

Taxonomy and Systematics of the Genus *Makatinus* Heyns, 1965 (Nematoda: Dorylaimida: Aporcelaimidae)

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Abstract: The taxonomy and the systematics of the genus *Makatinus* are discussed by means of the characterization of its morphological pattern and the first molecular (D2–D3 expansion segments of 28S rDNA) analysis of a representative of this taxon, *Makatinus crassiformis* from Costa Rica. The presence of two or more pairs of male ad-cloacal genital papillae is the most characteristic autapomorphy of the genus, but the status of its species on this concern differ among them. Both morphological and molecular data support a relationship with *Aporcelaimellus*, which, however, might not be as close as usually assumed. An emended diagnosis of the genus, a key to species identification, and a compendium of their morphometrics are provided. *Makatinus siddiqi* is regarded as *species inquirenda*, *Makatinus simus* is retained under *Eudorylaimus*, and *Makatinus tritici* becomes a junior synonym of *Aporcelaimellus tritici*.

Key words: Aporcelaimidae, compendium, diagnosis, key to species, *Makatinus*, molecular analysis, morphology, new synonym, systematics.

The genus *Makatinus* is an interesting dorylaimid taxon for several reasons. In spite of its worldwide spread, with occurrence in all the continents except Antarctica, it is a rather rare (infrequent) taxon. Nine of its 11 described species are only known from their type locality; one species (*Makatinus aquaticus*) was recorded twice in Europe, and another species (*M. crassiformis*) was found in four locations of two countries in tropical America. Among other features, its morphological pattern is characterized by the presence of at least two (occasionally more) pairs of male ad-cloacal genital papillae, an extraordinary (actually, exclusive) trait within the Dorylaimida, the male of their species having only one pair, this being an autapomorphy that separates the dorylaims from other nematode orders. After the original proposal by Heyns (1965) to accommodate two new species from South Africa, *Makatinus* has not been a matter of any taxonomical revision. It was (and currently is) classified under Aporcelaimidae (for instance, see Andrassy, 2009), probably an artificial (polyphyletic) family (cf. Holterman et al., 2008), but its relationships with other aporcelaimid genera have not been analyzed yet, and no molecular data of *Makatinus* species are hitherto available. The aim of this contribution is to analyze and discuss the diversity of *Makatinus* species and to update its taxonomy and systematics.

MATERIALS AND METHODS

Morphological analysis: The morphological pattern of the genus is studied through an exhaustive compilation of the available literature as relatively recent and ac-

ceptable descriptions are available for most species. These descriptions provide enough information and details to carry out a comparative analysis.

DNA extraction, polymerase chain reaction (PCR), and sequencing: Fresh material of *M. crassiformis* was obtained from a Costa Rican population (Varela et al., 2017) for sequencing and molecular analysis. Nematode DNA was extracted from single individuals as described by Castillo et al. (2003), and the D2 to D3 expansion segments of 28S rDNA were amplified using the D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (De Ley et al., 1999).

The PCR was performed with the addition of 2 µl of the extracted DNA to the PCR mix containing 1× PCR buffer (Dream Taq™ buffer), 200 µM of each dNTPs, 0.4 µM of each primer, 2 mM of MgCl₂, and 1.25 U of Dream taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA) to a final volume of 25 µl.

Amplification conditions consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 90 sec, and extension at 72°C for 2 min. A final extension was performed at 72°C for 5 min.

The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega), quantified using a Nanodrop spectrophotometer and used for direct sequencing in both directions using the abovementioned primers. The sequencing reactions were performed using the sequencing service from Macrogen Inc. (Seoul, Korea). The newly obtained sequences were submitted to the GenBank database under accession numbers KY492386, KY492387, KY492388, and KY492389.

Phylogenetic analyses: The obtained sequences were aligned with another dorylaimid 28S rRNA gene sequences available in GenBank using Muscle (Edgar, 2004). Outgroup taxa used for phylogenetic reconstruct were that used by Álvarez-Ortega et al. (2013) and Álvarez-Ortega and Peña-Santiago (2016). Sequence alignment was manually edited using Bioedit v7.2.5

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(Hall, 1999). Phylogenetic analyses were performed with Bayesian inference (BI) and Maximum Likelihood (ML) using MrBayes 3.1.2 (Ronquist et al., 2012) and MEGA 6 (Tamura et al., 2013), respectively. The best fit model of DNA evolution was obtained using jModelTest v2.1.10 (Darriba et al., 2012) with the Akaike Information Criterion (AIC). BI analysis was performed under the general time reversible model. The analysis was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 1×10^6 generations. The MCMC were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. ML analysis was implemented under the best-fitting evolutionary model GTR+G+I, obtained using the program MEGA 6, and 1000 bootstrap replications. The trees were visualized with the program Fig Tree v 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>) and drawn with Adobe Acrobat XI Pro 11.0.1.

RESULTS AND DISCUSSION

Historical outline

The genus *Makatinus* was proposed by Heyns (1965) under the family Aporcelaimidae to accommodate two new species, namely *Makatinus capensis* and *Makatinus punctatus*, both from South Africa, the latter of which became its type species. Other species were subsequently added to it, either as new forms or transferred from other genera. Heyns (1967) (see also Botha and Heyns, 1990) described a third South African species, *Makatinus macropunctatus*. Andr  ssy (1986) transferred *Dorylaimus crassiformis*, from Surinam. Ahmad and Ahmad (1992) described *Makatinus heynsi*, from India. Jim  nez-Guirado (1994) described *M. aquaticus*, from the Iberian Peninsula, renamed as *Makatinus goodeyi* the material described by Thorne and Swanger (1936) as *Dorylaimus tritici*, and transferred *M. macropunctatus* to *Aporcelaimellus*. Ahmad (1997) transferred *Aporcelaimus minor*, from Sri Lanka. Andr  ssy (2002) transferred *Dorylaimus simus*, from Bulgaria. Ahmad et al. (2003) described *Makatinus silvaticus*, from New Zealand. Shaheen and Ahmad (2004) described *Makatinus macrostylus*, from Costa Rica. Gantait et al. (2011) described *M. siddiqii*, from India. Very recently, Varela et al. (2017) have synonymized *M. macrostylus* with *M. crassiformis*.

Makatinus was originally characterized by having, among other features, cuticle without criss-cross lines but with superficial punctations, lips moderately amalgamated, strong odontostyle with aperture occupying about one-half its length, longitudinal vulva, caudal

region similar in both sexes, short and rounded, and male ventromedian supplements arranged in a double row. This concept (diagnosis) has not suffered relevant changes in more than five decades, but it has been refined in minor aspects by Jim  nez-Guirado (1994), Ahmad (1997) and Andr  ssy (2002, 2009).

Morphological pattern

Size: *Makatinus* are medium- to large-sized nematodes, whose body length ranges from 1.50 to 6.04 mm, but very often is up to 3.0 mm, and only three of its 11 species exceed, in total (*M. capensis* and *M. crassiformis*) or in part (*M. punctatus*), this limit.

Cuticle: Always two-layered, it consists of a thin outer layer with constant thickness throughout the body and a much thicker inner layer; this especially conspicuous at caudal region where it becomes even more widened. Both layers differ also in their aspect as the inner one is visibly more refringent. The outer layer bears fine transverse striation. Punctations are perceptible in several species (*M. crassiformis*, *M. minor*, and *M. punctatus*) as the inner layer has distinct radial striation, but they become weak or obscure in other species because radial striation is less conspicuous in them. Pores (dorsal, ventral, and lateral) abundant, although not always conspicuous, through the entire body.

Lip region: It shows some remarkable variation. Visibly tapering (one-sixth to one-fifth of the body diameter at the neck base) and nearly continuous with the adjacent body (Fig. 1E,K,L) in several species (*M. aquaticus*, *M. crassiformis*, *M. goodeyi*), nearly continuous but not visibly tapering (*ca* one-third of the body diameter at the neck base) in *M. heynsi* (Fig. 1D,J), *M. minor*, and *M. silvaticus*, and not tapering and offset by a weak but distinct constriction in *M. capensis* (Fig. 1A,B) and *M. punctatus* (Fig. 1C). Anterior margin truncate to slightly rounded. Lips always amalgamated and with hardly protruding papillae in those species displaying continuous lip region, barely more separated and with protruding papillae in the remaining forms. Oral aperture oval, dorso-ventral (Fig. 1B,J,K).

Odontostyle: Robust, with thick walls and large aperture. It is 17 to 40 μm long, but almost always between 20 and 30 μm , very exceptionally less, with only *M. crassiformis* surpassing this range (31 to 40 μm). It is almost always 4.0 to 6.0 times as long as wide, and longer (1.1 to 1.7 times) than the lip region diameter, but equal to lip region width in *M. minor*. Its walls appear unusually thick, in particular the anterior third of the ventral arm, becoming sometimes visibly massive (Fig. 1E). The aperture occupies *ca* one-half (42% to 55%) of the total length, seldom larger than this size.

Guiding ring: Simple, weakly refractive, and not very plicate.

Odontophore: Simple, rod-like in median view, and lacking any special differentiation. Its length about 1.5 (range 1.3 to 1.8) times the odontostyle.

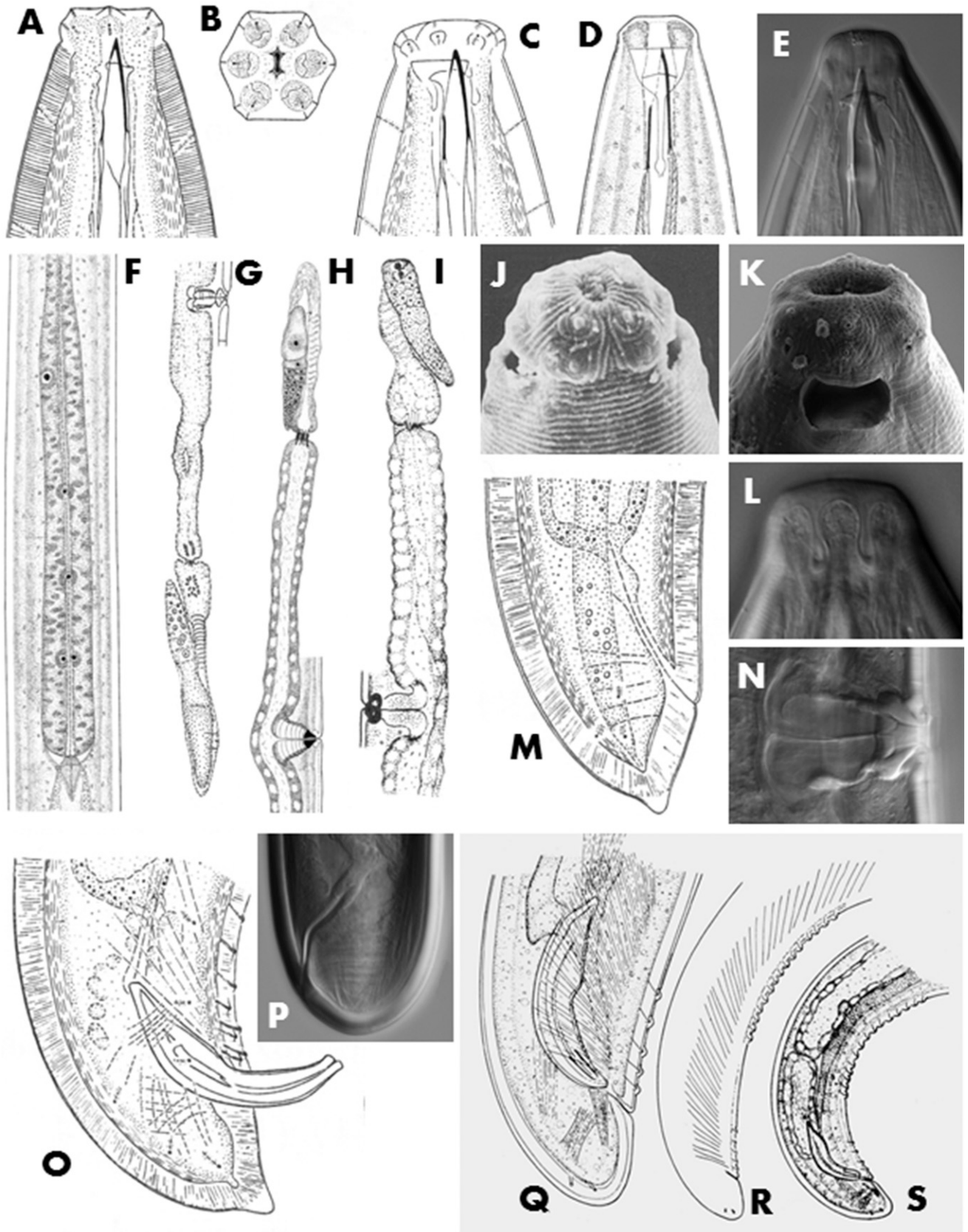


FIG. 1. General morphology of the genus *Makatinus* (Heyns, 1965). A to E, J to L, N. Lip region. F. Pharyngeal expansion, including gland nuclei. G to I. Female genital system. M, P. Female caudal region. N. Vagina. O, Q to S. Male posterior region. A, B, G, M, O. *Makatinus capensis* (after Heyns, 1965). C. *Makatinus punctatus* (after Heyns, 1965). D, H, J. *Makatinus heynsi* (after Ahmad and Ahmad, 1992). I, Q to S. *Makatinus aquaticus* (after Jiménez-Guirado, 1994). E, K, L, N, P. *Makatinus crassiformis*.

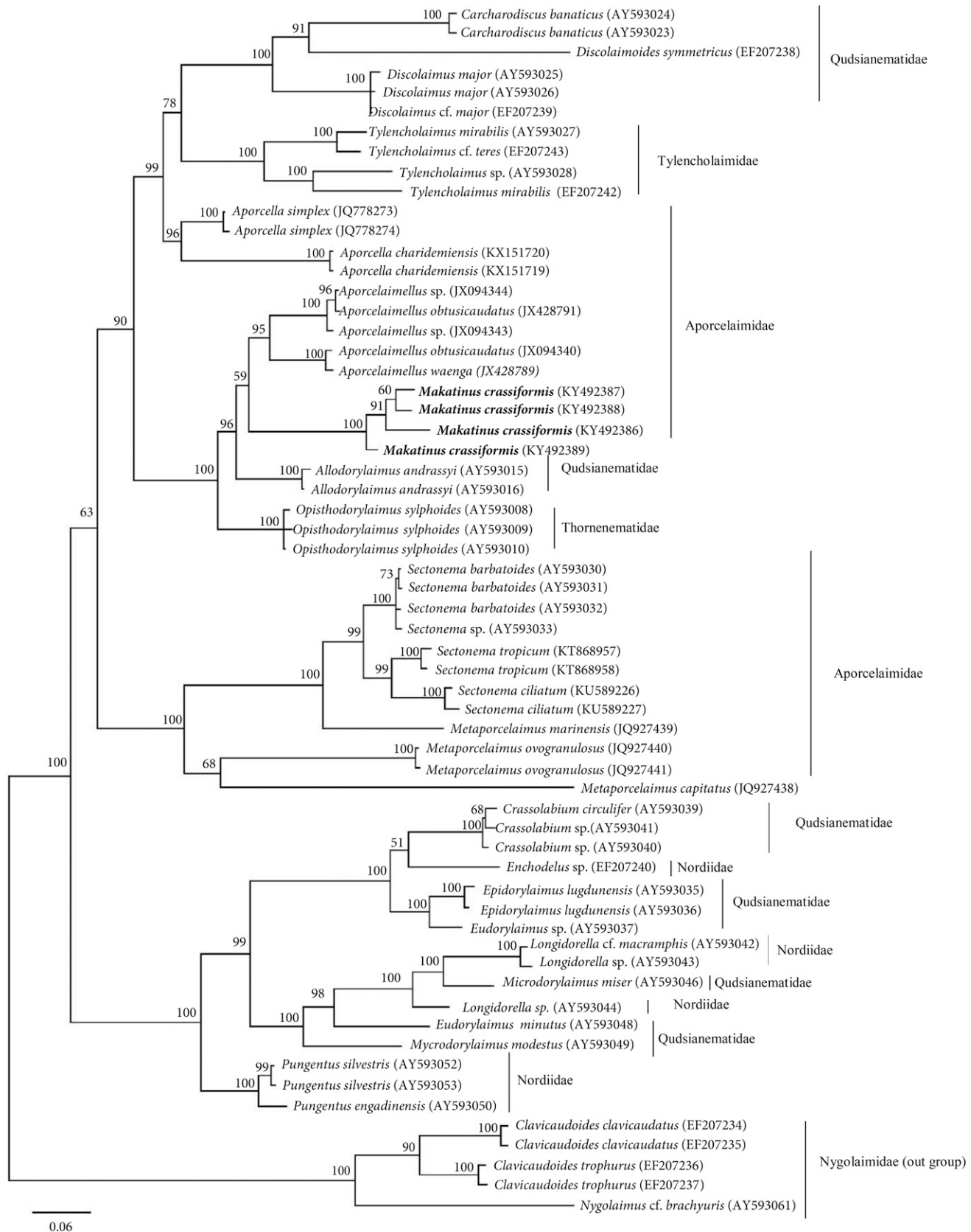


FIG. 2. Phylogenetic relationships of *Makatinus crassiformis*. Bayesian 50% majority rule consensus tree as inferred from D2 to D3 expansion segments of 28S rRNA gene sequence alignments under the GTR + G + I model. Posterior probabilities are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

Pharynx: Strongly muscular in its both sections, it enlarges very gradually (Fig. 1F). The basal expansion is comparatively long as it nearly always occupies more than half (52% to 62%) of the total neck length from the anterior end, but shorter (44% to 45% of total neck length) in *M. heynsi*. Gland nuclei: DO = 50 to 52, DN = 53 to 55 (62 in *M. minor*), S_1N_1 = 68 to 71 (61 to 66 in *M. silvaticus*), S_1N_2 = 75 to 77 (69 to 75 in *M. silvaticus*), S_2N = 84 to 87.

Pharyngo-intestinal junction: It basically consists of a conoid to conical cardia surrounded by intestinal tissue. A more or less developed ring-like structure (Fig. 1F) is present around the junction between the pharyngeal base and the intestine.

Female genital system: It is invariably didelphic-amphidelphic, with both genital branches equally and well developed. Oviduct with the usual two sections fairly distinct, and *pars dilatata* often differentiated. Uterus a tube-like structure, variably long and usually simple (Fig. 1H,I), but complex in *M. minor* and *M. punctatus* (Fig. 1G). *Pars refringens vaginae* present, exceptionally absent in *M. simus* (but see below), with conspicuous sclerotized pieces. Vulva transverse in general, but longitudinal in *M. crassiformis* (Fig. 1N).

Male genital system: Typical dorylamid. Genital papillae (supplements) with a singular arrangement. Ad-cloacal papillae two or more (up to five) pairs (Fig. 1O,Q–S). Ventromedian supplements a series of 8 to 23, contiguous (*M. aquaticus*), shortly spaced (*M. goodeyi*) or widely separate (*M. heynsi*, *M. punctatus*, and *M. silvaticus*), with (*M. aquaticus* and *M. goodeyi*) or without (*M. heynsi*, *M. punctatus*, and *M. silvaticus*) hiatus. Spicules typical dorylamid, 47 to 83 μ m long.

Caudal region: Similar in both sexes. It is short (c' -ratio under 1.0), convex conoid to rounded or nearly hemispheroid (Fig. 1P–S). Exceptionally, the type species (*M. punctatus*) displays a somewhat more conical tail because of the existence of a little terminal, hyaline projection (Fig. 1M,O). *Makatinus siddiqii* shows a typical conical, straight tail ($c' = 1.3$ to 1.4) and *M. simus* also a conical tail with a terminal digitation and dorsal concavity, but the status of these species as true *Makatinus* needs confirmation.

Taxonomy

Diagnosis (emended): Medium- to large-sized nematodes, 1.50 to 6.04 mm long, but very often under 3.0 mm. Cuticle two-layered, its inner layer very thick, visibly more refringent than the outer one, and occasionally bearing punctations. Lip region continuous with the adjacent body to offset by a weak constriction; lips amalgamate or fairly separate. Amphid fovea cup- to funnel-shaped, its aperture occupying about one-half of the corresponding body diameter. Odontostyle strong, with thick walls, especially the anterior half of its ventral arm, often somewhat longer than the lip region diameter and with wide aperture occupying about one-half of its total length. Odontophore linear, without any

singular differentiation. Pharynx very muscular, enlarging rather gradually, with the basal expansion occupying usually more (up to three-fifths) of the total neck length. Pharyngo-intestinal junction surrounded by a more or less developed ring-like structure. Female genital system didelphic-amphidelphic; uterus usually a variably long but simple tubelike structure, but tripartite in two species; *pars refringens vaginae* present; vulva transverse, occasionally longitudinal. Caudal region similar in both sexes, very often short and rounded to hemispheroid, exceptionally somewhat conical. Spicules 47 to 83 μ m long, typical dorylamid. Male genital papillae arranged in two to five ad-cloacal pairs and a series of 5 to 23 ventromedian supplements often widely spaced but occasionally contiguous, and with or without hiatus.

Comparison with other genera: *Makatinus* resembles *Aporcelaimellus* and *Aporcelaimus* Thorne and Swanger, 1936 in some aspects. Nevertheless, it differs from this two genera in its lip region very often continuous (vs. almost always offset by deep constriction), with amalgamate (vs. separate) lips; and two or more pairs (vs. only one pair) of male pre-cloacal genital papillae. Besides, it can be distinguished from *Aporcelaimellus* in the nature of their cuticle: two- vs. three-layered, and without vs. with a visible lacuna at the tail tip. From *Aporcelaimus* in the nature of the odontostyle aperture about one-half (vs. largely more than one-half) of its total length.

List of valid species

- M. aquaticus* Jiménez-Guirado, 1994
- M. capensis* Heyns, 1965
- M. crassiformis* (Kreis, 1924) Andrassy, 1986
= *Dorylaimus crassiformis* Kreis, 1924
= *Makatinus macrostylus* Shaheen & Ahmad, 2004
- M. goodeyi* Jiménez-Guirado, 1994
= *Dorylaimus tritici apud* Thorne and Swanger (1936), nec Bastian (1865)
- M. heynsi* Ahmad & Ahmad, 1992
- M. minor* (Loos, 1945) Ahmad, 1997
= *Aporcelaimus minor* Loos, 1945
- M. punctatus* Heyns, 1965
- M. silvaticus* Ahmad, Sturhan & Wouts, 2003

Species inquirenda

- M. siddiqii* (Gantait, Tanmay & Amalendu, 2011)

Key to identification of valid species

- 01a. Odontostyle very strong, more than 30 μ m long *crassiformis*
- 01b. Odontostyle not so strong, less than 30 μ m long 2
- 02a. Larger general size, body length 3.61 to 3.77 mm; lip region broader (30 μ m wide); male unknown *capensis*
- 02b. Smaller general size, body length less than 3.5 mm; lip region narrower (16 to 23 μ m broad); male known 3

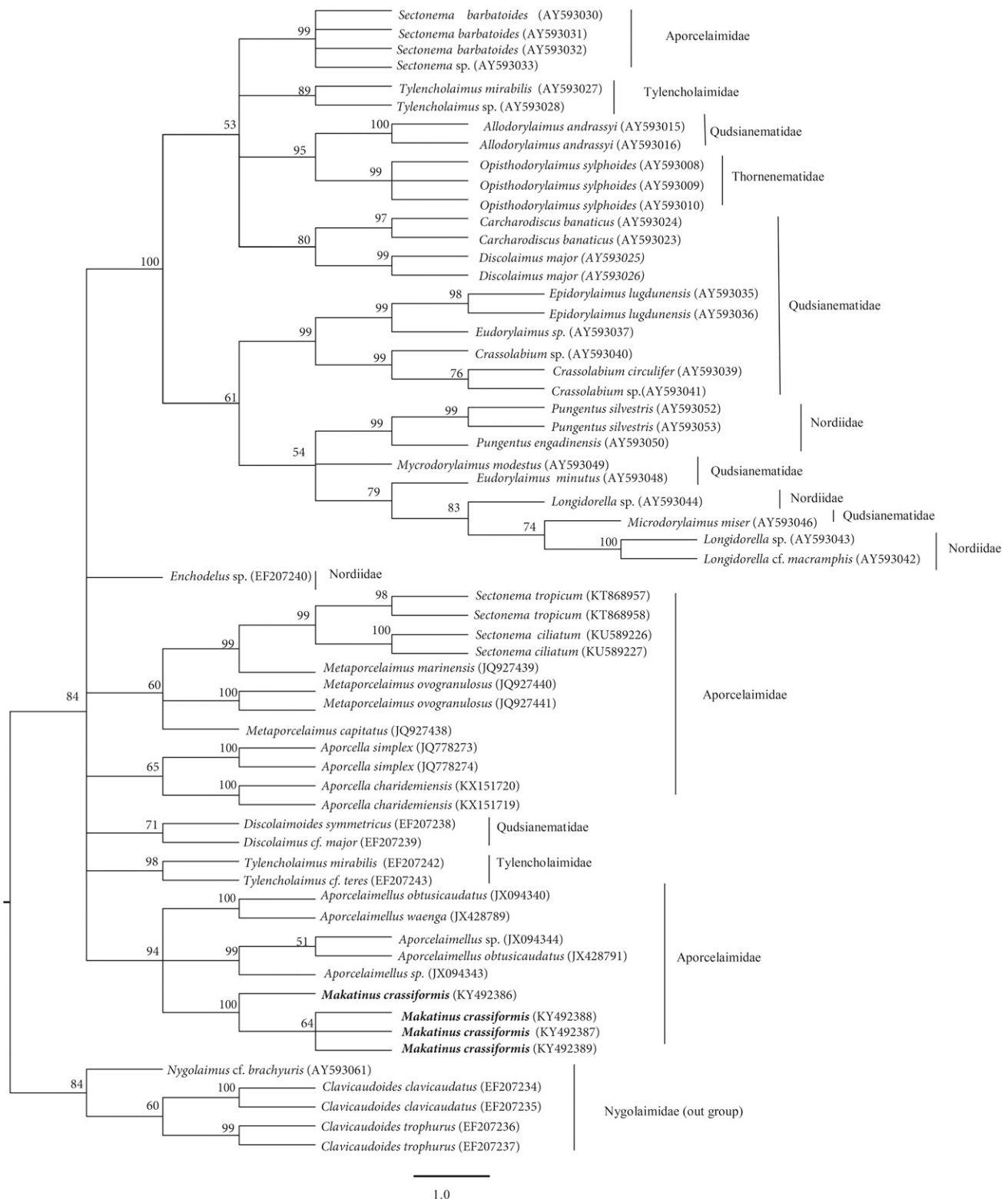


FIG. 3. Phylogenetic relationships of *Makatinus crassiformis*. Maximum likelihood tree as inferred from D2 to D3 expansion segments of 28S rRNA gene sequence alignments under GTR + G model. Bootstrap values are given for appropriate clades. Newly obtained sequences are indicated by bold letters.

03a. Lip region offset by weak constriction; tail nearly conical, with a short terminal digitation *punctatus*
 03b. Lip region continuous or offset by shallow depression; tail short and rounded 4

04a. Three to five pairs of ad-cloacal supplements; 15 to 17 ventromedian supplements *aquaticus*
 04b. Two pairs of ad-cloacal supplements; 8 to 12 ventromedian supplements 5

TABLE 1. Relevant morphometrics of *Makatinus* species as well their geographical distribution.

| Species | n | L | a | b | c | c' | V | lrw | odont. | neck | ph. exp. | abw | perect. | tail | spicul. | ve. sup. | geog. dis. | Refer. |
|---------------------|------|------------|--------|----------|---------|----------------------|-------|-----------------|--------------------|-----------------------|----------|-----------------|---------|---------------------|-----------------|----------|--------------|--------|
| <i>aquaticus</i> | 10♀♀ | 1.92–2.26 | 27–35 | 3.3–4.1 | 71–88 | 0.5–0.8 | 54–59 | 16–19 | 22–26 | 512–636 | 54%–66% | | 69–158 | 24–29 | 73–83 | 15–17 | Spain | 1 |
| | 5♂♂ | 2.02–2.58 | 25–31 | 3.5–4.0 | 63–86 | 0.7–0.9 | | 16–19 | 22–26 | 536–603 | 57–64 | | 95–127 | 29–34 | | | | |
| | 2♀♀ | 1.72–2.00 | 24–26 | 3.7–4.1 | 56–90 | 0.6–0.7 | 50–51 | 16 | 21–22 | 440–485 | | | | 25–26 | | | Hungary | 2,3 |
| | ♂ | 1.88 | 26 | 4.1 | 62 | 0.7 | | | | | | | | 30 | 70 | 16 | | |
| <i>capensis</i> | 4♀♀ | 3.61–3.77 | 32–34 | 4.4–5.0 | 103–127 | 0.5–0.6 ^a | 48–50 | 30 ^a | 24–28 ^a | 754 ^a | | 50 ^a | | 37 ^a | | | South Africa | 4 |
| <i>crassiformis</i> | 8♀♀ | 2.45–3.92 | 21–27 | 3.6–4.3 | 97–152 | 0.4 | 41–63 | | | 818 ^a | | | | 26 ^a | | | Surinam | 5, 6 |
| | 4♀♀ | 3.16–4.67 | 20–27 | 4.3–5.8 | 125–183 | | | | 31–40 | | | | | | | | Surinam | 7 |
| | 6♀♀ | 3.35–3.70 | 21–24 | 3.6–4.2 | 71–95 | 0.5–0.7 | 38–47 | 27–28 | 38–40 | 801–959 | 519–607 | 72–80 | 132–178 | 39–49 | | | Costa Rica | 9 |
| | 10♀♀ | 3.04–6.04 | 22–33 | 3.9–5.6 | 77–172 | 0.3–0.6 | 37–44 | 29–32 | 30–40 | 769–1161 | 429–661 | 61–101 | 107–190 | 22–47 | | | Costa Rica | 15 |
| <i>goodi</i> | ♂ | 2.2 | 30 | 4.5 | 71 | 0.6 ^a | | 18 ^a | 22 ^a | 484 ^a | | | | 26 ^a | 55 ^a | 9–12 | UK-England | 6 |
| <i>heynsi</i> | 10♀♀ | 1.96–2.42 | 37–43 | 4.0–5.0 | 82–110 | 0.6–0.7 | 46–52 | 15–16 | 21–25 | 472–518 | | 35–41 | 114–156 | 22–28 | | | India-Goa | 8 |
| | 5♂♂ | 2.04–2.29 | 39–43 | 4.3–4.8 | 71–90 | 0.6–0.8 | | | 20–23 | 462–501 | | 35–39 | 157–172 | 26–30 | 57–67 | 9–11 | | |
| <i>macrostylus</i> | 6♀♀ | 3.35–3.70 | 21–24 | 3.6–4.2 | 71–95 | 0.5–0.7 | 38–47 | 27–28 | 38–40 | 801–959 | 519–607 | 72–80 | 132–178 | 39–49 | | | Costa Rica | 9 |
| <i>minor</i> | 9♀♀ | 1.76–2.24 | 30–40 | 3.4–4.6 | 61–85 | 0.6 | 49–53 | 21–23 | 19–28 | 518–590 | 269 | 44–46 | 73–86 | 15–29 | | | | 10–12 |
| | 5♂♂ | 1.77–2.29 | 36–41 | 3.7–4.8 | 74–92 | | | | | | | | | | 50 | 8–9 | Sri Lanka | |
| <i>pundatus</i> | 7♀♀ | 2.40–3.17 | 36–42 | 4.2–4.4 | 84–99 | 0.7 ^a | 56–59 | 23 ^a | 25–27 | 729 ^a | | | | 31 ^a | | | South Africa | 4 |
| | 2♂♂ | 2.79, 3.37 | 40, 45 | 4.9, 4.7 | 91, 89 | | | | | 569, 717 ^a | | | | 31, 38 ^a | 75–80 | 21–23 | | |
| <i>siddiqi</i> | 9♀♀ | 1.5–1.9 | 31–34 | 3.3–3.9 | 39–41 | 1.3–1.4 | 56–67 | 13–15 | 18–20 | 478–490 | | 31–34 | 54–64 | 39–47 | | | India | 13 |
| <i>sitaticus</i> | 5♀♀ | 2.35–2.72 | 32–37 | 4.0–4.3 | 84–96 | 0.5–0.6 | 50–53 | 19–20 | 25–28 | 586–652 | 343–377 | 47–53 | 88–180 | 25–30 | | | New Zealand | 14 |
| | 4♂♂ | 2.29–2.50 | 34–38 | 3.8–4.4 | 70–73 | 0.6–0.7 | | 19–20 | 25–26 | 519–652 | 292–371 | 49–53 | 142–220 | 32–35 | 72–78 | 11–12 | | |

^a Morphometrics calculated from original illustrations and/or other morphometrics.Measurements in μm , except L in mm.

L, a, b, c, c', V = Dermanian ratios; lpr = lip region width; odont. = odontostyle length; neck = neck length; ph. exp. = pharyngeal expansion length; abw = anal body diameter; perect. = preectum length; tail = tail length; spicul. = spicules length; ve. sup. = number of ventromedian supplements; geog. dis. = geographical distribution (country); Refer. = corresponding bibliographic reference.

References: 1 - Jiménez-Guizado (1994); 2 - Andrassy (2002); 3 - Andrassy (2009); 4 - Heyns (1965); 5 - Kreis (1924); 6 - Thorne and Swanger (1936); 7 - Micoletzky (1925); 8 - Ahmad and Ahmad (1992); 9 - Shaheen and Ahmad (2004); 10 - Loos (1945); 11 - Ahmad (1997); 12 - Alvarez-Ortega and Peña-Santiago (2011); 13 - Cantait et al. (2011); 14 - Ahmad et al. (2003); 15 - Varela et al. (2017).

05a. Ventromedian series of supplements contiguous and distinctly separate (bearing large hiatus) from the ad-cloacal pairs *goodeyi*

05b. Ventromedian series of supplements widely spaced and not distinctly separated (lacking hiatus) from the ad-cloacal pairs 6

06a. Body 2.29 to 2.72 mm long; odontostyle 25 to 28 μ m long; spicules 72 to 78 μ m long *silvaticus*

06b. Body up to 2.40 mm long; odontostyle up to 25 μ m long; spicules up to 67 μ m long 7

07a. Lip region 15 to 16 μ m broad; uterus about three body diameters long *heynsi*

07b. Lip region 21 to 23 μ m broad; uterus 1.5 to 1.9 body diameters long *minor*

Table 1 provides a compendium of the most relevant morphometrics of *Makatinus* species as well their geographical distribution.

Status of the species: Five of them (*M. aquaticus*, *M. goodeyi*, *M. heynsi*, *M. punctatus*, and *M. silvaticus*) perfectly fit the *Makatinus* pattern as their males are known and bear more than one pair of ad-cloacal genital papillae. *Makatinus punctatus*, however, differs from the other species of this group by its more conical (vs. short and rounded to hemispheroid) tail. *Makatinus aquaticus* is distinguishable by its 3 to 5 (vs. 2) pairs of male ad-cloacal genital papillae. The specimens described by Andr  ssy (2002) as *M. aquaticus* from Hungary differ from the Iberian type population of this species in their more anterior ($V = 50$ to 51 , $n = 2$ vs. $V = 55$ to 59 , $n = 10$) and (more important) transverse (vs. longitudinal) vulva; thus, there is a doubt about the co-specificity of both populations.

Makatinus minor needs further studies to confirm its true identity as the existence of more than one pair of male ad-cloacal genital papillae was not mentioned in its original description.

Two species (*M. capensis* and *M. crassiformis*) lack male, a fact that come up with a question about its precise identity. Nonetheless, their general morphology fits well the pattern of the genus in other aspects.

There is no solid evidence to support the assignment of *M. siddiqii* to *Makatinus*. Leaving aside the absence of males in this species, several features (odontostyle not especially strong, very short uterus, and conical tail much longer than the anal body diameter) suggest it might be more compatible with *Metaporcelaimus* pattern. Thus, this species is maintained under *Makatinus* but regarded as *species inquirenda*.

The transference of *Aporcelaimellus simus* (Andr  ssy, 1958, 1986) to *Makatinus* by Andr  ssy (2002) is probably not justified. On one hand, at least two features, namely, vagina lacking *pars refringens* and male with only one pair of ad-cloacal genital papillae do not fit the genus pattern. On the other hand, the general morphology of this species, including nearly continuous lip region, relatively strong odontostyle, conical tail with

terminal short digitation dorsad bent—a dorsal concavity is easily perceptible in both sexes at its terminal part—and male with few (five) and widely separate ventromedian supplements is more compatible with that observed in some *Eudorylaimus* representatives; therefore, this species is herein retained under this genus.

  lvarez-Ortega and Pe  a-Santiago (2013) transferred *Aporcelaimellus tritici* (Bastian, 1865) Andr  ssy, 1986 [= *Dorylaimus tritici* Bastian, 1865; *Eudorylaimus tritici* (Bastian, 1865) Andr  ssy, 1959] to *Makatinus* mainly on the base of description of this species by Thorne and Swanger (1936), but overlooking Jim  nez-Guirado's (1994) previous action in the same sense. As Jim  nez-Guirado (1994) pointed out, the original material of *D. tritici* is not conspecific with the specimens described by Thorne and Swanger (1936) and certainly belongs to *Aporcelaimellus*. Thus, *M. tritici* becomes a junior synonym of *A. tritici* and the latter a valid species within *Aporcelaimellus*.

Systematics: Under a cladistics approach, *Makatinus* pattern is characterized by a very remarkable autapomorphy within the whole order Dorylaimida: the possession of more than one pair of male ad-cloacal genital papillae (see additional comments above). The totally fused lips should be interpreted as a synapomorphic condition too, only occasionally found in aporcelaims, for instance in a few *Sectonema* species. The morphology of the odontostyle (strong, with thick walls, especially the anterior third of its ventral arm, and with aperture occupying *ca* one-half of its length) might also be regarded as a synapomorphic condition, but this should be taken with more caution because of its less objective characterization. Thus, *Makatinus* probably is a natural (monophyletic) taxon, but the status of its species differs among them.

The analysis of four D2 to D3 28S rRNA, 735 to 780 bp sequences of *M. crassiformis* has allowed molecular exploration of the evolutionary relationships of this genus for the first time. The corresponding results are presented in Figs. 2 and 3. *Makatinus* sequences are grouped together with those of *Aporcelaimellus*, *Allodorylaimus* Andr  ssy, 1986, and *Opisthodorylaimus*, showing a closer, but not very robust (PP = 59) relationship with *Aporcelaimellus*, the other member of Aporcelaimidae in this group as *Allodorylaimus* and *Opisthodorylaimus* Ahmad and Jairajpuri, 1982 belong to the families Qudsianematidae and Thornenematidae, respectively. Besides, the results obtained confirm the previous ones (among others, Holterman et al., 2008;   lvarez-Ortega and Pe  a-Santiago, 2016) regarding the polyphyly of Aporcelaimidae and the problems to elucidate the relationships among the members of the suborder Dorylaimina.

Both morphological and molecular evidence suggests a (close) relationship among *Makatinus* and *Aporcelaimellus*, although with low PP in BI, which should be confirmed in the future, especially when new

Makatinus species are sequenced and/or sequences of other genes are available for both genera. The morphological differences among these genera are small in appearance, but probably more relevant than initially assumed, this being a possible cause that explains the low PP value obtained in the molecular analysis. Leaving aside the presence of more than one pair of adcloacal pair of male genital papillae, a very peculiar autapomorphy of *Makatinus*, the nature of lip region and odontostyle displays some differences among *Makatinus* and *Aporcelaimellus* in spite of the existence of an appreciable intrageneric variability in both genera concerning these two features.

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