, 1953; 19 *R. megamphidum* Boucher, 1974; 20 *R. moorea* Boucher, 1974; 21 *R. ornatum* Lorenzen, 1975; 22 *R. quemer* Boucher, 1974; 23 *R. scutatatum* Lorenzen, 1972; 24 *R. semiserratatum* Lorenzen, 1975; 25 *R. separatatum* Lorenzen, 1975; 26 *R. sieverti* Gourbault, 1982; 27 *R. subsetosum* Murphy, 1964; 28 *R. tomakinense* Stewart and Nicholas, 1995; 29 *R. tremendum* Lorenzen, 1975; 30 *R. veronicae* sp. n.; 31 *R. xiamenensis* Huang and Liu, 2002

Stewart, 1995) and China (one species; Huang and Liu, 2002). Gourbault (1982) also described a new sub-species: *Rhynchonema ornatum antillensis*, stating that the cuticle ornamentation of this sub-species differs from the one of *R. ornatum*. To date, the genus contains 29 validated species. Taking into account the two new species described herein, the genus *Rhynchonema* Cobb, 1920 has 31 valid species. There is no molecular data on the genus *Rhynchonema*. Preliminary results from the sequence analysis of *R. ornatum* Lorenzen, 1975 were presented by King et al. (2007) during the 46th Annual Meeting of the Society of Nematologists in San Diego, California, but no further information has been published.

In this article we describe two new species of *Rhynchonema* Cobb, 1920 from Olinda's Isthmus, Pernambuco, Brazil: *Rhynchonema cemaē* sp.n. and *R. veronicae* sp. n.. We report for the first time the presence of *Rhynchonema* Cobb, 1920 on the east coast of South America. A re-appraisal of the genus is presented since the most complete revision in print is in German (Lorenzen 1975) therefore not easily accessible to most nematologists. We also provide an updated polytomous key to *Rhynchonema* Cobb, 1920 species.

## Material and methods

Samples were taken at Olinda's Isthmus (8°2'24"S; 34°51'48" W) from November 1988 to October 1989 at low tide. The beach of Olinda's Isthmus is exposed and frequently used for surfing. It is directly affected by the organic and inorganic pollution of a nearby slum (Ilha do Maruim) and by the polluted flow that comes from the Beberibe River. The collecting stations were located along a transect parallel to the water line in the mid-intertidal zone. Seven stations located 300 m apart from each other covered about 2 km with three defined areas: (1) just in front of the slum; (2) a sheltered area, in front of the Brazilian Navy Base; (3) close to the mouth of the Beberibe River.

To extract the sediment we used a PVC corer (length 10 cm, diameter 5 cm) inserted to a sediment depth of 10 cm. For analysis, the core was divided in three slices corresponding to depths of 0–2, 2–5 and 5–10 cm, respectively. Sediment samples were fixed with a 4 % formaldehyde seawater solution. The meiofauna was extracted using the method of Boisseau (1957), as adapted by Fonsēca-Genevois (1987). Nematodes were extracted from the sand by first re-suspending the sand

in tap water and then allowing the sand to settle. The floating material was passed through sieves with a mesh size of 1.0 mm and 44  $\mu\text{m}$ , respectively. This procedure was repeated ten times for each sample. Nematodes were sorted out and transferred to permanent slides according to the method of De Grisse (1969).

Species descriptions were made from observing the glycerol slides by interferential contrast microscopy (Leica DMLS; Leica Microsystems, Wetzlar, Germany). Drawings were made with a camera lucida; images of the specimens were taken with a Leica DFC 420 digital mounted camera and processed with Leica LAS 3.3 imaging software.

All measurements are in micrometers and all curved structures were measured along the arc.

A polytomous key with the main characteristics of all valid species is provided. For this purpose we had to make a number of assumptions based on the species descriptions:

#### Position of the amphids:

- Although in the description of *R. kikuchii*, Aryuthaka (1989) says that the amphid is slightly behind the end of the pharyngostome, the drawing shows the anterior edge of the amphid before the end and the whole structure at the end of the pharyngostome. We used the information of the drawing since we considered the position of the anterior edge of the amphids.
- In *R. tremendum*, Lorenzen (1975) affirms that the amphids are positioned slightly before the end of the pharyngostome. He considers the position of the anterior edge of the amphids, since the posterior one is at the end of the pharyngostome. We agree with his interpretation.
- Huang and Liu (2002) state for *R. xiamenensis* Huang and Liu, 2002 that the amphid is positioned behind the pharyngostome, but the drawings show that in females they are situated before the end of the pharyngostome. The drawing also shows dimorphism in amphid size. We adjusted their information according to the drawings.

#### Symmetry of spicules:

- Boucher (1974) describes *R. falciferum* with just one straight spicule, although his drawing of *R. falciferum* shows similar shape when compared with *Rhynchonema* species with asymmetric spicules. Asymmetric spicules for *Rhynchonema* species were just described one year later by Lorenzen (1975). Without looking to the original slide it is not possible to affirm that is a case of asymmetric spicules.

#### Presence of capitulum:

- Lorenzen (1975) states that *R. tremendum* has one spicule with a “boat shape” and that the other one is always

normally constructed, built but he does not mention the presence of a capitulum. The figure also does not show a capitulum, so we assumed it is not present.

#### Distal end of spicules in a claw:

- Nicholas and Stewart, (1995) mention that the tips of the spicules of *R. collare* Nicholas and Stewart, 1995 and *R. tomakinense* Nicholas and Stewart, 1995 are turned up. We interpreted this as being claw-shaped. Boucher (1974) affirms that the distal ends of *R. megamphidum* Boucher, 1974 spicules are claw-shaped. Lorenzen (1975) was not sure if this feature was part of the spicule or of the gubernaculum in his specimens of *R. megamphidum* Boucher, 1974. We considered the original description of Boucher (1974).

#### Distal end of spicules bifid:

- Although Lorenzen (1975) does not mention that the right spicule of *R. tremendum* Lorenzen, 1975 is bifid, Gourbault (1982), in her comparison with *R. dispari* Gourbault, 1982 assumes that the spine-shaped structure on top of its convex curvature is part of the spicule distal end and considers this to be bifid. Gourbault (1982) also considers the sickle-shape of *R. dispari* as a bifid end. The left spicule of *R. tremendum* Lorenzen, 1975 is clearly bifid. We followed these assumptions.

#### Pre-cloacal supplements:

- When not mentioned in the text, we assumed there were none present.

#### Annulation around the vulva:

- We assumed there was a differentiation of the annulation around the vulva when mentioned in the text or visualized in the figure.

#### Presence of flap covering the vulva:

- Cobb (1920) states that the vulva of *R. cinctum* is broad and elevated. We assumed he was referring to a flap; Lorenzen (1975) affirms that *R. chiloense* has a thin flap.

Type specimens have been deposited at the Zoology Museum of the Ghent University–Universiteit Gent Museum voor Dierkunde (UGMD), K.L. Ledeganckstraat 35, 9000 Ghent, Belgium.

## Taxonomy and revision

**Systematics:** According to Fonseca and Bezerra (2014)

- Class Chromadorea Inglis, 1983
- Subclass Chromadoria Pearse, 1942
- Order Monhysterida Filipjev, 1929
- Superfamily Sphaerolaimoidea Filipjev, 1918
- Family Xyalidae Chitwood, 1951
- Genus *Rhynchonema* Cobb, 1920

**Diagnosis** (from Fonseca and Bezerra, 2014): Xyalidae. Cuticle coarsely striated. Amphids placed over or very close to the end of pharyngostome. Buccal cavity in two parts: cheilostome, short anterior chamber, at the level of cephalic setae; pharyngostome, a narrow tubular part extending along the cervical region. Male with two testes.

**Description:** Body strongly tapered until a finger-shaped area around the buccal cavity. The buccal cavity is at least 25  $\mu\text{m}$  long and less than 2  $\mu\text{m}$  wide and consists of two sections: the front section is short and lies in the region of the cephalic setae; the second one is elongated and tube-shaped. The cuticle is always clearly annulated, the individual *annuli* are often thick and “tire-like.” Six lip papilla are present but difficult to recognize. The 6+4 setae are all of the same length and are sometimes arranged in two distinct circles. In several species, only six setae are visible. Since the cephalic setae are often very delicate and too difficult to observe, an assumption of six setae is frequently made. Amphids are always circular, and the tail is always conical. All known species have a body size of <900  $\mu\text{m}$  and are usually between 450 and 650  $\mu\text{m}$ . In all known descriptions, males have two testes, with the anterior one lying to the left of the intestine and the posterior one lying to the right (except for *R. tremendum* with two anterior-facing testes). The female reproductive system is monodelphic prodelphic. In all known species the ovary lies on the left side of the intestine (Lorenzen, 1975).

**Differential diagnosis:** The general aspect of *Rhynchonema* is quite uniform and, with few exceptions, does not show striking morphological differences. The main features used to identify *Rhynchonema* species are: shape, size and symmetry of spicules and gubernaculum (Fig. 2) and the position of the amphids in relation to the end of the pharyngostome (Vitiello, 1967; Boucher, 1974; Lorenzen, 1975). Among the Xyalidae there are two genera which were described later than *Rhynchonema* Cobb, 1920, which also have an attenuated anterior end: *Enchonema* Bussau, 1993 and *Manganonema* Bussau, 1993. *Rhynchonema* Cobb, 1920 differs from *Enchonema* Bussau, 1993 by its more accentuated tapered anterior end and spicules shape, which in *Enchonema* Bussau, 1993 are unusually short and straight (Bussau, 1993); it differs from *Manganonema*, Bussau, 1993 by the less accentuated tapered anterior end. *Manganonema* Bussau, 1993 has a dummy-like head shape, which is unique in the family (Fonseca and Bezerra, 2014) and a male reproductive system

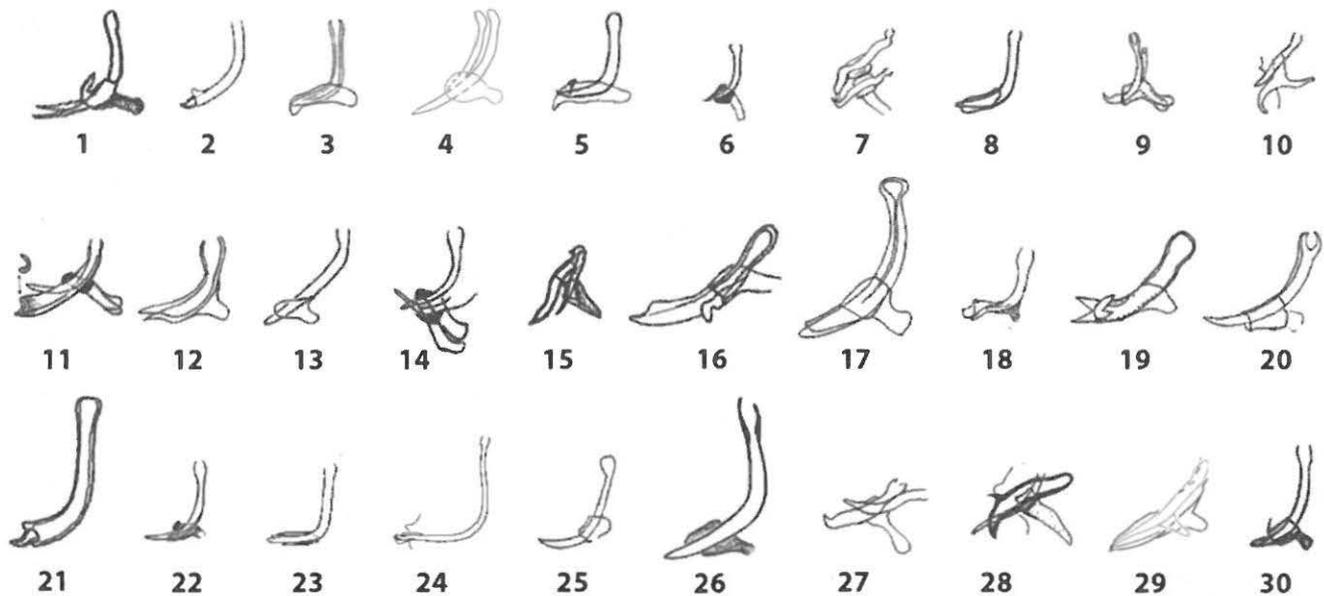
with two small straight spicules and weak gubernaculum without apophysis (Fonseca and Bezerra, 2014).

Until now the genus contained 29 valid species. Taking the two new species described here, *Rhynchonema* Cobb, 1920 has 31 valid species (Table 1), one *species inquirenda* (*R. wieseri* Hopper, 1961 pro *R. cintum* sensu Wieser, 1956) where just one female was analyzed and one *nomen nudum* species (*R. ronaldi*, described in a PhD thesis by Calles-Procel (2007)) that can be found using internet search engines but which was never published in a scientific journal.

Since the erection of the genus, 15 taxonomists have published articles on *Rhynchonema* Cobb, 1920. One of them published a misidentification (Allgen, 1940) of *R. lyngey*, incorrectly identified as *Leptolaimus lyngei*, and afterwards reviewed and renamed by Gerlach (1953a). Two of these publications contain a genus revision (Hopper, 1961; Lorenzen, 1975) and four contain an identification key to the species level: Hopper (1961), Vitiello (1967) and Boucher (1974) published dichotomous keys and Lorenzen (1975) published a polytomous key. An electronic key for *Rhynchonema* Cobb, 1920 species can be found at <http://www.marinespecies.org/deblauwehans/> (Peso, 2007). Six of the above-mentioned articles are written in English, five in German, three in French and one in Chinese, but the majority of descriptions as well the major revision (Lorenzen, 1975) are not in English. Therefore, we decided to compile the main features of all *Rhynchonema* Cobb, 1920 species described to date. The information contained in these descriptions only reflects that provided in the original descriptions.

*R. amakusanum* Aryuthaka, 1989

Cuticle strongly annulated. Two rows of lateral longitudinal ridges on each side of the body. Annuli and ridges are projected forwardly in the anterior part of the body and backwardly in the posterior part of the body. Inversion point at 47–50 % of the body length in males. Ridges are specially prominent between the region of the nerve ring and anus. Six cephalic setae. Amphids of 6  $\mu\text{m}$  in length, 5  $\mu\text{m}$  in width are located at the end of the stoma. From the anterior end to the anterior edge of the amphid, 26–28 *annuli*. Stoma 50–57  $\mu\text{m}$  long. Pharynx is slightly expanded at posterior end. Small cardia protruding into the cylindrical intestine. Testes are paired and opposed; anterior one lying to the left and posterior to the right side of the intestine. Spicules are of equal size, curved. Gubernaculum with a median cuticularized projection and a pair of symmetrical apophyses. *Annuli* of anal region are slightly inflated. Females are similar to males. Reproductive system is monodelphic, prodelphic, with outstretched ovary. Ovary lies to the left of the intestine. Vulva is 68–73 % of the body length. Two caudal glands are clearly visible. Tail is conical, ventrally curved with terminal spinneret. Species is closely related to *R. ornatum* Lorenzen, 1975 due to the presence of lateral longitudinal ridges, shape of anterior end, size of amphids and shape of spicules, but it differs from the latter in the number of *annuli* anterior to the amphid, body



**Fig. 2** Morphological differences in the shape and size of the spicules and gubernaculum. Adapted from: Gerlach (1953a, b), Vitiello (1967), Lorenzen (1975), Gourbault (1982), Aryuthaka (1989), Nicholas and Stewart (1995) and Huang and Liu (2002). 1 *R. amakusanum* Aryuthaka, 1989 (27–28  $\mu\text{m}$ ), 2 *R. ambianorum* Boucher, 1974 (23  $\mu\text{m}$ ), 3 *R. brevituba* Gerlach, 1953 (22  $\mu\text{m}$ ), 4 *R. cema* sp. n. (25–26  $\mu\text{m}$ ), 5 *R. ceramotos* Boucher, 1974 (23.5–25.5  $\mu\text{m}$ ), 6 *R. chilense* Lorenzen, 1975 (12  $\mu\text{m}$ ), 7 *R. collare* Nicholas and Stewart, 1995 (right 16–19  $\mu\text{m}$ , left 13–14  $\mu\text{m}$ ), 8 *R. deconincki* Vitiello, 1967 (16  $\mu\text{m}$ ), 9 *R. dispar* Gourbault, 1982 (right 14–15  $\mu\text{m}$ , left 12–13  $\mu\text{m}$ ), 10 *R. falciferum* Boucher, 1974 (14–15  $\mu\text{m}$ ), 11 *R. fossium* Lorenzen, 1975 (right 21–22  $\mu\text{m}$ , left 15.5–17  $\mu\text{m}$ ), 12 *R. gerlachi* Vitiello, 1967 (21  $\mu\text{m}$ ), 13 *R. hirsutum* Hopper, 1961 (26  $\mu\text{m}$ ), 14 *R. impar* Lorenzen, 1975 (right

20–22  $\mu\text{m}$ , left 14–16  $\mu\text{m}$ ), 15 *R. kikuchii* Aryuthaka, 1989 (right 20–22  $\mu\text{m}$ , left 14–16  $\mu\text{m}$ ), 16 *R. longituba* Gerlach 1953 (27  $\mu\text{m}$ ), 17 *R. lyngei* (Allgen 1940) Gerlach, 1953 (32  $\mu\text{m}$ ), 18 *R. megamphidum* Boucher, 1974 (16–18  $\mu\text{m}$ ), 19 *R. moorea* Boucher 1974 (22  $\mu\text{m}$ ), 20 *R. ornatum* Lorenzen 1975 (22  $\mu\text{m}$ ), 21 *R. quemer* Boucher, 1974 (28  $\mu\text{m}$ ), 22 *R. scutatatum* Lorenzen, 1972 (19.5  $\mu\text{m}$ ), 23 *R. semiserratatum* Lorenzen, 1975 (19  $\mu\text{m}$ ), 24 *R. separatatum* Lorenzen, 1975 (24–25  $\mu\text{m}$ ), 25 *R. sieverti* Gourbault, 1982 (18–20  $\mu\text{m}$ ), 26 *R. subsetosum*, Murphy, 1964 ( $\pm$ 28  $\mu\text{m}$ ), 27 *R. tomakinense* Stewart and Nicholas (11–19  $\mu\text{m}$ ), 28 *R. tremendum* Lorenzen, 1975 (right 20–21, left 18–20  $\mu\text{m}$ ), 29 *R. veronicae* sp. n. (right 16–21  $\mu\text{m}$ , left 15–17  $\mu\text{m}$ ), 30 *R. xiamenensis* Huang and Liu, 2002 (19  $\mu\text{m}$ ). Cobb (1920) did not provide a drawing of the spicules of *R. cinctum*, type species used for the genus description

length and longitudinal ridges in females which are nearly invisible in *R. ornatum* Lorenzen, 1975 and clearly visible in *R. amakusanum* (Aryuthaka, 1989).

#### *R. ambianorum* Boucher, 1974

Cuticle annulated. Edges of the *annuli* are slightly pronounced at the amphid region and totally absent in the rest of the body. Somatic setae are fine and spread out on the body. Six cephalic setae are a little bit smaller than the corresponding diameter. Male amphid at the end of the pharyngostome. Female amphid positioned far behind the pharyngostome. Amphids occupying 30 % of the correspondent diameter in both sexes. Internal amphid contour more evident than the external one. Buccal cavity is 46.5  $\mu\text{m}$  long. Spicules are arcuate, 1.15-fold the cloacal diameter. Distal part of spicule with a claw, capitulum absent. Gubernaculum is not evident, without dorsal apophyses and with two distal subventral hooks. Tail long, conical and 3.7 to 4.4-fold the cloacal diameter. This species is characterized by the absence of edges on the *annuli*, strong cervical region and shape of copulatory apparatus. It closely resembles *R. megamphidum* Boucher, 1974 in terms of the shapes of the spicules bearing distal hooks and the shape of the distal end of the gubernaculum. The anterior region is more similar to that of *R. hirsutum* Hopper, 1961 (Boucher, 1974).

#### *R. brevituba* Gerlach, 1953

Cuticle strongly annulated. *Annuli* more than 2  $\mu\text{m}$  wide. Somatic setae are long and fine. Swollen lip region forming a protruded ring surrounds the anterior end, anterior to the cephalic setae, but this is hardly visible in tiny specimens. Lip papillae and cephalic setae are slightly longer than the diameter of the head. Amphids are slightly larger in males than in females, situated at the end of the pharyngostome. Tubular stoma, with sclerotized walls 27–33  $\mu\text{m}$  long. Nerve ring of 26–29  $\mu\text{m}$  behind the anterior end. Pharynx expands at the base. Males spicules are relatively strongly built, nearly perpendicular curved with a proximal, slightly swollen capitulum. Gubernaculum with a dorsal apophyse. Females with one outstretched ovary. First two-thirds of the tail only slightly tapered, then it tapers more rapidly towards the end. Tail end is not annulated, bearing the spinneret. Similar to the genus type *R. cinctum* Cobb, 1920 but differs from this in the length of the mouth, position of the amphids and the more strongly built spicules (Gerlach, 1953).

#### *R. ceramotos* Boucher, 1974

Wide *annuli* cover the entire body. *Annuli* are forwardly directed at the anterior part of the body and backwardly at the posterior part of the body. The inversion point is at 50 % of the

**Table 1** List of validated species of *Rhynchonema* Cobb, 1920 with measurements, sampling location of the type species and type of sediment (taken from the original descriptions)<sup>a</sup>

Validated species of <i>Rhynchonema</i> Cobb, 1920	Length of male, in $\mu\text{m}$ ( <i>n</i> )	Locality	Habitat
<i>R. amakanum</i> Aryuthaka, 1989	456–506 (2)	Oniike, Shimoshima Island, Japan (32°33'N 130°12'E)	Lower intertidal area, seagrass bed Fine sand and clay
<i>R. ambianorum</i> Boucher, 1974	583	Eastern English Channel, Bay of Somme	Subtidal 5 m depth Fine sand
<i>R. brevituba</i> Gerlach, 1953	512–664 (2)	Mediterranean Sea, San Rossore, Pisa, Italy	Coastal groundwater maximum 2 m depth Sand
<i>R. cema</i> sp. n.	509–590 (4)	Olinda's Isthmus, Pernambuco, Brazil (8°2' 24"S; 34°51'48"W)	Intertidal Medium sand
<i>R. ceramotos</i> Boucher, 1974	487–574 (3)	Eastern English Channel, Bay of Somme	Subtidal 5 m depth Fine sand
<i>R. chilense</i> Lorenzen, 1975	400–430 (2)	Quellon Viejo, SW coast of Chiloé Island, Southern Chile	Mid-intertidal Coarse sand
<i>R. cinctum</i> Cobb, 1920 nec <i>R. Cinctum</i> sensu Gerlach, 1955 and Wieser, 1959: see <i>R. Hirsutum</i> ; nec <i>R. Cinctum</i> sensu Wieser, 1956: see <i>R. wieseri</i>	500	Salaverry, Peru	Low intertidal Sand
<i>R. collare</i> Nicholas and Stewart, 1995	412–579 (3)	Rosedale beach, New South Wales, Australia	Between low tidemarks to 40 cm depth. Sand
<i>R. deconincki</i> Vitiello, 1967	593–637 (2)	L'Aber, Roscoff, France	Location not mentioned Lugworm sand
<i>R. dispar</i> Gourbault, 1982	356–378 (3)	La Grande Anse, Deshaies, Basse-Terre, Guadeloupe	Location not mentioned Sand
<i>R. falciferum</i> Boucher, 1974	510–528 (3)	Pierres Noires, Bay of Morlaix, (western English Channel) and Bay of Somme Eastern English Channel	Subtidal 19 m depth and subtidal 5 m depth
<i>R. fossum</i> Lorenzen, 1975	430–450 (3)	Salamanca Island (Santa Marta), Columbia	Exposed surf beach, upper edge of the outgoing waves; 10–15 cm deep in the substrate. Sand
<i>R. gerlachi</i> Vitiello, 1967	747	Europa Island, Indian Ocean	Sandy reef area Sand
<i>R. hirsutum</i> Hopper, 1961 (syn <i>R. cinctum</i> sensu Gerlach, 1955 and Wieser, 1956)	533	Alabama Coast line, Gulf of Mexico	Below low tide mark Sand
<i>R. impar</i> Lorenzen, 1975	450–485 (3)	Santa Marta Airport, Columbia	Steep surf beach, low intertidal, 5–10 cm deep in the substrate Sand
<i>R. kikuchii</i> Aryuthaka, 1989	410–477 (3)	Oniike, Shimoshima Island, Japan (32°33'N; 130°12'E)	Lower intertidal area; seagrass bed. Fine sand and clay
<i>R. longituba</i> Gerlach, 1953	608–758 (2)	Mediterranean Sea, Pisa, Italy	Coastal groundwater max 2 m depth Sand
<i>R. lyngey</i> (Allgen, 1940) Gerlach, 1953	586	Tvärminne, Western Gulf of Finland, Finland	Coastal groundwater Sand
<i>R. megamphidum</i> Boucher, 1974	492–528 (3)	Pierres Noires, Bay of Morlaix (western English Channel), North Britain	Subtidal 19 m depth Fine sand
<i>R. moorea</i> Boucher, 1974	860	Barrier reefs of Lagoon Moorea, French Polynesia, Society Islands, Central Pacific	Subtidal 1.5 m depth Coarse coral sand
<i>R. ornatum</i> Lorenzen, 1975	525–615 (3)	Salamanca Island (Santa Marta), Columbia	Exposed surf beach, upper edge of the outgoing waves; 10–15 cm deep in the substrate. Sand
<i>R. quemer</i> Boucher, 1974	688	Bay of Somme, Eastern English Channel	Subtidal 5 m depth Fine sand

**Table 1** (continued)

Validated species of <i>Rhynchonema</i> Cobb, 1920	Length of male, in $\mu\text{m}$ ( <i>n</i> )	Locality	Habitat
<i>R. scutatum</i> Lorenzen, 1972	480	Northwest of Helgoland, North Sea	Subtidal 28 m depth Fine sand
<i>R. semiserratum</i> Lorenzen, 1975	560–600 (3)	Pelluco, Puerto Montt, Southern Chile	Extensive coarse sand flats near the spring tide low-water line, 10–25 cm deep in the substrate Coarse sand
<i>R. separatum</i> Lorenzen, 1975	470–500 (3)	Quellon Viejo, SW coast of Chiloé Island, Southern Chile	Lower intertidal Coarse sand
<i>R. sieverti</i> Goubault, 1982	440–485 (3)	Le Gosier, Grande-Terre, Guadeloupe	Location not mentioned Coral sand
<i>R. subsetosum</i> Murphy, 1964	610–770 (4)	Governor Patterson Memorial State Park, near Waldport, Oregon, North Pacific Coast	Intertidal zone Sand
<i>R. tomakinense</i> Stewart and Nicholas, 1994	438–551 (3)	Tomakin beach, New South Wales, Australia	Between low tidemarks to 40 cm depth Sand
<i>R. tremendum</i> Lorenzen, 1975	480–525 (3)	Quellon Viejo, SW coast of Chiloé Island, Southern Chile and Chinquihue Puerto Montt, Southern Chile	Mid-intertidal and loose bearing coarse sand near the low water line, 10–20 cm deep in the substrate. Coarse sand
<i>R. veronicae</i> sp. n.	485–565 (6)	Olinda's Isthmus, Pernambuco, Brazil (8°2' 24"S; 34°51'48"W)	Intertidal Medium sand
<i>R. xiamenensis</i> Huang and Liu, 2002	432–493 (3)	Xiamen Island Fujian, China (24°25'24"N; 118°06'40"E)	Low intertidal. Sand

<sup>a</sup> Updated from Gerlach, and Riemann (1973), Lorenzen (1975) and later literature

body. Somatic setae are extremely fine, not easily visible, displaced in eight rows spread out. Cephalic setae not well defined, apparently six. Long tubular stoma of 31.7  $\mu\text{m}$ . Elongated cheilostome not surrounded by annulations. From the amphids to the anterior end there are 20–21 large *annuli* in males, 22 in females. Circular amphids are located at the base of the pharyngostome. Pharynx expanded at the base. No excretory system was observed. Spicules curved forming a right angle, with a protruding capitulum slightly curved. Gubernaculum sleeve-shaped with two dorsal apophyses. Gubernaculum distal extremity with two fine, transparent, triangular projections and a strong hook central posterior. Vulva is just before two-thirds of the body. Just one anterior ovary. Close to *R. megamphidum* Boucher, 1974 but differs from that in the length of the vestibulum (3-fold the head diameter instead of 2-fold), number of *annuli* before the amphid (20–22 instead of 29–34), position of the amphid (at the end of the pharyngostome instead of behind the pharyngostome), body length, distribution of the somatic setae, size and shape of the spicules (pointed instead of claw shaped), gubernaculum ending in a hook in one piece instead of forked and a strong cuticle annulation at the caudal region (Boucher, 1974).

#### *R. chiloense* Lorenzen, 1975

Body cuticle annulated, annulations stronger at the middle of the body. Edges of the rings are forwardly directed at the

anterior half of the body and backwardly at the posterior half. Inversion point is just behind the half of the body. Only six cephalic setae could be observed. Strong sexual dimorphism on the number of annulations from the anterior end to the anterior edge of the amphids: 46 in the male and 54 in the female. Buccal cavity is 38–45  $\mu\text{m}$  long. Spicules symmetrical. Gubernaculum is paired, with apophyses less sclerotized than the rest of the spicule apparatus. It was not possible to detect if there are one or two testes. Cuticle sclerotization significantly stronger at the vulva level than at the rest of the body, covered by a thin cuticle flap. Ovary lies to the left side of the intestine. Caudal glands were not detected. Tail end bent to the left (Lorenzen, 1975).

#### *R. cinctum* Cobb, 1920

Cuticle *annuli* change direction from anterior to posterior directed. Lateral differentiation is about one-third as wide as the body but more or less indistinct. Pharynx is elongated, with colorless musculature and no indication of glands. Nerve ring at two-fifths of the pharynx length. No cardia. Intestine is set off, with a refractive lumen. Male spicules are slender, rather frail and sub-acute; seen in profile they appear to have their proximal ends about opposite or a little dorsal from the body axis. Gubernaculum is slender and frail. Three minute papilloid supplementary organs are present. They hardly more

than accentuate the *annuli* on which they occur but are rendered visible by carmine staining. In the vicinity of the vulva, about eight *annuli* present simply a crenate, instead of a serrate contour. The broadly elevated vulva is rather conspicuous, particularly anteriorly. Vagina small. A mass of sperm cells has been noted near the vulva. Tail conical, sub-arcuate, tapering from the anus to the non-striated spinneret. At the base of the spinneret there are always about three minute nuclei that stain strongly. The ellipsoidal caudal glands form a tandem in the anterior two-fifths of the tail (Cobb, 1920).

*R. collare* Nicholas and Stewart, 1995

Cuticle strongly annulated, annule profiles sharply angled forward at the anterior half of the body, backward at posterior half; inversion point at 56 % of the body length. Very thin somatic setae are spaced uniformly between amphid and anus. Six cephalic setae are at base of buccal cup; extremely large elongated amphids enclose posterior 40 % of narrow cervical region. Amphids are strongly dimorphic—they are relatively large in females but are much smaller than those in males, separated by a strong non-annulated cuticle. Buccal cavity is a shallow cup leading into long narrow parallel-sided tube with strongly cuticularized walls extending the length of the narrow cervical region to the level of the middle of the amphid. Pharynx is cylindrical, cardia is heart-shaped. Spicules are unequal and weakly cephalated, lack a rectangular bend, with tips turned up. Large gubernaculum encloses the mid region of the spicules, strong dorso-caudal apophysis. Vulva with operculum, on which annulations are greatly reduced or absent. Terminal vaginal canal cuticular. The species can be distinguished from a number of other species by its sharply angled annulations along the whole body and its sexually dimorphic amphids. Resembles *R. chiloense* Lorenzen, 1975 and *R. scutatatum* Lorenzen, 1972 but has quite differently shaped spicules. The male amphids are larger than those of either of these species. It differs from *R. tomakinense* Nicholas and Stewart, 1995 in the larger male amphid, absence of annulations between amphids and its cuticular vaginal canal. The tail is rather long, broadly curved and conical, bent at tail tip (Nicholas and Stewart 1995).

*R. deconincki* Vitiello, 1967

Cuticle annulated. Six rows of somatic setae, about 6  $\mu\text{m}$  long. Cephalic region is elongated, very constricted. Head diameter is 35 % of the diameter at the level of the amphids and 26 % of the diameter of the pharynx base. The first annule is wider than the following ones and clearly separated from the rest of the body. Pappilae are not visible. It would appear that there are six cephalic setae 2–3  $\mu\text{m}$  long. Amphid is slightly oval with an open spiral structure situated on a very long annule. The distance from the amphid to the anterior end is 65–71  $\mu\text{m}$ ; its anterior edge is situated at 4 *annuli* behind the buccal cavity. Buccal cavity 50 to 56  $\mu\text{m}$  long, formed by a cheilostome, continuing with a long pharyngostome of a very small diameter (<2  $\mu\text{m}$ ). Pharynx straight, fairly narrow with no bulb. Excretory system

not detected. Nerve ring at 63 % from the anterior end to the base of the pharynx. Spicules are curved and inflated at the anterior end. Small gubernaculum, not easily visible, tubular, without apophysis. No pre-cloacal supplements. Tail conical, tapering slowly, 4.6–5.6-fold the anal diameter. No caudal glands are observed. This species differs from the others by the shape of its amphid, positioned on an elongated annule (Vitiello 1967).

*R. dispari* Gourbault, 1982

Cuticular *annuli* with dentate edges. Annulation starts at 5  $\mu\text{m}$  behind the anterior end. Inversion point at 45–55 % of the body length. *Annuli* overlapping, except at the anterior region where they are finer. Eight rows of a few, thin, long somatic setae of 10–12  $\mu\text{m}$  that are not regularly distributed. Just six cephalic setae, the same size as the body diameter, are visible. Presence of a few cervical setae in two separated circles. Amphids are small, circular and occupy 63 % of the corresponding diameter; three *annuli*, 40  $\mu\text{m}$  distant from the anterior end; posterior edge always before the base of the buccal cavity. Short lip area. Cheilostome is as high as wide. Tubular part of the long and narrow buccal cavity of 46  $\mu\text{m}$  extends over 47 rings. Pharynx is inflated at the base. Short cardia. Male with two testicles: anterior to the left and ventral to the intestine, posterior to the right and dorsal to the intestine. Spicules with clear asymmetry, length is equal to anal diameter: left spicule, 2  $\mu\text{m}$  smaller than the right one, bent with globular capitulum; right spicule is more massive, regularly arched, also with a globular capitulum, extremity bifid sickle-shape. Gubernaculum is thick and sleeve-like with two asymmetrical apophyses. Females are the same size as males or slightly bigger. Inversion point of *annuli* at 51 % of body length. Ovary lies to the left of the intestine, starting high, behind the cardia. Posterior edge of the vulva is strongly sclerotized, without operculum. Tail is tapered, 4-fold longer than the cloacal diameter in males and 5-fold in females. *Rhynchonema dispari* can be compared to *R. tremendum* Lorenzen, 1975 but the position of the amphid in *R. tremendum* Lorenzen, 1975 is different; the animal has a larger total body size, and the asymmetry of the spicules is much more pronounced; the right spicule is more developed, bifid, with a backwardly bent tip and a secondary tip, like a spine, above the convex curvature of the distal end. *Rhynchonema fossum* and *R. impar*, also described by Lorenzen, 1975, are closer to *R. dispari*, but they have a larger size and differ mainly in the shape of the right spicules (sickle shape in *R. dispari*, also more developed than the left) and the gubernaculum. Vulvas are comparable, but that of *R. dispari* is not covered by a flap as in *R. fossum* (Gourbault, 1982).

*R. falciferum* Boucher, 1974

Cuticular *annuli* overlapping strongly throughout the body length. *Annuli* forwardly directed in the anterior half and backwardly in the posterior part. Inversion point of *annuli* at 58–64 % of the body from the anterior end. Eight rows of somatic setae 12  $\mu\text{m}$  in length. Cephalic setae are not well

defined, apparently six. Before the amphids 36–37 rings slightly overlapping and some short setae are present. Amphids longitudinally oval in male, occupying 89–100 % of the corresponding diameter; round in females, occupying 87 % of the corresponding diameter. The center of the amphids is situated just at the level of the base of the pharyngostome. Sudden increase in the body diameter at level of the amphids. Presence of four sub-lateral seta on the first or second ring which are behind the lower edge of the amphid. Buccal cavity is long, tubular, 36–37 % of the length of the pharynx in males and 36–40.8 % in females. Pharynx ends in a slight enlargement. Presence of a cardia between pharynx and intestine. Excretory organs are not observed. Spicule straight, capitulum slightly protruding, length close to the cloacal diameter. The distal end of the gubernaculum presents a posterior well-developed sickle hook. Two dorsal apophyses in the median part of the gubernaculum. Long, tapered tail, 4.1–4.3-fold the cloacal diameter, bearing six rows of somatic setae. *Rhynchonema falciferum* is characterized by the presence of 36–37 rings before the upper edge of the amphid, by pronounced cuticular rings which overlap along the entire length of the body and, especially, by the copulatory apparatus with one spicule not bended and gubernaculum with a posterior, hyper-developed, ventral hook. Female is determinable by the number of rings before the amphid (Boucher, 1974).

*R. fossum* Lorenzen, 1975

Cuticle annulated. Edges of *annuli* forwardly inclined at the anterior half of the body and backwardly at the posterior half of the body. Inversion point of the *annuli* is in the middle of the body. Head with 6+4 cephalic setae in two circles, close to each other. The six-lip papillae are hardly recognizable. Amphids are of equal size in both sexes and always before the end of the pharyngostome. There are 44–45 body *annuli* between the anterior end and the anterior edge of the amphids. Buccal cavity is 53–54  $\mu\text{m}$  long, of irregular diameter along the length and only fairly weakly cuticularized. Spicules are asymmetrical: the right one is always larger than the left one and its posterior half is U-shaped (in cross-section). The right and left apophysis of the gubernaculum have different shapes. There are two testes: the anterior one lies to the left and the posterior one to the right of the intestine. At the top of the posterior part of the testes are structures that might represent ejaculatory glands. The strong sclerotized vulva is always positioned slightly to the left of the ventral line and has a thin-skinned cover that overlaps. The vagina appears to be muscular. The proximal part of the ovary is located to the left of the intestine, the distal portion is ventral in relation to the intestine. Just two caudal glands were observed (Lorenzen, 1975).

*R. gerlachi* Vitiello 1967

Cuticle annulated. Between the end of the pharynx and the anus the *annuli* are slightly larger and closer to each other. Anterior to the amphids, in males, there is a series of three

seta. Body is covered with rather long somatic setae arranged regularly in eight longitudinal rows. Amphid is circular, located 22  $\mu\text{m}$  from the anterior end. There are 39 *annuli* before the amphid in males, 37 in females. Head has a small diameter (33 % of the body diameter at the amphid region). Papillae are not visible. Cephalic setae are very small, difficult to distinguish; there seem to be six. Nerve ring is located about 70 % of the distance anterior to the cardia. Excretory organs are not observed. Buccal cavity is 53–55  $\mu\text{m}$ , with a large cheilostome, extending to a pharyngostome whose width varies very little along its length. The buccal cavity ends at the middle of the amphid. Pharynx is cylindrical, widened slightly but without forming a bulb. Cardia small. Nerve ring is located 68–72 % far from the anterior end to the cardia in females and 70 % in males. Males spicules curved with proximal end swollen and distal end bifurcated. Gubernaculum with rectangular-caudal apophyses. Anterior to the cloaca are three light papilla. Female is monodelphic and prodelphic. Vulva is situated at three-quarters of the body length. Tail conical, 3.9 and 4.1–4.7-fold the anal diameters in males and females, respectively. *Rhynchonema gerlachi* is close to *R. longituba* Gerlach, 1952 and *R. hirsuta* Hopper, 1961, but differs from the latter by the structure of the spicules. The buccal cavity of *R. gerlachi* is shorter than that of *R. longituba* and its amphids are larger than those of *R. hirsuta* (Vitiello, 1967).

*R. hirsutum* Hopper, 1961

Cuticle strongly annulated. Scattered somatic setae. Head with six small papilla around the buccal cavity and six cephalic setae, about one head diameter from the anterior end. Amphids are located at the base of the pharyngostome. Stoma is 53–55  $\mu\text{m}$  long. Pharynx is conoid, wide at its base. Spicules are arcuate at proximal cephalation. Gubernaculum is small, with flanking spicula on both sides and caudally directed apophysis. Anterior to the cloaca, on the ventral side, five breaks in the hypodermis and musculature appear to be present. The three anterior ones are clearly supplementary organs, while the remaining two perhaps represent rudiments of such organs. There are no external indication of these supplements, i.e., no papilloid structures as described by Cobb (1920) for *R. cinctum*. Females are monodelphic, prodelphic with outstretched ovary. *Rhynchonema hirsutum* is closely related to *R. lyngei* (Allgen, 1940) Gerlach, 1953. These two species differ in (1) the location of the amphids (at the base of the pharyngostome in *R. hirsutum* and posterior to the pharyngostome in *R. lyngei* (Allgen, 1940) Gerlach, 1953); (2) number of cephalic setae (6 vs. 10 for *R. lyngei* (Allgen, 1940) Gerlach, 1953); (3) length of spicules, with those of *R. hirsutum* being shorter (26 vs. 32  $\mu\text{m}$ , respectively) and less arcuate than those in *R. lyngei* (Allgen, 1940) Gerlach, 1953. The only difference between *R. hirsutum* and *R. cinctum* Gerlach, 1955 is the length of the spicules (26 vs. 21  $\mu\text{m}$ ,

respectively). As only a single male specimen of each is known, their exact relationship is dubious (Hopper 1961).

*R. impar* Lorenzen, 1975

Body with thick *annuli* forwardly inclined at the anterior half of the body and backwardly at the posterior half of the body. Inversion point is about 55 % of the body length, the ventral inversion point is slightly anterior than the dorsal one. The cuticle of the *annuli* shows irregular vacuoles. Head with 6+4 cephalic setae (just visible at very high-quality preparations) of which the two lateral ones are more anterior than the rest. Amphids in both sexes are of same size and always before the end of the pharyngostome. Between the anterior end and the anterior edge of the amphids there are 51 *annuli* in males and 55 in females. Stoma 62–69  $\mu\text{m}$  long. Spicules are very sclerotized and asymmetrical: the right spicule is always bigger and shaped differently than the left one. The two apophyses of Gubernaculum are always shaped differently. Pre-cloacal supplements are represented by ventral setae on a papilla and three tiny papillae. There are two testes directed towards the anterior; the anterior one lies to the left side of the intestine and the posterior one to the right side of the intestine. Vulva is strongly sclerotized and not covered by a flap. The unpaired ovary lies proximally to the left side of the intestine and more distally to the dorsal side of the intestine. Caudal glands not visualized (Lorenzen, 1975).

*R. kikuchii* Aryuthaka, 1989

Cuticle strongly annulated; *annuli* anteriorly orientated at the anterior part and posteriorly at the posterior part of the body. Inversion point is about 54–60 % of the body length, the reversal point of the dorsal side being slightly posterior to that of the ventral side. Body *annuli* with one or two rows of vacuoles. Eight rows of 4 to 17  $\mu\text{m}$ -long somatic setae are present. They do not appear to be arranged in pairs, but are slightly staggered over the entire body surface. The head bears six cephalic setae of about one head diameter. Lip region is weakly outlined, but in some specimens, there seem to be six lips. Between the anterior end of the body and the anterior edge of the amphids there are 32–37 *annuli*. Amphids are located just behind the base of the stoma. The oval-shaped amphids occupy about 84 % of the corresponding body diameter. Pharynx is cylindrical and expands a little in its posterior region. The small cardia protrudes slightly into the cylindrical intestine. The tip of the anterior testis is located at 46–51 % of the body length lying to the left of the intestine, while the posterior one lies to the right side of the intestine. The structure of the testes is not clear. Spicules are asymmetric; the right one is always bigger and longer than the left one, having a curved bow-like shape and a thick wall which is bent at the proximal end. The left one is notably more slender, shorter and slightly bent in its distal part. Gubernaculum is also asymmetric with a cuticularized postero-dorsal apophysis. Females are very similar to males in body size, structure and measurements of the various parts of the body and general shape. In contrast

to males, the amphids of females are smaller, and the tail is slightly shorter. The reproductive system is monodelphic, prodelphic, with outstretched ovary. Vulva at 68–70 % of the body length, covered with a single flap. Only two caudal glands are clearly observed. Tail conical, ventrally curved, with the spinneret terminally located. Although there are slight morphological differences, sexual dimorphism is not pronounced. Morphological variation among the specimens of the sex is also very small. *R. kikuchii* differs from the other species described till now in terms of the asymmetry and shape of spicules (*R. tremendum* Lorenzen, 1975, *R. impar* Lorenzen, 1975, *R. fossum* Lorenzen, 1975 and *R. dispar* Goubault, 1982). In all cases, the right spicule is larger than the left one (Aryuthaka, 1989).

*R. longituba* Gerlach, 1953

Cuticle annulated. Numerous somatic setae are present. Anterior part of the head is a bit offset, with the lip region very well defined. Cephalic setae very fine. Stoma 65–67  $\mu\text{m}$  long, slightly expanded anteriorly. Amphids are of different sizes in males and females (10 vs. 6  $\mu\text{m}$ , respectively). The front edge of the amphids is located just behind the end of the pharyngostome. Pharynx extends at its base. Spicules are 27  $\mu\text{m}$  long. Females are monodelphic, prodelphic. Tail is four anal diameters long. *Rhynchonema longituba* is close to *R. lyngei* (Allgen 1940 here synonym for *Leptolaimus lyngei*) but differs from the latter by the sexual dimorphism of its amphids (Gerlach 1953a).

*R. lyngei* (Allgén, 1940) Gerlach, 1953

Cuticle annulated. Long somatic setae, particularly in the cervical region. Head carries tiny lip papillae, followed by a circle of ten cephalic setae which can be easily overlooked because they are very delicate. The front edge of the amphids is located 57 and 62  $\mu\text{m}$  from the anterior end in males and females, respectively. The buccal cavity is 52–55  $\mu\text{m}$  long and consists of two indistinctly separate departments: the anterior one, the vestibule, has no sclerotized walls and it is slightly wider than the second tubular section, which clearly has sclerotized walls. Spicules with a strong capitulum followed by an evenly rounded arc. Gubernaculum with a small rectangular dorsal apophysis. Female is monodelphic, prodelphic with outstretched ovary. Tail cylindro-conical tapering to the end, about 3.5 cloacal diameters long. *Rhynchonema lyngei* type species, was identified by Allgen (1940) from the beach zone of Norway as *Leptolaimus lyngei* with a very limited description, although it cannot, by no means, be a representative of the genus *Leptolaimus* and even not a member of the family Araeolaimidae due to the presence of one single ovary. Although it matches *Rhynchonema* descriptions from various points of European and non-European coasts, where the animals are very similar with just different proportions, buccal cavity and amphids, the specimen on which Allgen's description is based is 835  $\mu\text{m}$  long and more than 200  $\mu\text{m}$  wide. It is bigger than the type species of *Rhynchonema* Cobb, 1920, but

since the length and width of the buccal cavity, as well as the location and size of the amphids agree very well with the animals from both localities, the author refrained from drawing up a new species (Gerlach, (1953b).

*R. megamphidum* Boucher, 1974

Body diameter increases abruptly at the level of the amphids. Somatic setae are well-developed, rather thick, disposed alternating in eight rows. Cuticle *annuli* are overlapping only in the cervical and esophageal area. Edge of the *annuli* is directed forwardly at the anterior region of the body and absent at the posterior region. Cephalic setae are not well defined, apparently six. Buccal cavity tube measures 30–31.8 % of the pharynx length. The contour of the body at the vestibulum funnel in front of the rings is set off. From the amphids to the anterior end there are 29–34 *annuli* bearing some cervical setae, especially at the dorsal side of the 27th annule. Amphid is circular, more than 100 % of the corresponding diameter wide and 125 % long in males; 58–64 % of the corresponding diameter in females, occupying six *annuli*. Amphid anterior limit is behind the pharyngostome. Pharynx is slightly enlarged at its base. No excretory pore observed. Spicules are short arcuate, and the length is the same as the cloacal diameter; capitulum slightly protruding, distal extremity claw-shaped. Gubernaculum is compact with two short dorsal apophysis and distal part with sharp points oriented to the anterior part of the body. Female monodelphic, Ovary reaches almost the base of the pharynx. Vulva at 74 % of the body, from the anterior end. Tapered tail, 3.7–4.2-fold longer than the anal diameter, bearing six rows of setae where the lateral rows just show two setae. *Rhynchonema magamphida* Boucher, 1974 closely resembles *R. ceramotos*, but differs from the latter by the non-annulated part of the head set off and the presence of 29–34 *annuli* before the amphid, instead of 21–22; by the size of the amphids; by the more numerous somatic setae; by the size and shape of the spicules and the gubernaculum ending with an anterior hook instead of a posterior one (Boucher, 1974).

*R. moorea* Boucher, 1974

Cuticle with *annuli* slightly overlapping. Somatic setae are extremely numerous, arranged in eight rows over the entire length of the body from the amphids to the posterior part of the body. In general, a long setae alternates with a short one, while in the next row a short setae alternates with a long one. Cephalic setae not well defined, apparently six. Buccal cavity is tubular and 29–31 % of the length of the pharynx. The non-annulated area of the cuticle at the vestibulum measures 1.2–1.5-fold the cephalic diameter. The anterior part of the head is abruptly set off, just behind the cephalic setae. Presence of 33–34 rings before the amphids in both sexes. Few short setae at the 23th annule. Amphids are circular, 63–73 % of the corresponding body diameter wide and are located at the base of the pharyngostome. First row of setae on the third annule behind the amphid. Spicules are almost straight, same length as the

cloacal diameter, capitulum slightly globular; distal end is bifurcate with divergent branches. Gubernaculum in one piece with proximal extremity forming the dorsal apophysis and the distal extremity with two bifurcated claws situated at the point of the spicules. No pre-cloacal supplements observed. Vulva situated at three-quarters of the body, from the anterior extremity. One ovary. The length of the tapered tail is 4.1–4.4-fold the anal diameter; the tail bears eight rows of setae. *Rhynchonema moorea* Boucher, 1974 closely resembles *R. gerlachi* Vitiello, 1967, but differs from the latter in the number of *annuli* before the amphids (33–34 vs. 37–39, respectively), by smaller amphideal diameter (63–73 % depending on the sex vs. 73–87 %, respectively), by the lack of pre-cloacal papillae and, especially, by the shape of copulatory apparatus; the spicules are nearly straight instead of arcuate and the gubernaculum ends in bifurcated claws absent in *R. gerlachi* (Boucher, 1974).

*R. ornatum* Lorenzen, 1975

Cuticle annulated. *Annuli* forwardly directed at the anterior half of the body and backwardly directed at the posterior half. Inversion point of *annuli* at 50 % of the body. On the ventral side the inversion occurs further forward than dorsally. From the pharynx until just before the anus, the *annuli* edges are projected in tongue-like processes. These projections are indistinctly pronounced in most animals and in females are usually not recognizable. Only six cephalic setae are visible. Amphids are strongly sclerotized, only slightly larger in males than in the females and are always located at the end of the pharyngostome. Between the anterior end and the anterior edge of the amphids there are 33 body *annuli*. Buccal cavity is 50–52  $\mu\text{m}$  long. The symmetrical spicule apparatus is strongly sclerotized. Two testes: the anterior one lies to the left side of the intestine and the posterior one to the right side. Female is monodelphic, ovary lying to the left side of the intestine. Just two indistinct caudal glands were detected (Lorenzen, 1975).

*R. ornatum antillensis* Goubault, 1982

Cuticle annulated. Reversion point of the *annuli* at 50 % of body length in males. Round amphids comprise 75 % of the corresponding body diameter, occupying a height of three *annuli* and situated at the end of the pharyngostome. Anterior edge of the amphid is 48  $\mu\text{m}$  from the anterior end. Quite long cervical setae in three circles along the tubular portion of the anterior end. Short lip area. Cheilostome is as high as wide, followed by a long and narrow tubular part. Two testes, anterior one to the left and posterior one to the right of the intestine. Symmetrical spicules are regularly arcuate, a bit longer than the anal diameter (1.2- to 1.3-fold) with globular capitulum; distal part with simple and afunilated end. Gubernaculum in one piece, sleeve-shaped with ventral sclerotized hook and two short rounded dorsal apophyses. Female with amphids of smaller diameter than in males; inversion point of the *annuli* at the level of the vulva or 68 % of the body

length. Ovary lies to the left of the intestine. Vulva is narrow, not well sclerotized and not covered by flap. Tail is conical with a length 4-fold the anal diameter. The morphological characters of *R. ornatum antillensis* resemble those of *R. ornatum* Lorenzen, 1975, excluding the cuticular ornamentation characteristic of males. A cross section of the body is not perfectly circular due to the existence of a slight flattening at the side faces. Moreover, the inversion point of the *annuli* shows a sexual dimorphism: in females it is at the vulva (68 %) and in males at the middle of the body (Gourbault, 1982).

*R. quemer* Boucher, 1974

Cuticle annulated. *Annuli* overlapping just at the anterior and posterior ends of the body. Presence of an amphideal plate. Six long cephalic setae are about the same size as the corresponding body diameter. Few cervical setae. There are 32 *annuli* between the anterior end and the amphids. The first body annule is much wider than the next ones. Amphideal plate is 9  $\mu\text{m}$  high, formed by the welding of four rings. Amphid is longitudinally oval with a spiral structure, occupying 47 % of the corresponding body diameter; its upper part is connected to the anterior edge of the plate. Cylindrical buccal cavity, 47  $\mu\text{m}$  long. Spicules are arcuate, with a slightly globular capitulum and claw-shaped distal ends. Gubernaculum is indistinct with distal ends set off. Dorsal apophysis is absent. Tapered tail, with a length 4.1-fold the cloacal diameter. This species is characterized by the presence of an amphideal plate, the spiral shape of its amphids and the shape of the distal end of the copulatory apparatus. *Rhynchonema quemer* closely resembles *R. deconincki* Vitiello, 1967, but differs from the latter by the number of fused *annuli* forming the amphideal plate (4 vs. of 8, respectively), by the position of the amphid on the plate (anterior vs. median, respectively) and by the size of spicules. When compared with the type specimen of *R. deconincki* Vitiello, 1967, the distal end of the spicules are the same and the gubernaculum without dorsal apophysis also show distal set-off ends (Boucher, 1974).

*R. scutatatum* Lorenzen, 1972

Cuticle strongly annulated. Body *annuli* facing forwardly in the anterior part of the body and backwardly in the posterior part of the body. Inversion point of *annuli* at 45–50 % of the body length. Somatic setae 6–8  $\mu\text{m}$  long are scattered. Head bears six cephalic setae. Lip region is poorly contoured. Amphids and surrounding neck region show sexual dimorphism. In males the contour of the amphids is slight. Females amphids have a strong pronounced inner contour, while the outer contour is slight and the body *annuli* are not interrupted. Buccal cavity extends up to the middle of the amphids. Pharynx widens a bit at its base. A small cardia is present. Spicules are more sclerotized proximally than distally. Gubernaculum consists of one centerpiece from which arises a soft sleeve, to pull the spicules, at its right

and left sides forming a cuff at the ventral side of the spicule. A paired apophysis feeble. Female with vulva is covered by a flap. *Rhynchonema scutatatum* is close to *R. deconincki* Vitiello, 1967, but the buccal cavity of the latter ends 15  $\mu\text{m}$  before the anterior edge of the amphids; amphids are built differently. In his description, Vitiello (1967) limits the description of the female to the imaging of the tail. Up to this description there was no female described with vulva covered by a flap (*R. scutatatum* Lorenzen, 1972).

*R. semiserratum* Lorenzen, 1975

Body *annuli* edges dentate at the first and last third of the body. *Annuli* of increased thickness at the middle third of the body, without dentate edges. Head carries six cephalic setae, which are very difficult to visualize. Amphids show a strong sexual dimorphism. In males the anterior edge lies at the end of the pharyngostome, while in females it is behind the pharyngostome. Between the anterior end and the anterior edge of the amphids there are 40 *annuli* in males and 46 in females. Buccal cavity is 48–52  $\mu\text{m}$  long. Spicules are symmetrical and strongly sclerotized, with two distal tips each. Two testes: the anterior one lies to the left side of the intestine and the posterior one to the right side. To the right and the left side of the testes there are ejaculatory glands, with a coarsely granulated content, situated 30–40  $\mu\text{m}$  anterior to the cloaca. Females are monodelphic, ovary lying to the left of the intestine. Tail is always bent to the left. In some animals two caudal glands were detected (Lorenzen, 1975).

*R. separatum* Lorenzen, 1975

Cuticle annulated. Body *annuli* not dentate. Head with six tiny papillae and 6+4 cephalic setae in two widely separated circles. Amphids show a strong sexual dimorphism. In males the anterior edge of the amphid lies always at the end of the pharyngostome, while in females it is always significantly behind. Between the anterior end and the anterior edge of the amphid there are 38–39 *annuli* in males and 53 in females. Buccal cavity is 37–45  $\mu\text{m}$  long. Pharynx is extended at the end somewhat like a bulb. Spicules are unusually fine. It was not possible to affirm whether all distal structures of the spicule apparatus belonged to the spicules or to any existing gubernaculum. Two testes are present: the anterior one is shorter, lies to the left side of the intestine; the posterior one is longer and lies to the right side of the intestine. In one male, two inconspicuous thin-skinned pre-anal papillae were observed slightly to the left of the ventral line. These could not be detected with certainty in all males. Females are monodelphic. Ovary lies to the left of the intestine. Only two caudal glands were present, with two separated ducts. Tail end is always clearly bent to the left (Lorenzen, 1975).

*R. sieverti* Gourbault, 1982

Cuticle strongly annulated. *Annuli* overlapping and giving an rough aspect, except at the anterior end where they are narrower. Inversion point of the *annuli* at 53 % of the body length, with a small difference between the ventral and dorsal

sides. Few and very fine somatic setae 10  $\mu\text{m}$  long in length. Ten cephalic setae are inserted just behind the lip region. Buccal cavity is 56  $\mu\text{m}$  long, longer than larger, prolonged by a tubular portion which is characteristic of the genus. Amphids are ovoid and often not pronounced in males, 57  $\mu\text{m}$  from the anterior end reaching the base of the pharyngostome; they occupy 77 % of the body width and four cuticle *annuli*. Females have circular amphids, slightly smaller than those of males but much more pronounced; they occupy the same position on three cuticle *annuli* only. Pharynx is cylindrical surrounded by many glands. Very small cardia. Two testes. Two symmetrical spicules, curved, with a large capitulum and tapered at its distal ends. Gubernaculum with a feeble dorsal apophysis and one ventral piece well sclerotized. Presence of five pre-cloacal papillae. Females are monodelphic, with outstretched ovary lying to the left side of the intestine. Vulva is slightly sclerotized and covered with a punctuated flap. Tail is conical, 4.5-fold the anal diameter, with long setae on the dorsal side and short spines on the ventral side. It is very close to *R. ornatum antillensis*: both have a similar copulatory apparatus with gubernaculum bearing an unpaired sclerotized ventral piece. The presence of pre-anal papillae does not seem frequent in the genus *Rhynchonema*. Up to this description they have been reported: five in *R. hirsutum* Hopper, 1961 (single gubernaculum with dorsal apophyses), three in *R. cinctum* Cobb, 1920 and one in *R. subsetosum* Murphy, 1961. In addition, the vulva-shaped “madreporite” characterizes *R. sieverti* (Gourbault, 1982).

*R. subsetosum* Murphy, 1964

Cuticle coarsely annulated; *annuli* forwardly directed at the anterior portion of the body and backwardly at the posterior half. Six lips, each bearing one setose papilla. Six cephalic setae, slightly less than one-half head diameter in length, and three circles of subcephalic setae located between the amphid and the anterior circle of setae. Long, thin, cervical and somatic setae are located at regular intervals. Anterior region of the head bears papillae and cephalic setae. It is readily differentiated from the rest of the cephalic region by the appearance of a denser, more solid construction. Circular amphids of 4.5–5.0  $\mu\text{m}$  in diameter (slightly > 50 % of the corresponding head diameter) and positioned immediately above the base of the stoma. Pharynx is cylindrical, terminating in a moderate bulb; cardia is oval, long axis is directed dorso-ventrally. Nerve-ring at 35 % of the esophagus, measured from the base of the stoma. Excretory pore not observed. Male has one genital papilloid supplement that is one anal diameter anterior to the cloacal opening. Spicula sharply bent about midway in their length. Gubernaculum is plate-like with a dorsal apophysis. Female is monodelphic, ovary reflexed. Tail is conical, curved ventrally: female tail is 4.8-fold the anal diameter long; the male tail is 3.4-fold the cloacal diameter long. *Rhynchonema subsetosum* is most closely related to *R. hirsutum* Hopper, 1961 collected from the Gulf of Mexico; these two species are

the only described species of the genus with six cephalic setae rather than ten. The Oregon species differs in having a distinct bulb, males with but one pre-cloacal supplement (vs. 3 or 5? in *R. hirsutum*) and spicula with a sharp bend centrally (vs. a moderate bend proximally in *R. hirsutum*). Of possible taxonomic significance is that the new species, in contrast to *R. hirsutum*, possesses three circles rather than one circle of subcephalic setae and has a reflexed rather than outstretched ovary in the females. The latter in particular may only be a reflection of variation in stages of development (Murphy, 1964).

*R. tomakinense* Nicholas and Stewart, 1995

Strongly annulated, sharp border forwardly angled, especially in amphidial region, direction sharply inverted 54 % from the anterior end. Six very small cephalic setae inserted on the base of the vestibulum, from which extends a narrow, parallel-sided cuticular buccal tube throughout the length of the cervical region. Large circular amphids located over the end of the pharyngostome. The annulation continues between amphids. Amphids are dimorphic and smaller in the female. Cylindrical pharynx, heart-shaped cardia. Thin setae are spaced along the body from the cervical region to the anus. Spicules are cephalated, asymmetric, slightly unequally sized, without strong rectangular curvature, tips turned up. Gubernaculum encloses the middle of spicules, strong dorso-caudal apophysis. Vulva with operculum, vaginal canal not cuticularized. This species is very close to *R. collare*. The two are sibling species from the same beaches but can be clearly distinguished by several features. Annulation continues between amphids in both sexes, whereas there is smooth cuticle in *R. collare*. The male amphids are not as large. The vaginal canal is not cuticular and the spicules show greater asymmetry. *Rhynchonema tomakinense* resembles *R. chiloense* Lorenzen, 1975, and *R. scutatum* Lorenzen, 1972, but has quite differently-shaped spicules (Nicholas and Stewart, 1995).

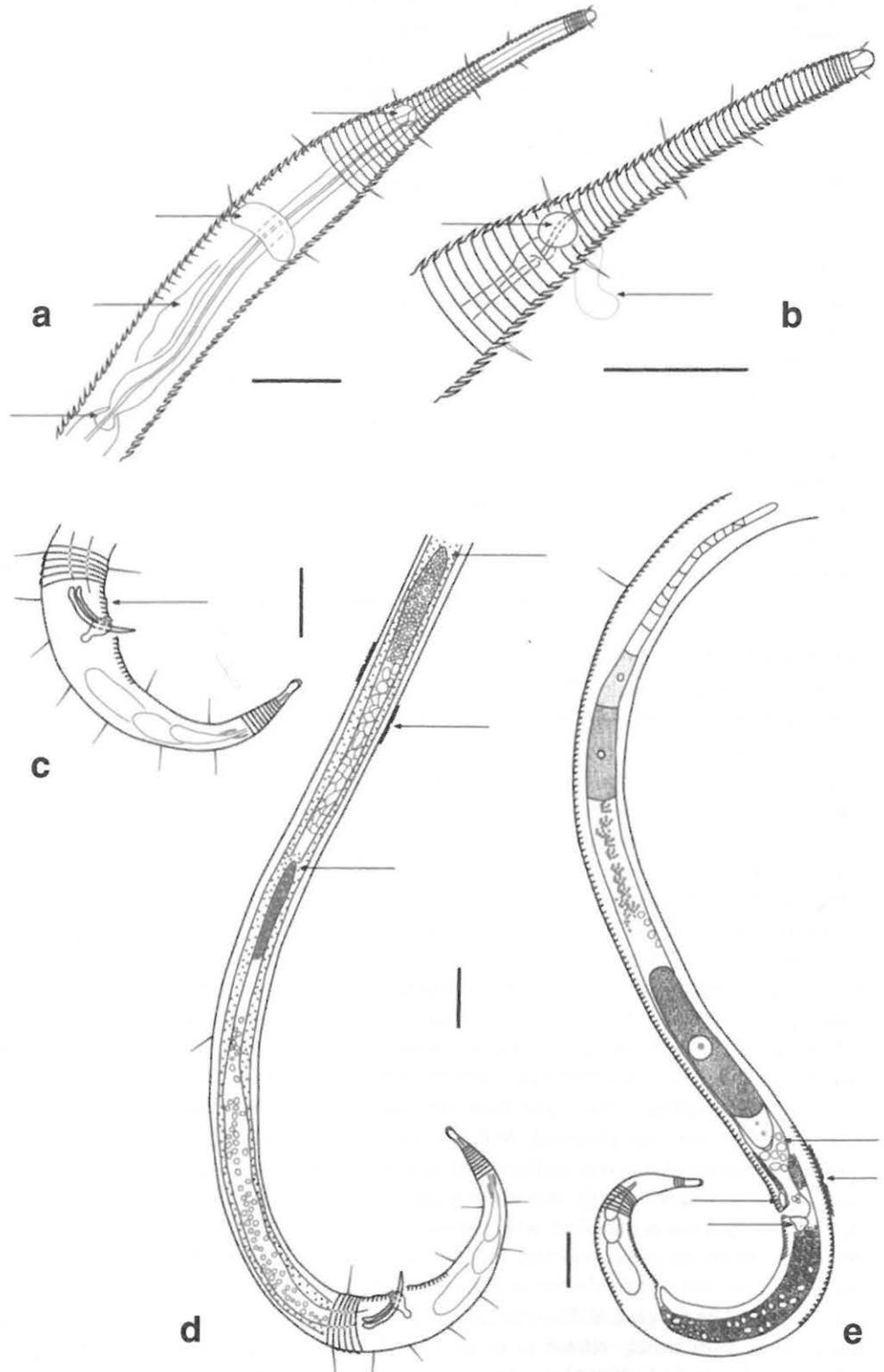
*R. tremendum* Lorenzen, 1975

Body cuticle annulated. *Annuli* forwardly inclined at the anterior half of the body and backwardly at the posterior half of the body. Inversion point of *annuli* shortly after the middle of the body, slightly anterior at the ventral side and posterior at the dorsal side. *Annuli* are ornamented with vacuoles, one or two rows per ring. The orientation of the ornamentation is particularly evident in the esophagus and tail region, but only slightly on the rest of the body. Just six cephalic setae were observed. Male amphids are clearly larger than those of the females, and in both sexes the posterior edge is at the level of the end of the pharyngostome. Between the anterior end and the anterior edge of the amphids are 38 body *annuli* in males and 35 in females. Buccal cavity is 45–55  $\mu\text{m}$  long. The very strong sclerotized spicule apparatus is strongly asymmetric. The right spicule is always very thick-walled and has the shape of a boat; the left one is always built normally and

terminates distally in two peaks. The gubernaculum is also asymmetrical and possesses an unpaired apophysis. About 80  $\mu\text{m}$  anterior to the cloaca are three body rings strikingly thickened. Testes always lie to the left of the intestine. No posterior testis was observed, rather there are two anterior-

facing testes. Since no germinal zone could be recognized, it is not possible to decide whether both branches are testes or if only the longer one is a real testis. In females the ovary is to the left of the intestine, built quite complicated in the uterus. Vagina is designed in an unusual and broadly conical shape.

**Fig. 3** *Rhynchonema cema* sp. n. **a** Holotype male head showing the position of the amphid, nerve ring, a gland and the cardia, **b** male head showing receptor of the sensory cell and the extruded *corpus gelatum*, **c** male tail showing one of the cuticular bumps, **d** male posterior end showing the end of the anterior and the posterior testes and the inversion point of the cuticle, **e** paratype female reproductive system, arrows showing spermatozoa in the spermatheca, the perivulvar glands and the inversion point of the cuticle. Scale bars 20  $\mu\text{m}$



Vaginal canal difficult to see. Tail tip is always bent to the left. Only two caudal glands were detected (Lorenzen, 1975).

*R. xiamenensis* Huang and Liu, 2002

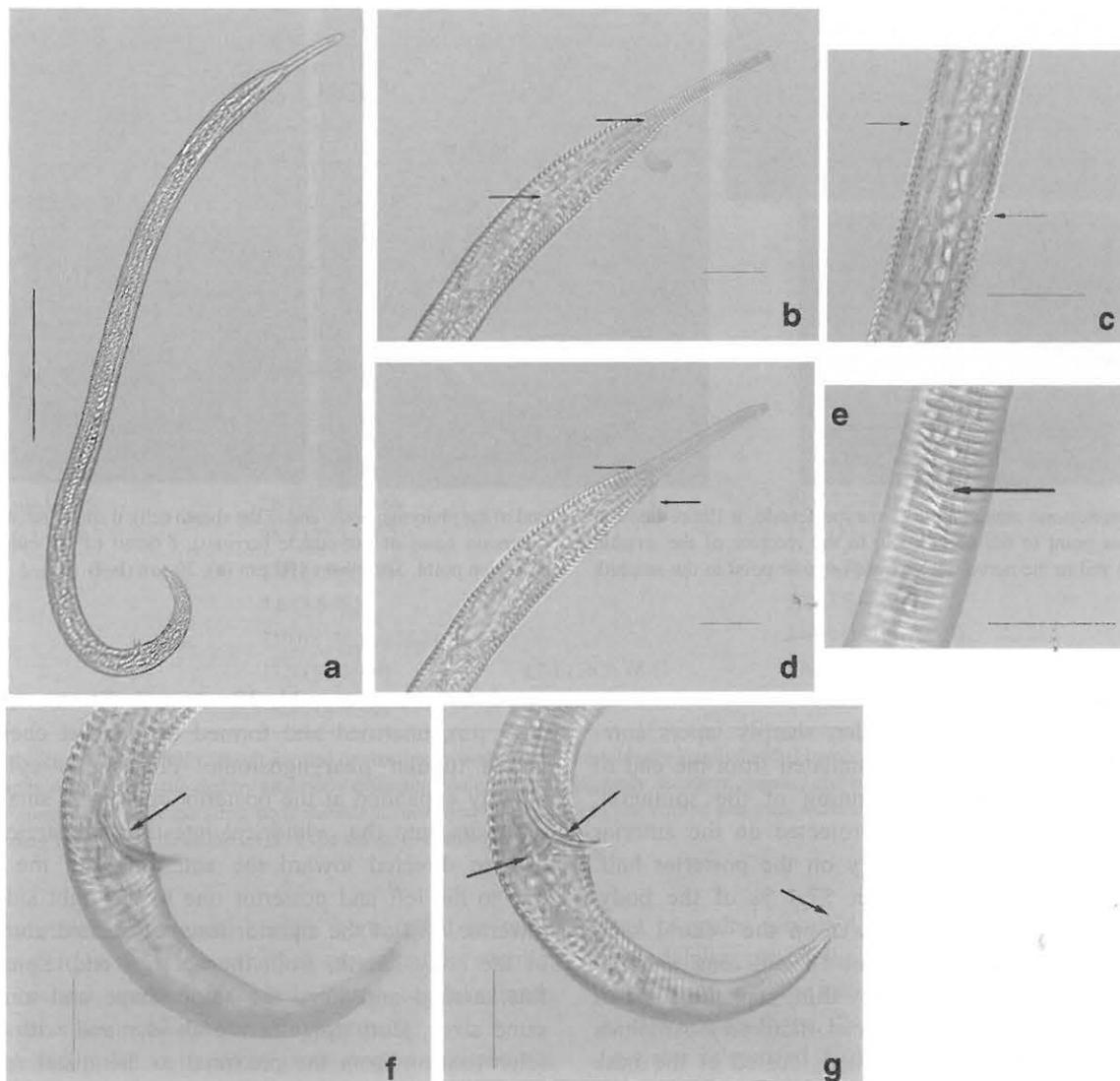
Cuticle strongly sclerotized, especially at the anterior half of the body. Annulations are weak at the level of the tail. Mouth is cylindrical, elongated. Head with ten (6+4) long setae, 1.08-fold the head width. Amphids are circular, located behind the pharyngostome. The distance from the anterior end to the anterior edge of the amphids is 39  $\mu\text{m}$ . Pharynx gradually swells until its base, without forming a bulb. Males have two outstretched testes, two symmetrical arched spicules. Gubernaculum is slightly curved, with a small proximal handle-like projection. This appearance is unique among the known species. Females are similar to males. Strong sclerotization of the cuticle at the level of the vulva. Ovary is

outstretched. Tail conical. Closely related to *R. ornatum* but differs in size and position of the amphids, which in *R. xiamenensis* are behind the pharyngostome in males and at the end of the pharyngostome in *R. ornatum*, by the shape of the gubernaculum and cuticle annulation at the vulva region (Huang and Liu, 2002).

*Rhynchonema cema* sp. n. (Figs. 3, 4, 5; Tables 1, 2, 3)

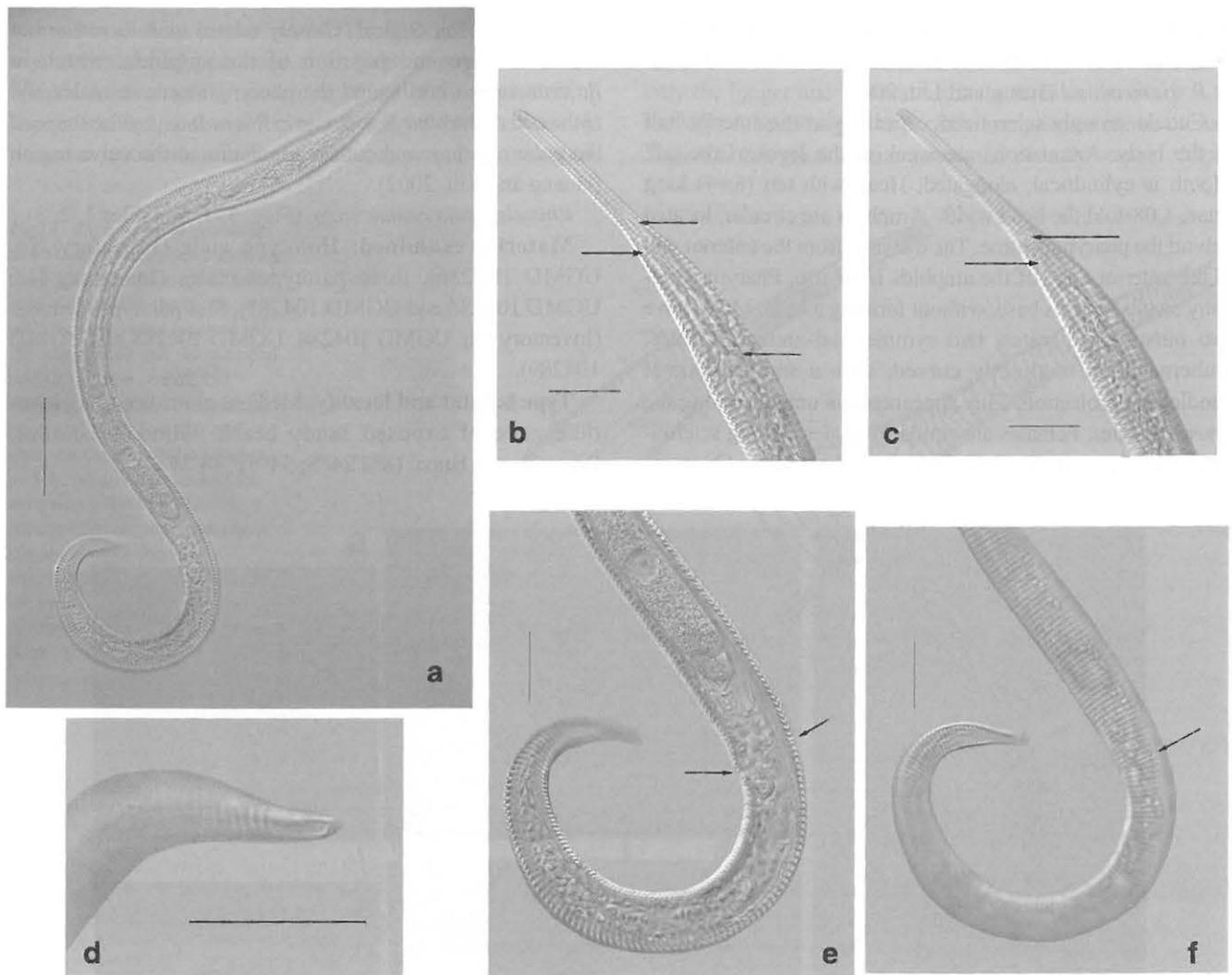
**Material examined:** Holotype male (Inventory No. UGMD 104286), three paratypes males (Inventory No. UGMD 104286 and UGMD 104287), four paratypes females (Inventory No. UGMD 104284, UGMD 104288 and UGMD 104289).

**Type habitat and locality:** Medium grain size sand, intertidal zone of exposed sandy beach, Olinda's Isthmus, Pernambuco, Brazil (8°2'24"S; 34°51'48"W).



**Fig. 4** *Rhynchonema cema* sp. n. Holotype male. **a** Entire body, **b** head (arrows point to the receptor of the amphid sensory cell and to the nerve ring), **c** inversion point of the cuticle, **d** head (arrows point to the amphid and its extruded *corpus gelatum*, secreted by the sheath cell), **e** detail of

the cuticle at the inversion point shifted from ventral to dorsal side, **f** left spicule, **g** right spicule (arrow points to the spicule, dorsal posterior apophyses and spinneret. Scale bars: 50  $\mu\text{m}$  (a), 20  $\mu\text{m}$  (b–g)



**Fig. 5** *Rhynchonema cemaie* sp. n. Paratype female. **a** Entire body, **b** head (arrows point to the socket cell, to the receptor of the amphid sensory cell and to the nerve ring) **c** head (arrows point to the amphid/

end of the pharyngostome and to the sheath cell), **d** spinneret, **e** vulva and inversion point of the cuticle (arrows), **f** detail of the cuticle at the inversion point. Scale bars 100  $\mu\text{m}$  (**a**), 20  $\mu\text{m}$  (**b–f**)

### Description

**Males:** Body elongated, slender, sharply tapers anteriorly to the amphids. Cuticle annulated from the end of the cheilostome until the beginning of the spinneret. Edge of the *annuli* forwardly projected on the anterior half of the body and backwardly on the posterior half. Inversion point of the *annuli* at 57.4 % of the body. Inversion occurs five *annuli* lower on the ventral side. Very thin somatic setae of about 10  $\mu\text{m}$  long are distributed along the body. Six, very thin, cephalic setae of 2  $\mu\text{m}$  are present (about 0.5 head diameter). Amphids are circular (diameter 4.8  $\mu\text{m}$ ) and located at the base of the pharyngostome (40  $\mu\text{m}$  from the anterior end), occupying 55.2 % of the corresponding body diameter. From the anterior end to the anterior edge of the

amphids there are 31–32 *annuli*. Stoma of about 42.5  $\mu\text{m}$ , unarmed and formed by a short cheilostome and a tubular pharyngostome. Pharynx is cylindrical, slightly expanded at the posterior region. A small cardia protrudes into the cylindrical intestine. Outstretched testes are directed toward the anterior, with the anterior one to the left and posterior one to the right side of the intestine. Tip of the anterior testis is located about 40 % of the body length, from the anterior end. Spicules are fine, arched and have the same shape and almost the same size. Both spicules are slender and with uniform sclerotization from the proximal to the distal region. A small capitulum is present at the proximal end of the spicules. Distal end of the spicules is surrounded by the gubernaculum, which bears a dorsal sclerotized projec-

**Table 2** Morphometrics of *Rhynchonema cema* sp. n. and *R. veronicae* sp. n.<sup>a</sup>

	<i>Rhynchonema cema</i>		<i>Rhynchonema veronicae</i>	
	Males (n=4)	Females (n=4)	Males (n=6)	Females (n=6)
L	548.6 (509–590)	546 (512.3–565.9)	540.2 (485–565)	512.8 (490.4–533.4)
a	28 (24.5–32.6)	27.3 (27.2–28.9)	30 (27–31.7)	25.4 (24.5–26.4)
b	4.4 (4.3–4.7)	4 (3.9–4.2)	4 (4.0–4.4)	4.2 (3.8–5.0)
c	7 (6.6–7.3)	7.7 (7.4–8.2)	7 (6.7–7.3)	7.6 (7.3–8.2)
c'	4 (3.9–4.6)	4.2 (3.8–4.4)	4.4 (4.0–4.7)	4 (3.9–4.0)
Head diam.	4 (3.6–4.9)	3.6 (3.2–4.0)	3.6 (3.5–3.8)	3.8 (3.2–4)
First part of stoma	4.2 (3.0–5.8)	3.4 (3.0–4.0)	4.4 (3.8–5.0)	3.4 (2.9–4.2)
Buccal cavity length	42.4 (36.2–46.6)	48.4 (46.4–50.2)	45 (38–48.8)	43.8 (37.4–49)
Cephalic setae	2 (1.8–2.0)	1.9 (1.0–2.4)	1.6 (1.4–1.7)	1.9 (1.3–2.5)
Somatic setae	8.2 (6.7–9.0)	8.5 (7.9–8.7)	9.4 (8.4–9.9)	9.3 (8.5–9.7)
Inversion point of the cuticle	311.2 (285–323)	Vulva level	310 (267–318)	Vulva level
Annules before the amphid	31.2 (31–32)	33 (31–35)	31.5 (31–32)	31 (31)
Amphid width	4.8 (4.4–5.0)	4.2 (3.9–4.5)	4.6 (4.0–5.0)	4 (3.5–4.7)
Amphid length	4.8 (4.6–5.0)	4 (4–4.2)	5 (4.5–5.4)	4 (3.5–4.3)
Amphid cbd	8.7 (8.2–9.0)	9 (8.5–9.9)	8.8 (8.5–9.2)	8.7 (8–9.5)
Amphid dist ant end	39.8 (35.7–43.8)	46.5 (42.7–49.8)	43.3 (36.4–47.3)	41.5 (35.5–46.7)
Pharynx length	118.3 (100.4–137.0)	137.2 (129.5–144.2)	130.2 (120.5–142.7)	123.3 (105–137.3)
Pharynx width base	9.7 (9.0–10.3)	10 (7.6–11.6)	8.9 (7.8–9.8)	10.9 (10–12.8)
Pharynx cbd	17.8(15.5–18.6)	19.6 (19.0–20.4)	17.5 (16–18.4)	19 (17.9–19.8)
Nerve ring from ant. end	73 (62.5–86.0)	86.5 (78.0–95.3)	81.3 (71.4–87.2)	75.2 (60.3–84.7)
Nerve ring cbd	18.2 (17.8–18.4)	19.2 (19.0–19.3)	17.8 (17.3–18.2)	18.7 (17.4–19.4)
mbd	18.5 (17.9–19.2)	20 (19.5–20.7)	18 (17.7–18.5)	123.3 (105–137.3)
Distance ant end-anus	449 (400–510.8)	475 (450–491.5)	469 (423–491)	445 (425.3–463.2)
v		379.4 (369.8–391)		354 (339–374)
v cbd		18 (18–19.6)		18 (17–20.5)
V		69.6 (67.2–73.3)		69 (68–70)
Right spicule length	26.7(22.8–32.6)		19 (17.2–22)	
Left spicule length	25.7 (23.3–31.2)		17.5 (15–19.3)	
Gubernac. length	11.7 (9.9–12.9)		7.7 (6.3–8.9)	
Apophyses	4.6 (3.6–6.3)		3.8 (3.5–4.0)	
Testes	210.6 (146.5–258.2)		244.4 (161.7–298.9)	
abd	17.9 (17.2–19)	17.1 (16.5–18.1)	17.4 (17.2–17.6)	16.9 (16.2–17.3)
Tail length	74.1 (70.7–80)	71.3 (62.4–74.4)	77 (70.2–81.5)	67.9 (69.2–70.2)

L Total body length, a total body length divided by maximum body width, b total body length divided by pharyngeal length, c total body length divided by tail length, c' tail length divided by anal body diameter, cbd corresponding body diameter, Amphid cbd corresponding body diameter at level of the amphid, Pharynx cbd corresponding body diameter, measured at the level of the end of pharynx, v distance from the anterior end to vulva, v cbd corresponding body diameter at the level of the vulva, abd anal body diameter

tion. Four small pre-cloacal bumps were found in some specimens; it is not clear whether they contain papilla. Tail is conical, ventrally curved. Three caudal glands opening in a terminal spinneret are present.

Females: Similar to males in most respects, sometimes slightly smaller but always a bit more robust. Inversion point of the annuli at the level of the vulva. Amphids are smaller and more oval shaped than those of males. Buccal cavity is longer

than in males (about 48.5  $\mu$ m). Reproductive system is monodelphic, prodelphic, with outstretched ovary. Ovary lies to the left side of the intestine. Spermatozoa frequently found in the spermatheca. Perivulvar gland cells are present. Ovejeter sclerotized. Vulva without operculum or particular sclerotization. Terminal spinneret with same morphology than in males.

Juveniles: Similar to adults in most morphological aspects.

**Table 3** Polytomous key<sup>a</sup> based on the somatic diagnostic characteristics<sup>b</sup> of *Rhynchonema* Cobb, 1920 species

<i>Rhynchonema</i>	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
<i>Amakusanum</i>	1	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0
<i>Ambianorum</i>	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	?	0	?
<i>Brevituba</i>	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Cemae</i>	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Ceramotos</i>	1	0	1	0	0	1	1	1	0	0	0	1	0	0	0	0	0	?	0	?	
<i>Chiloense</i>	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1
<i>Cinctum</i>	1	0	1	0	0	?	0	0	1	0	0	0	1	0	0	1	1	0	?	0	1
<i>Deconincki</i>	1	0	1	0	0	1	0	?	?	?	0	0	1	0	1	0	0	0	0	0	?
<i>Gerlachi</i>	1	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	?	0	?
<i>Hirsutum</i>	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0	?	0	?
<i>Longituba</i>	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Lyngei</i>	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	?	0	?
<i>Megamphidum</i>	1	0	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	?
<i>Moorea</i>	1	0	0	0	1	1	1	0	1	0	0	1	0	0	0	0	0	?	0	?	
<i>Ornatum</i>	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	0	?
<i>Ornatum antillensis</i>	1	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Quemer</i>	1	0	0	1	0	1	0	?	?	?	0	0	1	0	1	0	0	0	?	0	?
<i>Scutatatum</i>	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0
<i>Semiserratatum</i>	1	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	?
<i>Separatum</i>	1	0	?	?	?	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	?
<i>Sieverti</i>	1	0	1	0	0	1	1	1	0	1	0	0	1	0	0	1	0	0	1	1	1
<i>Subsetosum</i>	1	0	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0
<i>Xiamenensis</i>	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Falciferum</i>	?	?	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	?	0	?
<i>Collare</i>	0	1	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0
<i>Dispar</i>	0	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1
<i>Fossum</i>	0	1	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1
<i>Impar</i>	0	1	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	0	0	1
<i>Kikuchii</i>	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	1	0	0
<i>Tomakinese</i>	0	1	1	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0
<i>Tremendum</i>	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	?
<i>Veronicae</i>	0	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1

<sup>a</sup> 0 Absent, 1 present, ? not mentionend/not clear

<sup>b</sup> Labels: *A* Spicules symmetric, *B* Spicules asymmetric, *C* Distal end of spicules pointed, *D* Distal end of spicules in a claw, *E* Distal end of spicules bifid, *F* Presence of capitulum, *G* Gubernaculum with apophysis, *H* Amphid shape/size showing sexual dimorphism, *I* Amphids of equal size, *J* Amphids only slightly larger in males, *K* Amphids before the end of the pharyngostome, *L* Amphids at the end of the pharyngostome, *M* Amphids behind the end of the pharyngostome, *N* Amphids position showing sexual dimorphism, *O* Amphids with spiral structure, *P* Presence of pre-cloacal supplements, *Q* Lateral differentiation (ridges), *R* Annuli with vacuoles, *S* Vulva covered with flap/operculum, *T* Vulva flap “madreporite”, *U* Vulva annulation stronger than the rest of the body

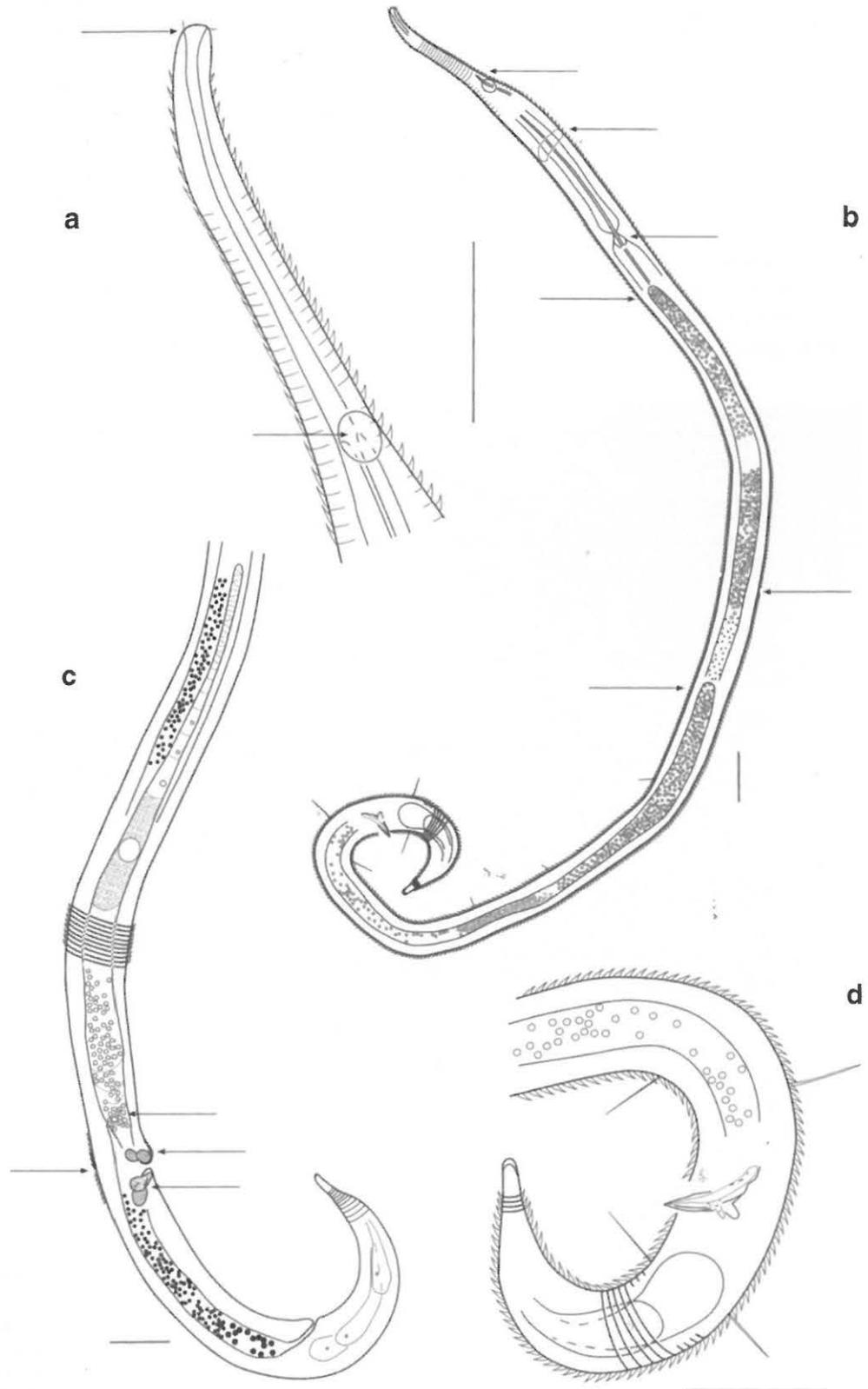
**Differential diagnosis:** This species is very close to *R. veronicae* sp. n. and *R. sieverti* Gourbault, 1982. It is clearly distinguishable from *R. veronicae* by the size and appearance of the cephalic setae, which are longer and much finer than those of *R. veronicae* sp.n.; by the *annuli* disposition at the inversion point of the cuticle, with nine *annuli* lower on the ventral side in contrast with just two *annuli* of *R. veronicae* sp.n.; by the shape of the spicules, which are slender and

uniform; by the symmetry of the spicules (symmetric); by the size of the spicules (average of 26.7  $\mu\text{m}$  for the right spicule and 25.7  $\mu\text{m}$  for the left one vs. 19  $\mu\text{m}$  for the right spicule and 17.5  $\mu\text{m}$  for the left one in *R. veronicae* sp.n.); by the symmetry of the gubernaculum (symmetric); by the size of the gubernaculum (average of 11.7  $\mu\text{m}$  vs. 7.7 in *R. veronicae* sp.n.); by the annulations around the vulva (not particularly stronger than the rest of the body vs. stronger than the rest of

the body in *R. veronicae* sp. n.). It differs from *R. sieverti* Gourbault, 1982 by the position of the amphids in relation to the pharyngostome (at the end of the pharyngostome in *R. cemaie* and behind the pharyngostome in *R. sieverti*); by

the absence of supplements in contrast with *R. sieverti* which shows four clear pre-cloacal supplements. In some specimens of *R. cemaie* sp. n. it was possible to see four bumps on the cuticle anterior to the cloaca but it is not clear if they bear papilla. In

**Fig. 6** *Rhynchonema veronicae* sp. n. **a** Male head showing the cheilostome and the amphid/end of the pharyngostome, **b** entire male head showing the position of the amphid, the receptor of the amphid sensory cell, the nerve ring, the cardia, the inversion point of the annules and the end of the anterior and the posterior testes, **c** female reproductive system (arrows point to spermatozoa in the spermatheca, the perivulvar glands and the inversion point of the cuticle), **d** male spicules and tail enlarged  
Scale bars: 20  $\mu$ m



addition, *R. cernae* has a simple vulva opening, instead of a vulva covered with a plate as in *R. sieverti* Gourbault, 1982. Although there is a small difference in the size of the spicules, their shapes are the same in these two species and we did not consider the small difference in size as an asymmetry.

**Ethymology:** The species is named in honor of Iracema de Melo Campina, aunt of the first author.

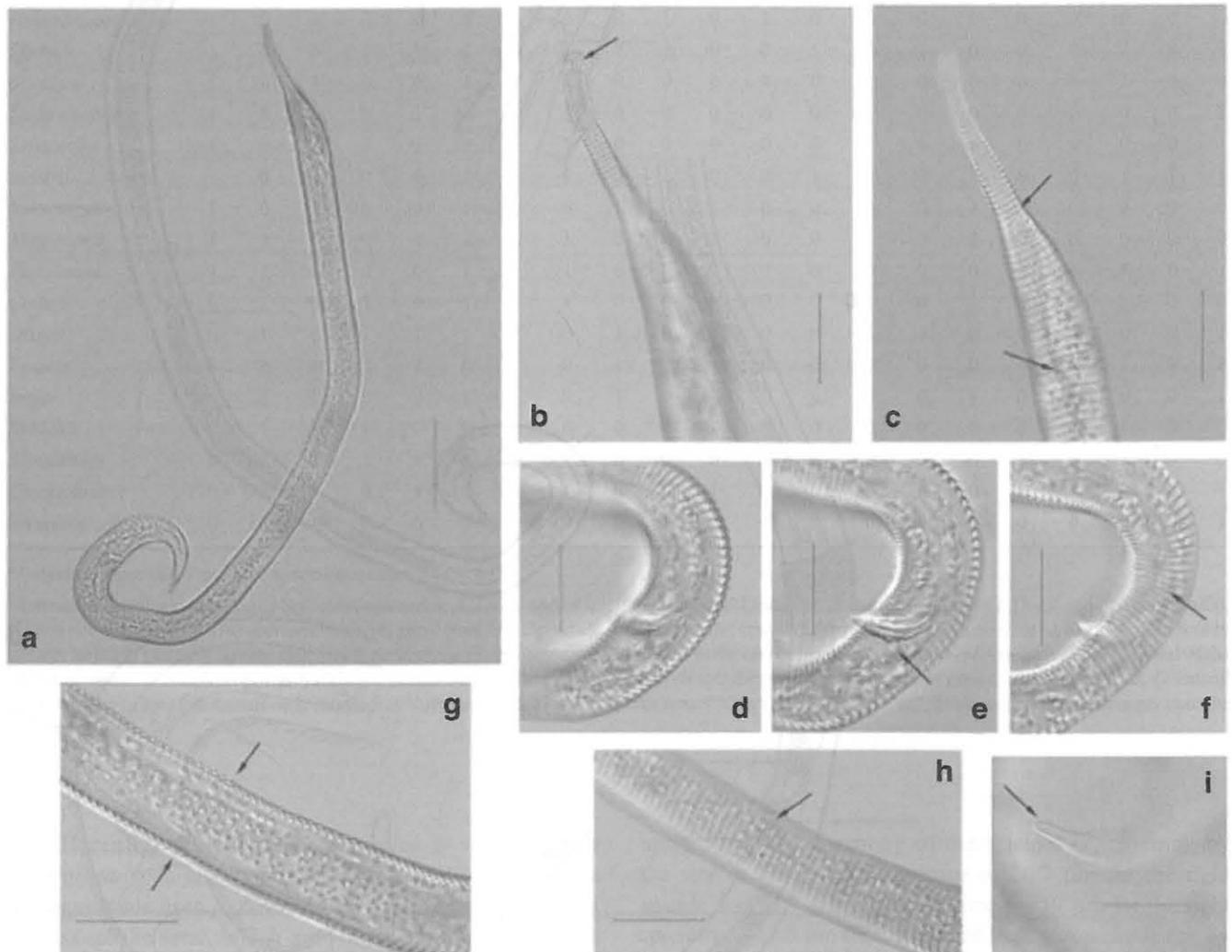
*Rhynchonema veronicae* sp. n. (Figs 6, 7, 8; Tables 1–3)

**Material examined:** Holotype male (Inventory No. UGMD 104282), five paratypes males (Inventory No. UGMD 104282 and UGMD 104283), six paratypes females (Inventory No. UGMD 104284 and UGMD 104285).

**Type habitat and locality:** Medium grain size sand, intertidal zone of exposed sandy beach, Olinda's Isthmus, Pernambuco, Brazil (8°2' 24"S; 34°51'48"W).

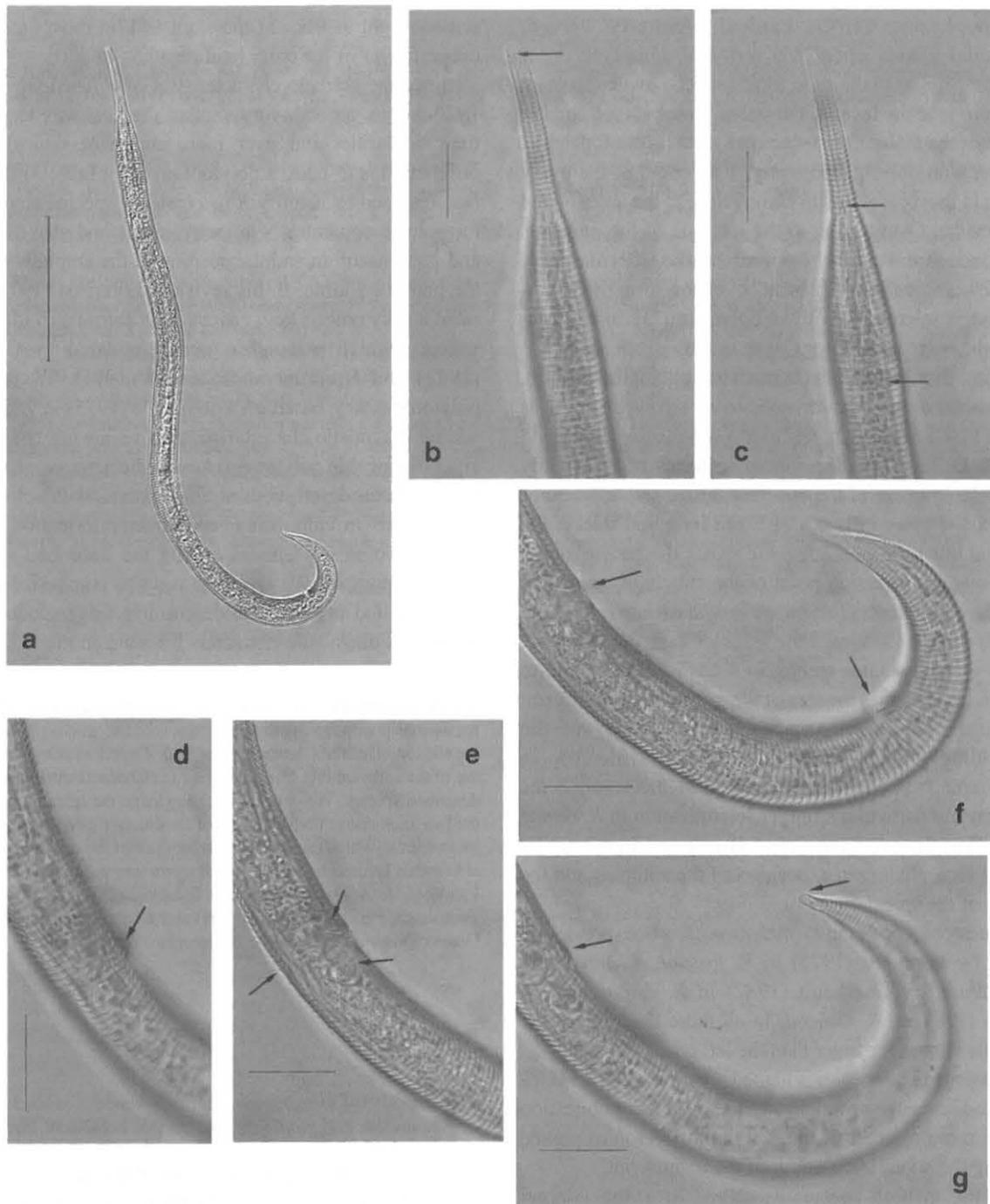
#### Description

**Males:** Body elongated, tapering sharply anteriorly to the amphids. Cuticle strongly annulated with annulations reaching the end of the cheilostome. *Annuli* showing a dentate shape laterally, sharply angled forward on the anterior half of the body and backward on the posterior half of the body. The inversion point of the *annuli* is located about 58 % of the body. The inversion point is two *annuli* lower at the ventral side. Somatic setae are about 11  $\mu\text{m}$  long, very thin and are distributed along the body in six longitudinal rows. Six cephalic setae of about 1.5  $\mu\text{m}$  length are present. Other sensilla are not visible. Amphids are almost circular (diameter 4.6  $\mu\text{m}$ , length 5.0  $\mu\text{m}$ ) and occupy 52.3 % of the corresponding body diameter; they are located at the base of the pharyngostome at a distance of 43  $\mu\text{m}$  from the anterior end. From the anterior end to the anterior border of the amphids there are 31–32 *annuli*. Unarmed stoma is about 45  $\mu\text{m}$  long in males, shows low lips, a short cheilostome and a narrow tubular pharyngostome.



**Fig. 7** *Rhynchonema veronicae* sp. n. Holotype male. **a** Entire body, **b** head (arrow points to the cheilostome, **c** head (arrows points to the receptor of the amphid sensory cell and to the nerve ring, **d** left spicule, **e** right spicule (arrow points to the dorsal posterior apophyses, **f** details of

the cuticle at the cloacal region, showing a local lateral differentiation, **g** inversion point of the cuticle, **h** detail of the cuticle at the inversion point, showing shift just by one annule form ventral to dorsal side, **i** spinneret. Scale bars 50  $\mu\text{m}$  (**a**), 20  $\mu\text{m}$  (**b–i**)



**Fig. 8** *Rhynchonema veronicae* sp. n. Paratype female. **a** Entire body, **b** head (arrow points to the cheilostome), **c** head (arrows point to the receptor of the amphid sensory cell and to the nerve ring), **d** arrow points to the cuticle sclerotization at the vulva level, **e** arrows point to the

perivulvar glands and to the inversion point of the annules on the dorsal side, **f** arrows point to the vulva and anus, **g** arrows point to the vulva, with stronger sclerotization than the rest of the body annules and the spinneret. Scale bars 50  $\mu\text{m}$  (**a**), 20  $\mu\text{m}$  (**b–g**)

Pharynx is cylindrical, slightly expanded at the posterior region. Cardia is of a small size, protruding into the cylindrical intestine. Outstretched testes are directed towards the anterior, with the anterior one lying to the left side of the intestine. Tip of the anterior testis is located around 45 % of the body length, from the anterior end. Posterior testis is to the right of the intestine. Spicules

are asymmetric, having almost the same length (average of right spicule 19  $\mu\text{m}$ ; left one 17.5  $\mu\text{m}$ ). The right spicule is more robust, with strong sclerotization and is also more curved than the left one. Posterior extremity of spicules has an arrow shape; capitulum of globular shape with open extremities resembles a sickle. Gubernaculum bears a sleeve with asymmetric postero-

dorsal apophyses. Tail is conical, ventrally curved. Three caudal glands open in a terminal spinneret.

Females: Similar to males in most aspects. Inversion point of the *annuli* is at the level of the vulva. Amphids are smaller than those of the males. Reproductive system is monodelphic, prodelphic, with outstretched ovary. The length of the ovary varies, but in most cases the tip comes close to the anterior end of the intestine. Ovary lying to the left side of the intestine. Spermatozoa are regularly found in the spermatheca. Perivulvar gland cells are present, ovejeter strongly sclerotized. Vulva is sclerotized without operculum. Terminal spinneret has the same morphology as in males.

Juveniles: Similar to adults in most morphological aspects.

**Differential diagnosis:** *Rhynchonema veronicae* sp. n. is very close to *R. cemaie* sp. n. and to *R. xiamenensis* Huang and Liu, 2002. Differs from the former in terms of its slightly stronger sclerotization of the cuticle; the size and appearance of the cephalic setae in males (1.5  $\mu\text{m}$  long and thicker vs. 2.0  $\mu\text{m}$  and much finer in *R. cemaie* sp.n.); the cuticle *annuli* disposition at the inversion point of the cuticle in males; the symmetry of the spicules (asymmetric with a more robust and curved left spicule vs. symmetric spicules in *R. cemaie* sp.n.); the shape of the spicules ending in a arrow versus uniform shape in *R. cemaie* sp.n.; the size of the gubernaculum (7.7  $\mu\text{m}$  vs. 1.7  $\mu\text{m}$  in *R. cemaie* sp.n.); the symmetry of the gubernaculum (apophyses asymmetric vs. symmetric in *R. cemaie* sp.n.); stronger sclerotization at the level of the vulva versus no particular stronger sclerotization in *R. cemaie* sp.n.. It differs from *R. xiamenensis* Huang and Liu, 2002 by the size of its cephalic setae, position of the amphids and the symmetry of the spicules.

Asymmetry of spicules in *Rhynchonema* has been previously described by Lorenzen (1975) in *R. fossum*, *R. impar* and *R. tremendum*, by Gourbault (1982) in *R. dispar* and by Aryuthaka (1989) in *R. kikuchii*. In all these descriptions the right spicule is stronger/larger than the left one.

**Ethymology:** The species is named after Prof. Dr. Verônica da Fonsêca-Genevois, originally co-author of this publication and the former promoter of the first author. Veronica passed way during the submission phase of the manuscript.

**Remarks:** In both species described here, the internal structures of the amphids were clearly visible. We were able to recognize the socket cell, sheath cell and the receptor of the sensory cell. In the male holotype of *R. cemaie*, the *corpus gelatum* was extruded from the amphideal fovea. In none of the previous descriptions is this mentioned, but it might have been overlooked.

*Rhynchonema* Cobb, 1920 was occasionally frequent in the soil samples collected from Olinda's Isthmus, reaching a maximum of 20 % of the frequency of occurrence of the total nematofauna. During a 1-year sampling period, *R. veronicae* was always more abundant than *R. cemaie*. Both species were found in all seven stations but they were more frequent at the

stations right in front of the slum and, in most cases, in the deepest layer of the corer (soil depth 5–10 cm).

Since the somatic characteristics of *Rhynchonema* Cobb, 1920 are, in general, very similar, the best way to point out their similarities and, even more important, their diagnostic differences is to build a polytomous key (Table 3). The main features used to identify *Rhynchonema* species are based on the spicule apparatus: symmetry, shape and size of spicules and gubernaculum and the position of the amphids related to the pharyngostome. In his revision, Lorenzen (1975) elaborated a polytomous key, generating a formula to identify the species, which was subsequently updated by Gourbault (1982) and Nicholas and Stewart (1995). We built this polytomous key based on Lorenzen's (1975) key, changing/adding diagnostic characteristics. In reviewing the literature and building this polytomous key we became aware that most of the species descriptions are incomplete, with some species descriptions providing only very limited information. The lack of information on females among the described species is quite remarkable. We therefore want to emphasize the need for a detailed taxonomical description that includes a large number of diagnostic characters for male and females.

**Acknowledgments** We thank Pierre Misseghers and Lisa Mevenkamp for their help with the translations of the German articles; Xiuqin Wu for the translation of the Chinese article; Sofie Derycke for her help with the use of the software PAUP in an attempt to elaborate a cladogram with the described species. We gratefully acknowledge the helpful comments of the two anonymous reviewers and of Dr. Gustavo Fonsêca, editor of this journal issue, which improved an earlier draft of this article. The research at Olinda's Isthmus by Tania Nara Bezerra was supported by FACEPE-Fundação de Amparo à Ciência e Tecnologia, Secretaria de Ciência e Tecnologia, PE (BPD-0088-2.05/94) and CNPq-Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (200720/95-3).

## References

- Allgen C (1940) Über einige neue freilebende Nematoden von der Nordwest- und Nordküste Norwegens. Folia Zool Hydrobiol 10: 443–449
- Aryuthaka C (1989) Two new species of the genus *Rhynchonema* (Nematoda, Xyalidae) from Amakusa, south Japan. Hydrobiologia 171(1):3–10. doi:10.1007/BF00005719
- Boisseau JP (1957) Technique pour l'étude quantitative de la faune interstitielle des sables. C R Congr Soc Savantes Paris Dep Sci 117 (119)
- Boucher G (1974) Six especes nouvelles du genre *Rhynchonema* (Rhynchonematinae–nematoda). Cah Biol Mar XV:447–463
- Bussau C (1993) Taxonomische und ökologische Untersuchungen an Nematoden des Peru-Beckens. Dissertation zur Erlangung des Doctorgrades der Mathematisch-Naturwissenschaftlichen. Fakultät der Christian-Albrechts-Universität zu Kiel, Kiel
- Calles-Procel AK (2007) Spatial and temporal patterns of meifauna along Ecuadorian sandy beaches, with a focus on nematode biodiversity. PhD thesis. Faculty of Sciences. Ghent University, Ghent
- Cobb N (1920) One hundred new nemas (type species of 100 new genera). Contrib Sci Nematol 9:1–64

- De Grisse AT (1969) Redescription ou modification de quelques techniques utilisées dans l'étude de nematodes phytoparasitaires. Meded Rijksfakulteit Landbouwwet Gent 34:351–369
- Fonseca G, Bezerra TN (2014) Order Monhysterida. Filipjev, 1929. In: Schmidt-Rhaesa A (ed) Handbook of zoology, 1st ed. De Gruyter, Berlin, pp 435–465
- Fonsêca-Genevois V (1987) Ecologie des méio-et-mixofaunes d'une vasière de l'estuaire de la Loire. Correlations avec le milieu sédimentaire et ses eaux interstitielles. Thèse de Doctorat d'Etat. Sciences Naturelles, Université de Nantes, Nantes
- Gerlach S (1953a) Die Nematodenbesiedlung des Sandstrandes und des Küstengrundwassers an der italienischen Küste I. Systematischer Teil. Archo Zool Ital 37:517–640
- Gerlach S (1953b) Die Nematodenfauna der Uferzonen und des Küstengrundwassers im Finnischen Meerbusen. Acta zool. Fenn Societas pro fauna et flora Fennica 73:1–32
- Gerlach S (1955) Zur kenntnis der freilebenden marinen nematoden von San Salvador. Z wiss Zool 158:249–303
- Gerlach SA, Riemann, F (1973) The Bremerhaven checklist of aquatic nematodes. A catalogue of nematoda Adenophorea excluding the Dorylaimida. Veröffentlichungen des Instituts für Meeresforschung Bremerhaven Suppl. Bremerhaven, Institute of Marine Research, pp 1–736
- Gourbault N (1982) Nematodes marins de Guadeloupe. I. Xyalidae nouveaux des genres *Rhynchonema* Cobb et *Prorhynchonema* nov. gen. Bull Mus natn Hist Nat., Paris 4, Section A n<sup>os</sup> 1-2:78–87
- Hopper B (1961) Marine nematodes from the coast line of the Gulf of Mexico. Can J Zool 39:1–7
- Huang H, Liu S (2002) One new species of free-living marine nematodes from southeastern beach of Xiamen Island. J Oceanogr. Taiwan Strait (Taiwan Haixia) 21(2):177–180
- King IW, Waumann D, De Ley P (2007) New record and SEM of *Rhynchonema ornatum* (Lorenzen, 1975) from the Sea of Cortez, Mexico, with notes on *Rhynchonema amakusanum* (Aryuthaka, 1989). In: Society of Nematologists 46th Annual Meeting. J Nematol 39(1):67–104
- Lorenzen S (1972) Die Nematodenfauna im Verklappungsgebiet für Industriabwasser nordwestlich von Helgoland I. Araeolaimida und Monhysterida. Zool Anz Leipzig 187:223–248
- Lorenzen S (1975) Rynchonema-Arten (Nematodes, Monhysteridae) aus Südamerika und Europa. Mikrofauna Meeresbodens 55:1–29
- Murphy D (1964) *Rhynchonema subsetosa*, a new species of marine nematode, with a note on the genus *Phylolaimus* Murphy, 1963. Proc Helminthol Soc Wash 31(1):26–28
- Nicholas W, Stewart A (1995) New genera, species and a new subfamily of Xyalidae (Nematoda, Monhysterida) from ocean beaches in Australia and Thailand. Trans R Soc S Aust 119(2):47–66
- Peso, M (2007) Electronic key for the species of the genus *Rhynchonema*. Available at: <http://nemys.ugent.be>. Version 1/2007. Accessed at [http://www.marinespecies.org/deblauwehans/aphia.php?p=idkeys\\_redirect&page=licence&taxon=400&keyid=65](http://www.marinespecies.org/deblauwehans/aphia.php?p=idkeys_redirect&page=licence&taxon=400&keyid=65)
- Vitiello P (1967) Deux nouvelles especes du genre *Rhynchonema* (Nematoda, Monhysteridae). Bull Soc Zool Fr 92(1):1–9
- Warwick R, Platt H, Somerfield P (1998) Free-living marine nematodes: part III Monhysterids: pictorial key to world genera and notes for the identification of British species. Synopses of the British fauna (New Series), 53. Field Studies Council, Shrewsbury
- Wieser W (1956) Free-living Marine Nematodes III. Axonolaimoidea and Monhysterioidea. Acta Univ Lund (NF 2) 52:1–115
- Wieser W (1959) Reports of the Lund University Chile expedition 1948–1949. 34. Free-living marine nematodes IV: General part. Lunds Universitets Årsskrift. Ny Följd Avd 2 55(5):1–112