

# *Encotyllabe spari* do not live in Brazil: Morphological and molecular evidence confirm two new species of *Encotyllabe* (Monogenea: Capsalidae) from Brazil

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## Research

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# Abstract

## Background

Currently, 24 species of *Encotyllabe* Diesing, 1850 (Monogenea: Capsalidae) are listed in WoRMS, but the validity of many species has been questioned due to deficient or incomplete descriptions. On the other hand, almost all species in the genus have been described from one host species or closely related host species, suggesting host specificity, but other species, specifically *Encotyllabe spari* Yamaguti, 1934, have been reported at least from 19 species belonging to nine families in two orders (Perciformes and Scorpaeniformes) from Japan, the Arabian Gulf and Brazil. Concerning Brazilian records of *Encotyllabe spari* (but also as *Encotyllabe* cf. *spari*); seven host species belonging to four families and two orders have been reported as hosts for this species. The aim of this study was to describe two new species of *Encotyllabe* from Brazil, previously considered as *E. spari*.

## Methods

During 2016, we examined specimens of *Orthopristis ruber* (Cuvier) (Haemulidae) and *Pagrus pagrus* (Linnaeus) (Sparidae) caught off the coast of Cabo Frio, Rio de Janeiro, Brazil by local fishermen. Specimens of *Encotyllabe* were collected from the pharyngeal plates of the hosts. Morphological and morphometric (multivariate analysis of proportional measurements standardized by total length) and molecular analysis (*LSU* rRNA and *cox1* gene) were performed in order to identify the collected monogenea.

## Results

The presence of two new species of *Encotyllabe*, *Encotyllabe yamagutii* n. sp. and *Encotyllabe haemuli* n. sp., parasitizing the pharyngeal plates of *Pagrus pagrus* and *Orthopristis ruber*, respectively, is strongly suggested by the three approaches used in this study. The main morphological differences from the most related species include a combination of body size, shape of the penis, and size and position of the testes.

## Conclusions

Specimens of *Encotyllabe*, hitherto recorded as *E. spari* or *E. cf. spari*, belong to two new species. Our results suggest that the host specificity for members of *Encotyllabe* and specimens registered as *E. spari*, other than those from the original description, must be revisited.

# Background

To date, 24 species of *Encotyllabe* Diesing, 1850 (Monogenea: Capsalidae) are listed in WoRMS [1]; of those, *E. masu* Ishii and Sawada, 1938, described from a Salmonid, should not be taken into consideration [2], and *Encotyllabe callaoensis* Tantalean, 1974 was described in an unavailable, short-lived journal (just a single issue). Thus, both species are not considered herein as valid. The first four species described in the genus, namely, *E. nordmanni* Diesing, 1850, from *Brama rail* (Bonnaterre) in the Mediterranean Sea; *E. pagelli* Van Beneden and Hesse, 1863, from *Pagellus centrodontus* (Brünnich) in Brest, France; *E. paronae* Monticelli, 1907, from *Crenilabrus pavo* (Linnaeus), in Geneve; and *E. valleii* Monticelli, 1907, from *Chrysophrys aurata* (Linnaeus) in Trieste, were so briefly described or illustrated that their similarities to, and differences from, other species are difficult to assess, and thus they should be considered provisional species. For instance, and as previously noted [3], *E. nordmanni* was described as “longit. Corp 11/2, latit 1/2, longit. pedic. Acet 1/2.” The same applies for *E. pricei*, *E. chironemi*,

*E. lintoni*, *E. monticelli*, *E. pricei*, *E. fotedari* and *E. punctatai*, which have been considered species inquirenda because they were poorly described and are based on only one or two specimens [3–9]. *E. lintoni* was described based on one anomalous specimen bearing one testis [10] and redescribed [11] based on the same specimen from the original description. *E. chironemi* was described from one specimen from *Chironemus spectabilis* (= *Cheilodactylus spectabilis* Cheilodactylidae) caught in Cook Strait, New Zealand [9] and redescribed on the basis of ten measured specimens obtained from the related *Nemadactylus macropterus* caught in Coffs Harbour, Australia, more than 2200 km apart from the type locality [8].

The status of those provisional species must be confirmed following redescrptions based on specimens from the type host and locality due to the expected host specificity of Capsalids [12] as well as the appreciable geographic variation observed in some species of monogeneans [13, 14]. Consequently, 12 species of *Encotyllabe* are considered herein as valid: *E. antofagastensis* Sepúlveda, González & Oliva, 2014, *E. caballeroi* Velasquez, 1977, *E. carangis* Pillai and Pillai, 1976, *E. caranxi* Lebedev, 1967, *E. cheilodactyli* Sepúlveda, González & Oliva, 2014, *E. embiotocae* Noble, 1966, *E. kuwaitensis* Khalil and Abdul-Salam, 1988, *E. lutjani* Tripathi, 1959, *E. pagrosomi* MacCallum, 1917, *E. suozalimae* Carvalho and Luque, 2012, *E. spari* Yamaguti, 1934 and *E. xiamenensis* Li, Yan & Wang, 2004. The most reliable taxonomic criteria for mature specimens of *Encotyllabe* are, among others, body shape, relative size of several organs, shape and position of testes, distance between the center of the ovary and center of the testis, penis shape and length of pedicel when extended [3–9]. However, multivariate analysis of proportional measurements standardized by total length as well as molecular studies will strongly improve the taxonomy of *Encotyllabe* [3, 15].

The following species of *Encotyllabe* have been recorded from marine fish in Brazil: *Encotyllabe spari* in *Haemulon sciurus*, *Orthopristis ruber*, *Anisotremus surinamensis*, *Conodon nobilis* (Haemulidae), *Pagrus pagrus* (Sparidae), *Menticirrhus americanus*, *Micropogonias furnieri* (Sciaenidae) and *Dactylopterus volitans* (Dactylopteridae); *E. cf. spari* in *O. ruber*, *E. lintoni* in *P. pagrus*, and *E. suozalimae* in *Trichiurus lepturus* (Trichiuridae) and *Thyrsitops lepidoides* (Gempylidae) [7, 15–26].

Since *Encotyllabe spari* have been reported from Brazilian fish belonging to seven species in four families and two orders (Perciformes and Scorpaeniformes), we searched for this species in *P. pagrus* and *O. ruber*, the most common reported hosts in Brazil, in order to confirm their identity. Fresh material allowed us to perform molecular as well as morphological and morphometric analyses. Our results indicated that specimens previously reported as *E. spari* in *P. pagrus* and *O. ruber* belong to two new species. These are described and differentiated below.

## Methods

### Sample collection and processing

During 2016 and aperiodically, 26 specimens of *Pagrus pagrus* and 9 specimens of *Orthopristis ruber* were obtained from local fishermen off Cabo Frio, Rio de Janeiro, Brazil. In the laboratory, fish were dissected; gills and pharyngeal plates were removed and examined under a stereomicroscope for monogeneans. Thirty-four specimens of two species of *Encotyllabe* obtained from *P. pagrus* (n = 16) and *O. ruber* (n = 18) were selected for morphological and molecular analyses.

Some worms were fixed in 4% neutral buffered formaldehyde and then transferred and stored in 70% ethanol for further morphological studies (light microscopy). Selected specimens from each of the two host species were

transferred to 96% ethanol for DNA analysis.

Population descriptors, prevalence and mean intensity [27] were recorded.

### **Morphological and statistical analysis**

Fixed specimens were stained with carmine, cleared with clove oil (Sigma-Aldrich, Taufkirchen, Germany) and mounted in Eukitts® (O. Kindler GmbH, Freiburg, Germany). Drawings were made with the aid of an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan) equipped with a drawing tube. Measurements are given in micrometers unless otherwise stated with a range followed by the mean in parentheses. Type-material was submitted to the Helminthological Collection Instituto Oswaldo Cruz (CHIOC) – Rio de Janeiro – Brazil.

To comply with the regulations set out in article 8.5 (version 2012) of the International Code of Zoological Nomenclature (ICZN), details of the paper have been submitted to ZooBank. The LSID (Life Science Identifier) is

### **Morphometric analysis**

Multivariate morphometric analyses were performed on 16 specimens from *O. ruber* and nine from *P. pagrus*. Principal component analysis (PCA) was used for proportional morphometric measurements [28]. Because of the expected correlation between the size of different organs and body length (BL, excluding peduncle), we used proportion rather than raw measurement [3]. The proportion measurements were as follows: (1) maximum body width/BL, (2) length of the peduncle/BL, (3) diameter of haptor/BL, (4) diameter of the anterior attachment organs/BL, (5) pharynx width/BL, (6) length of the ovary/BL, (7) ovary width/BL, (8) length of the testes/BL, (9) width of the testes/BL, (10) length of the large hamulus/BL, (11) length of the small hamulus/BL, and (12) length of the marginal hooks/BL. PCA was performed using the Statistic 7.0 software (Statsoft Inc., Tulsa, Oklahoma).

### **Molecular data and phylogenetic analyses**

DNA was isolated from each individual following a modified protocol [29] involving treatment with sodium dodecyl sulphate, digestion with proteinase K, NaCl protein precipitation and subsequent ethanol precipitation. DNA was eluted in nuclease-free water and quantified in a Biospec-nano spectrophotometer. For molecular analyses, the nuclear *LSU*rDNA and mitochondrial gene cytochrome *c* oxidase 1 (*cox1*) were used. The *LSU*rRNA gene was amplified with the forward primer C1 (5'-ACCCGCTGAATT-TAAGCAT-3') and reverse primer D2 (5'-TGGTCCGTGTT-TCAAGAC-3') [30]. The *cox1* gene was amplified with the forward primer ASmit1 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and reverse primer ASmit2 (5'-TAAAGAAAGAACATAATGAAAATG-3') [31]. Each PCR had a final volume of 35 µl, including five standard units of GoTaq DNA polymerase (Promega, Madison, USA), 7 µl 5× PCR buffer, 5.6 µl MgCl<sub>2</sub> (25 mM), 2.1 µl BSA (10 mg/ml), 0.7 µl of deoxynucleotide triphosphate (dNTP) (10 mM), 10 pM of each primer, 3 µl template DNA and sufficient nuclease-free H<sub>2</sub>O to reach a total volume of 35 µl. A Boeco Ecogermany M-240R Thermal Cycler (Boeckel, Hamburg, Germany) was used to carry out PCR. Amplification for each molecular marker follow [30] and [32] for the *LSU*rRNA and *cox1* gene, respectively. Both DNA strands were directly sequenced (Macrogen, Seoul, Korea; <http://www.macrogen.com>). Sequences were edited and contigs assembled using ProSeq v 2.91 beta [33]. New sequences were deposited in GenBank (see Table I for accession numbers).

For each gene, a database was constructed in FASTA format (new generate sequences + sequences of *Encotyllabe* spp. from GenBank) and aligned with Clustal X [34]. Then, a visual inspection was performed with

ProSeq v.2.91 [33] in order to edit the length of the final data set.

Phylogenetic reconstructions were conducted in a concatenate gene matrix (*LSU*rRNA + *cox1*) performed in Mesquite v.2.75 [35]. Bayesian inference (BI) was analyzed with MrBayes [36] and maximum likelihood (ML) analysis with W-IQ-TREE [37].

BI analyses were executed with the following parameters: nst = 6, rates = invgamma according to the evolutionary model determined by jModeltest 0.1.10 for each gene; that is, TVM+ G for *LSU*rRNA and TIM1+I for *cox1* and replaced by GTR+G+I for MrBayes. The analysis was performed for 5,000,000 generations. Analyses included two runs of four chains and sampling every 100 generations. Support for nodes in the BI tree topology was obtained by posterior probability burn-in of the initial 25% of samples. Visual inspection of log likelihood scores against generation time was performed in TRACER v.1.7 [38]. Statistical support for ML analyses was performed with 1,000 bootstraps. The trees were visualized and edited in FigTree v.1.4.4 [38]. The pairwise p-distances and numbers of nucleotide differences between *Encotyllabe* species were calculated using MEGA v6 [39] (Table 2). Two species of *Neobenedenia* (Capsalidae) were used as outgroups (Table 1).

For comparative purposes, vouchers of *Encotyllabe spari* from Brazil deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) from *P. pagrus* (CHIOC 34531), *Haemulon sciurus* (Shaw) (CHIOC 32019, 32064, 32068), *Anisotremus virginicus* (Linnaeus) (CHIOC 37947), and *Conodon nobilis* (Linnaeus) (CHIOC 37948) were studied.

## Results

### Class Monogenea (van Beneden, 1858)

### Family Capsalidae Baird, 1853

### Genus Encotyllabe Diesing, 1850

#### *Encotyllabe yamagutii* n. sp.

**Type-host:** *Pagrus pagrus* (Linnaeus) (Perciformes: Sparidae), red porgy.

**Type-locality:** Cabo Frio, Rio de Janeiro, Brazil (22°55'S, 41°58'W).

**Type-material:** Holotype (CHIOC xxxxxxx) and 3 paratypes (CHIOC xxxxxx – xxxxxx) were submitted to the Helminthological Collection Instituto Oswaldo Cruz.

**Site in host:** Pharyngeal plates.

**Prevalence and intensity:** Prevalence: 69%; mean intensity 1.5.

**Representative DNA sequences:** MT968928 (*LSU*rRNA)

**Etymology:** This species is named in honor of Dr. Satyu Yamaguti for his distinguished contributions to the study of monogeneans.

### Description

[Measurements based on nine mounted and stained adult worms]. Body ellipsoidal 1.77–2.50 (1.94) long; 700–1120 (890) wide. Anterior attachment organs bearing two muscular suckers, 150 (130–180) in diameter, in each anterolateral margin of the head region and surrounded by an incomplete membrane. Haptor pedunculate, bell-shaped 450–590 (490) in diameter, with thin marginal membrane 27–39 (35) long, peduncle 620 (560–650) long (Fig. 1A). Haptor armed with a pair of large hamulus, 210–270 (250) long; small hamulus 29–32 (30) long and 14 homogeneously distributed marginal hooklets 11–15 (13) long (Fig. 1 C-E). Mouth surrounded by digitiform processes leading to a pharynx of irregular shape and 170–240 (230) in diameter. Intestinal caeca branched not confluent posteriorly. Two pairs of eyespots at the level of pharynx. Testes oval, side by side and anteriorly midlevel of body proper 250–360 (300) long and 140–240 (210) wide. Goto's glands not observed. Vas deferens sinistral winding anteriorly, entering at the base of the penis and enlarging to form an internal seminal vesicle. Prostatic duct joins ejaculatory duct and opens at the tip of the penis. Penis muscular 240–340 (300) long; 90–110 (100) in wide, penis with prolongation oriented to the anterior region of the body. Genital pore ventral on the left side of the pharynx. Ovary oval, pretesticular, immediately posterior to the vitelline reservoir 90–160 (120) long, 130–190 (170) wide, with an intraovarian seminal receptacle (Fig.1 B). Uterus extends anterolaterally along the posterior wall of the penis bulb. Ootype not observed, apparently hidden by Mehlis' gland, slender uterus opens at genital pore. Vaginal pore on ventral side at level of vitelline reservoir; ducts not observed. Vitelline reservoir preovarian on the left side. Vitelline follicles extensive laterally and median fields, from penis to base of peduncle. Eggs not observed.

### Differential diagnosis

*Encotyllabe yamagutii* n. sp. resembles those species with testes side by side, located anterior to the midlevel of the body, and a peduncle larger than the haptor diameter, namely, *E. spari* and *E. antofagastensis*. The main differences between the new species and the abovementioned species are the shape of the penis with a projection near the genital pore in the new species and the body size, with *E. yamagutii* being smaller (1.94 mm) than *E. spari* (3.14 mm) and *E. antofagastensis* (2.43 mm). Moreover, the vitelline receptacle is definitively preovarian in *E. spari* and *E. antofagastensis* but anterolateral in the new species. Molecular analysis of concatenated genes shows that *E. antofagastensis* and *E. yamagutii* are well discriminated species (Fig. 3). Unfortunately, molecular data for *E. spari* are not available in GenBank,

The type host for *E. spari* is *Sparus microcephalus* (Sparidae), but it is also found in two non-related hosts in the Inner Sea (Japan): the haemulid *Plectorhynchus pictus* and serranid *Epinephelus akaara*. The type host for *E. antofagastensis* is the haemulid *Anisotremus scapularis* from the southeastern Pacific. The new species is also a parasite of a sparid (*Pagrus pagrus*) but from the coast of Brazil.

Examination of specimens from *P. pagrus* identified by several authors as *E. spari* (CHIOC 34531) has revealed that they in fact belong to the new species.

### *Encotyllabe haemulii* n. sp.

**Type-host:** *Orthopristis ruber* (Cuvier) (Perciformes: Haemulidae), corocoro grunt.

**Type-locality:** Cabo Frio, Rio de Janeiro, Brazil (22°55'S, 41°58'W).

**Type-material:** Holotype (CHIOC xxxxxxx) and 3 paratypes (CHIOC xxxxx – xxxxxx) were submitted to the Helminthological Collection Instituto Oswaldo Cruz.

**Site in host:** Pharyngeal plates.

**Prevalence and intensity:** Prevalence: 100%; mean intensity 4.75.

**Representative DNA sequences:** MT968927 (*LSU* rRNA), MT967362, MW000907-MW000909 (*cox1*)

**Etymology:** This species is named after the host family.

## Description

### *Encotyllabe haemuli* n. sp.

[Measurements based on 16 stained and mounted adult worms]: Body bell-shaped, tapered anteriorly and wide posteriorly 2.74–3.60 (3.19) long; 810–1.65 (1.23) wide. Anterior attachment organs bearing two muscular suckers 180–300 (250) in diameter, in each anterolateral margin of the head region and surrounded by an incomplete membrane. Haptor pedunculate, bell-shaped 470–780 (660) in diameter, with a thin marginal membrane 45–76 (62) long, peduncle 650–400 (540) long (Fig. 2A). Haptor armed with a pair of large hamuli 250–340 (280) long; small hamulus 24–30 (28) long and 14 homogeneously distributed marginal hooklets 10–13 (12) (Fig. 2 D-F). Mouth surrounded by digitiform processes leading to a globular pharynx 270–450 (390) in diameter. Intestinal ceca branched not confluent posteriorly. Two pairs of eyespots at the level of the pharynx. Testes oval, side by side and anteriorly midlevel of body proper 130–180 (150) long and 100–170 (120) wide. Goto's glands not observed. Vas deferens sinistral winding anteriorly, entering at the base of the penis, enlarging to form an internal seminal vesicle. Prostatic duct joins ejaculatory duct and opens at the tip of the penis. Penis muscular 180–230 (210) long and 89–120 (110) wide. Genital pore ventral on the left side of the pharynx. Ovary oval, pretesticular, immediately posterior to the vitelline reservoir, 170–210 (180) long and 180–260 (220) wide, with an intraovarian seminal receptacle (Fig. 2B). Uterus extends anterolaterally along the posterior wall of the penis. Vaginal pore on ventral side of the vitelline reservoir; ducts not observed. Vitelline reservoir preovarian, sinistral. Vitelline follicles extensive laterally and in median fields, from the pharynx to the base of peduncle. Eggs pyramidal, with 4 long and twisted filaments (Fig. 2C).

## Differential diagnosis

Only two species of *Encotyllabe* have been described with testes smaller than the ovary, namely, *E. embiotocae* and *E. caranxi*. Of those, *E. caranxi* is the longest species described in the genus (11.26 mm); the type host is a carangid (*Caranx lutescens*) from the Great Barrier (Australia), whereas the type host for *E. haemuli* is a haemulid from the coast of Brazil. The relationship between large and small haptor hooks in *E. embiotocae* varies between 6.8:1 on average, whereas this value reaches 10:1 in the new species, with the smaller hooks in the new species being proportionally smaller.

Specimens of *Encotyllabe* previously identified as *E. spari* from *Anisotremus virginicus*, *Conodon nobilis* and *Haemulon sciurus* deposited in CHIOC and identified as *E. spari* in fact belong to the new species. Specimens identified as *E. spari* [17] also belong to *E. haemuli*.

## Morphometric analysis

Figure 3 presents the plot of specimens in the bidimensional axis of the PCA. The first and second components explain 59.5% of the total variance. The first component explains 37.3% of the variance and was mainly

associated with the proportional morphometric measurements of the ovary width/BL, attachment organs average/BL, testes length/BL, testes width/BL, large hamulus length/BL, and marginal hooks length/BL. The second component explains 22.2% of the variance and was associated with haptor diameter/BL and body width/BL. The new species, *E. yamagutii* and *E. haemuli*, showed some overlap but were clearly differentiated.

### Molecular and phylogenetic analyses.

For the *LSU*rRNA, five sequences were obtained, three from *E. haemuli* (852 bp) and two from *E. yamagutii* (865 bp). The intraspecific genetic variability for both species was 0%. Four sequences for *cox1* were obtained from *E. haemuli* (437 bp long). Concatenated analysis based on ML and BI produced trees with similar topology (Fig. 4). Each of the new species was statistically supported in independent clades (ML>90; BI>0.9). Table 2 indicates pairwise genetic divergence for *LSU*rRNA and the *cox1* gene.

## Discussion

In general, monogeneans are among the most host-specific parasites and may be the most host-specific of all fish parasites [40]. When broad host specificity, i.e., many hosts for the same species, of Monogenea is studied under molecular scrutiny, that broad specificity is questioned. For instance, the “cosmopolitan” capsalid *Neobenedenia melleni*, recorded from more than 100 host species in five orders, may be a complex of species, as suggested by molecular studies [12], and the related *Benedenia seriola*, recorded as a parasite of natural populations of *Seriola* spp. in Japan, Australia, and Chile as well as in farmed conditions around the world, is also a species complex, as demonstrated by molecular evidence [41,42]. When the hosts for the herein recognized species of *Encotyllabe* were analyzed, an interesting picture became evident: five species (*E. anisotremi*, *E. carangis*, *E. cheilodactyli*, *E. lutjani*, and *E. xiamenensis*) have been recorded from one host species [3, 5, 44], and two species have been recorded from two different but related host species. *E. caranxi* was found in three species of *Caranx* and one of the related *Pseudocaranx* (Carangidae) [5], and *E. embiotocae* was found to be a parasite of *Cymatogaster aggregata* and *Amphistichus argenteus* (Embiotocidae) [4]. Exceptions are *E. caballeroi*, reported as a parasite of three species of *Lethrinus*, *Gymnocranius audleyi* (Lethrinidae) and *Scolopis monogramma* and *Scolopis* sp. (Nemipteridae) from Australia, New Caledonia, the Philippines and Vietnam [5, 45, 46]; *E. kuwaitensis*, a parasite of *Caranx sexfasciatus*, *Caranx* sp. (Carangidae) and *Plectorhinchus schotaf* (Haemulidae) from the Arabian Gulf and the Mediterranean sea [6, 47]; and *E. souzalimae*, which has been reported from two nonrelated hosts from Brazil: *Trichiurus lepturus* (Trichiuridae) and *Thyrsitops lepidopoides* (Gempylidae) [7, 24]. *E. pagrosomi* has been recorded from seven host species belonging to three families from the Galapagos Islands, Mexico (Pacific coast), Australia, Venezuela and Peru [5, 48, 49]. Finally, *E. spari* has been recorded from at least 25 host species in nine families and two orders in Japan, the Arabian Gulf, Brazil, Vietnam, Venezuela, Argentina and the Mediterranean sea [5-7, 15-26, 47, 49, 50, 51]. With regard to the Brazilian records, *E. spari* (but also *E. cf. spari*) has been recorded from at least four species of Haemulidae, *Conodon nobilis*, *Anisotremus surinamensis*, *Haemulon sciurus* and *Orthopristis ruber*, one species of Sparidae, *Pagrus pagrus* (Sparidae); two species of Sciaenidae (*Menticirrhus americanus*, *Micropogonias furnieri*); and one species of Dactylopteridae (*Dactylopterus volitans*) [15,16,20,22,25,26] (Table 3). Our data challenge the low host specificity of *Encotyllabe* from Brazil; worms from the Sparid *Pagrus* and those from the Haemulid *Orthopristis ruber* belong to different species, as demonstrated by molecular tools (Fig. 3). Surprisingly, the two species from Haemulidae (*E. haemuli* and *E. antofagastensis*) form a well-supported clade, presenting additional evidence of high host specificity.

The taxonomic status of members of *Encotyllabe* is not easy to clarify, as traditional taxonomy is based on body shape, the relative sizes of several organs, the shape and relative position of the testes, the penis shape, the extension of the vitellaria, and the size and shape of the anchors as well as the relative distances between different organs [3-9]. Additional characteristics, such as the relative position of the testes, have also been suggested, but multivariate analysis (i.e., principal component analysis) of proportions of different organs in relation to body size, rather the analysis or comparison of raw measurements, will be an adequate tool to discriminate species in this genus [3] as well as in the Monopisthocotylea *Acanthocotyle* [43], as suggested by our results (Fig. 4). Our results confirming the host specificity of *Encotyllabe* suggest that previous records of *E. spari* in hosts other than *Pagrus pagrus* and *Orthopristis ruber* could represent new species. This finding also applies to other species, particularly *E. spari*, *E. pagrosomi* and *E. caballeroi* with nonrelated hosts and from different geographical localities.

## Declarations

## Ethics approval and consent to participate:

This study was conducted under the protocol of the Ethical Commission of the Universidad de Antofagasta, Antofagasta, Chile.

### Consent for publication:

Not applicable

### Availability of data and materials:

Data supporting the conclusions of this article are included within the article. The sequences generated in this study were deposited in the GenBank database under the accession numbers MT967362 (*E. haemuli*) (*cox1* gene), MT968927 (*E. haemuli*), MT968928 (*E. yamagutii*), MT982166 (*E. antofagastensis*), MT982167 (*E. cheilodactyli*), MT982168 (*Neobenedenia* sp.1) (*LSU* rRNA gene)

### Competing interests:

The authors declare that they have no competing interests

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## Authors' contributions:

NLT and JLL initiated the research and obtained the samples, NLT, JLL, RE and MEO performed the descriptions and morphological analyses. FAS conducting molecular analyses. All authors read and approved the final manuscript.

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## Conclusions

Two new species of the genus *Encotyllabe* are described from the pharyngeal plates of two teleosts from Cabo Frio, Rio de Janeiro, Brazil. Both species were previously reported as *Encotyllabe spari* or *Encotyllabe cf. spari* from seven species belonging to four families and two orders from Brazil. The status of some species must be confirmed after redescriptions based on materials from the type host and locality. *Encotyllabe* seems to be highly specific, and additional studies using molecular and multivariate morphometric analyses must be performed, specifically for species reported from nonrelated hosts and different geographical localities.

## Abbreviations

CHIOC: Colección Helminológica Instituto Oswaldo Cruz; PCA: Principal component analysis, *LSU*rRNA: *cox1*: cytochrome c oxidase subunit 1 gene; PCR: polymerase chain reaction; BI: Bayesian inference; ML: maximum likelihood; TVM+ G: transversion model + Gamma distribution; TIM1+I = transition model + Invariant site; GTR+G+I = General Time Reversible Model + Gamma distribution + invariant sites.

## Declarations

**Ethics approval and consent to participate:** This study was conducted under the protocol of the Ethical Commission of the Universidad de Antofagasta, Antofagasta, Chile.

**Consent for publication:** Not applicable

**Availability of data and materials:** Data supporting the conclusions of this article are included within the article. The sequences generated in this study were deposited in the GenBank database under the accession numbers MT967362 (*E. haemuli*) (*cox1* gene), MT968927 (*E. haemuli*), MT968928 (*E. yamagutii*), MT982166 (*E. antofagastensis*), MT982167 (*E. cheilodactyli*), MT982168 (*Neobenedenia* sp.1) (*LSU*rRNA gene)

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**Authors’ contributions:** NLT and JLL initiated the research and obtained the samples, NLT, JLL, RE and MEO performed the descriptions and morphological analyses. FAS conducting molecular analyses. All authors read and approved the final manuscript.

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## Tables

**Table 1.** GenBank accession numbers for sequences (*LSU* rRNA gene and *cox1* gene) for *Encotyllabe* spp. and the species of the outgroup used in phylogenetic analyses.

Specie	Host	Family	Country	GenBank ID		Reference
				<i>cox1</i>	<i>LSU</i> rRNA	
<i>E. haemuli</i> n. sp.	<i>Orthopristis ruber</i>	Haemulidae	Brazil	MT967362 MW000907-09	MT968927	This study
<i>E. yamagutii</i> n. sp.	<i>Pagrus pagrus</i>	Sparidae	Brazil	-	MT968928	This study
<i>E. antofagastensis</i>	<i>Anisotremus scapularis</i>	Haemulidae	Chile	JQ782838	MT982166	[3] / This study
<i>E. caballeroi</i>	<i>Gymnocranius audleyi</i>	Lethrinidae	Australia	-	AF026112	[52]
<i>E. caranxi</i>	<i>Pseudocaranx dentex</i>	Carangidae	Australia	-	FJ971990	[53]
<i>E. cheilodactyli</i>	<i>Cheilodactylus variegatus</i>	Cheilodactylidae	Chile	JQ782842	MT982167	[3] / This study
<i>E. chironemi</i>	<i>Chironemus marmoratus</i>	Chironemidae	Australia	-	AF382054	[54]
<i>Neobenedenia</i> sp. 1	<i>Cheilodactylus variegatus</i>	Cheilodactylidae	Chile	JQ782846	MT982168	[41] / This study
<i>Neobenedenia</i> sp. 2	<i>Paralabrax humeralis</i>	Serranidae	Chile	MG735627	MK202450	[55]

**Table 2.** Percent pairwise genetic distances between *Encotyllabe* spp. for *LSU* rRNA gene (under the diagonal) and the mitochondrial *cox1* gene (above the diagonal). Bp pairwise differences between parentheses.

Species	1	2	3	4	5	6
1 <i>Encotyllabe haemuli</i> n. sp.		-	-	-	7 (18.8)	9.2 (24.8)
2 <i>Encotyllabe yamagutii</i> n. sp.	0.9 (7)		-	-	-	-
3 <i>E. caballeroi</i>	1.3 (10)	1.4 (11)		-	-	-
4 <i>E. caranxi</i>	1.6 (6)	1.9 (7)	2.5 (9)		-	-
5 <i>E. antofagastensis</i>	0	0.9 (7)	1.3 (10)	1.6 (6)		9.5 (25.6)
6 <i>E. cheilodactyli</i>	0.6 (5)	1.1 (9)	1.3 (10)	1.6 (6)	0.6 (5)	
7 <i>E. chironemi</i>	0.4 (3)	0.8 (6)	1.1 (9)	1.6 (6)	0.4 (3)	0.5 (4)

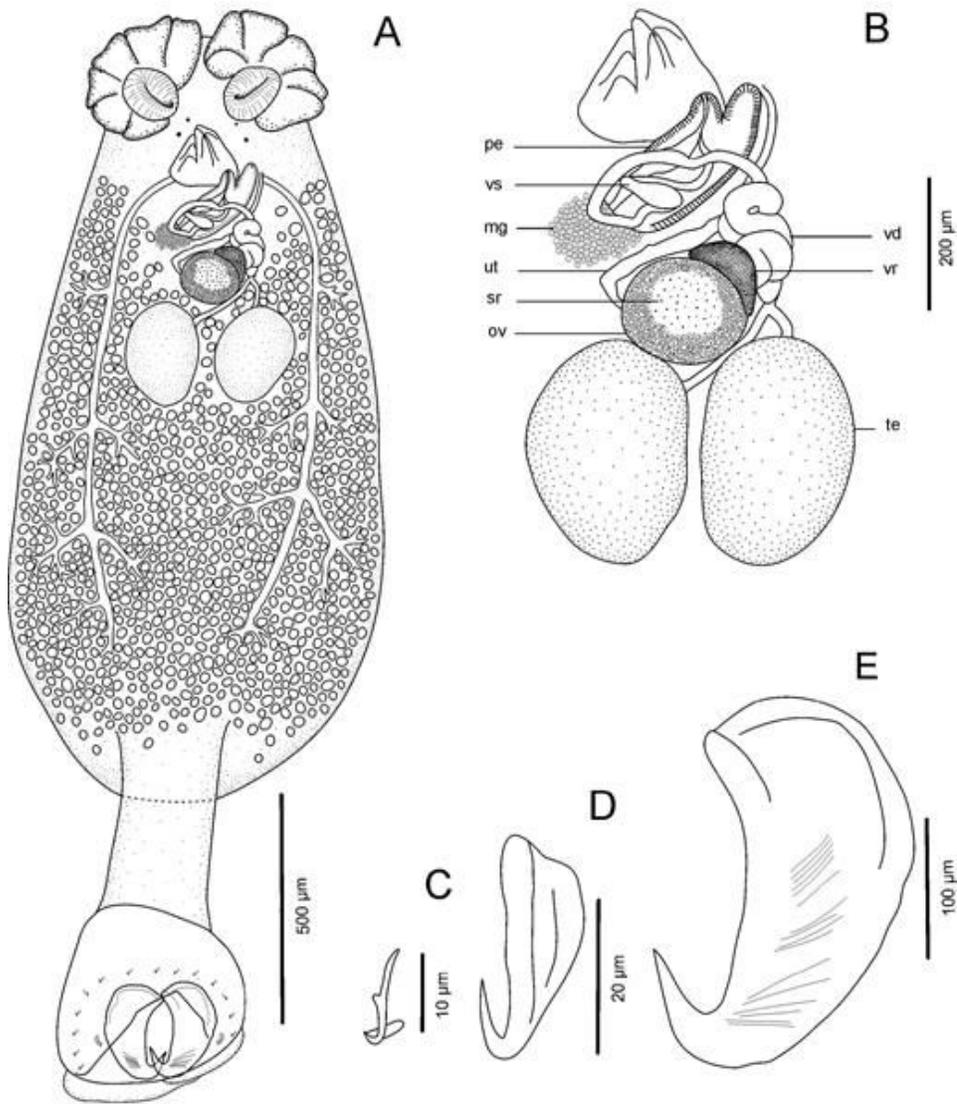
Table 3. *Encotyllabe* spp. accepted as valid in this study, recorded hosts, host family, locality and reference. Most of the references previous 2000, follow Egorova (2000) [5].

Species	Recorded hosts	Host Family	Locality	Reference
<i>E. antofagastensis</i>	<i>Anisotremus scapularis</i>	Haemulidae	Chile	3
<i>E. caballeroi</i>	<i>Gymnocranius audleyi</i>	Lethrinidae	Australia	5
<i>E. caballeroi</i>	<i>Lethrinus miniatus</i> (*)	Lethrinidae	Australia; New Caledonia	5, 46, 47
<i>E. caballeroi</i>	<i>Lethrinus nebulosus</i>	Lethrinidae	Philippines	5
<i>E. caballeroi</i>	<i>Scolopsis monogramma</i>	Nemipteridae	Australia	5
<i>E. caballeroi</i>	<i>Scolopsis</i> sp.	Nemipteridae	Vietnam	5
<i>E. caranxi</i>	<i>Caranx lutescens</i>	Carangidae	Australia	5
<i>E. caranxi</i>	<i>Caranx sexfaciatus</i>	Carangidae	Mediterrano	5
<i>E. caranxi</i>	<i>Caranx</i> sp.	Carangidae	Australia	5
<i>E. caranxi</i>	<i>Pseudocaranx dentex</i>	Carangidae	Australia	5
<i>E. cheilodactyli</i>	<i>Cheilodactylus variegatus</i>	Cheilodactylidae	Chile	3
<i>E. embiotocae</i>	<i>Amphistichus argenteus</i>	Embiotocidae	California USA	5
<i>E. embiotocae</i>	<i>Cymatogaster aggergata</i>	Embiotocidae	California USA	5
<i>E. haemuli</i>	<i>Orthorpistis ruber</i>	Haemulidae	Brazil	This study
<i>E. kuwaitensis</i>	<i>Caranx sexfasciatus</i>	Carangidae	Mediterrano	5
<i>E. kuwaitensis</i>	<i>Caranx</i> sp.	Carangidae	Arabian Gulf	5
<i>E. kuwaitensis</i>	<i>Plectorhinchus shotaf</i>	Haemulidae	Arabian gulf	47
<i>E. lutjani</i>	<i>Lutjanus johni</i>	Lutjanidae	India	5
<i>E. pagrosomi</i>	<i>Caulolatilus princeps</i>	Malacanthidae	Peru	48
<i>E. pagrosomi</i>	<i>Caulolatilus</i> sp.	Malacanthidae	Galapagos Islands	5
<i>E. pagrosomi</i>	<i>Chrysophrys auratus</i>	Sparidae	Australia	5
<i>E. pagrosomi</i>	<i>Haemulon steindachneri</i>	Haemulidae	Venezuela	49
<i>E. pagrosomi</i>	<i>Orthopristis ruber</i>	Haemulidae	Venezuela	49
<i>E. pagrosomi</i>	<i>Pagrosomus auratus</i>	Sparidae	Australia	5
<i>E. pagrosomi</i>	<i>Pomadasys macracanthus</i>	Haemulidae	Mexico	5
<i>E. souzalimae</i>	<i>Thyrsitops lepidopoides</i>	Gempylidae	Brazil	24
<i>E. souzalimae</i>	<i>Trichiurus lepturus</i>	Trichiuridae	Brazil	7
<i>E. spari</i>	<i>Acanthopagrus bifasciatus</i>	Sparidae	Arabian Gulf	47

<i>E. spari</i>	<i>Anisotremus surinamensis</i>	Haemulidae	Brazil	25
<i>E. spari</i>	<i>Argyrops spinniofer</i>	Sparidae	Arabian Gulf	47
<i>E. spari</i>	<i>Carangoides bajad</i>	Carangidae	Arabian Gulf	47
<i>E. spari</i>	<i>Conodon nobilis</i>	Haemulidae	Brazil	25
<i>E. spari</i>	<i>Epinephelus akaara</i>	Serranidae	Japan	5
<i>E. spari</i>	<i>Gymnocranius griseus</i>	Lethrinidae	Vietnam	5
<i>E. spari</i>	<i>Haemulon sciurus</i>	Haemulidae	Brazil	5
<i>E. spari</i>	<i>Lethrinus nebulosus</i>	Lethrinidae	Japan	5
<i>E. spari</i>	<i>Menticirrhus americanus</i>	Sciaenidae	Brazil	20
<i>E. spari</i>	<i>Micropogonias furnieri</i>	Sciaenidae	Brazil	25
<i>E. spari</i>	<i>Nemipterus virgatus</i>	Nemipteridae	Japan	5
<i>E. spari</i>	<i>Orthopristis ruber</i>	Haemulidae	Brazil	22
<i>E. spari</i>	<i>Pagrus major</i>	Sparidae	Japan	5
<i>E. spari</i>	<i>Pagrus pagrus</i>	Sparidae	Brazil	26
<i>E. spari</i>	<i>Parapristipoma trilineatus</i>	Haemulidae	Japan	5
<i>E. spari</i>	<i>Plectorhinchus cinctus</i>	Haemulidae	Arabian Gulf	6
<i>E. spari</i>	<i>Plectorhinchus pictus</i>	Haemulidae	Arabian Gulf	5,6
<i>E. spari</i>	<i>Plectorhinchus schotaf</i>	Haemulidae	Arabian Gulf	6
<i>E. spari</i>	<i>Plectorhinchus</i> sp.	Haemulidae	Vietnam	5
<i>E. spari</i>	<i>Plectorhinchus</i> spp.	Haemulidae	Arabian Gulf	47
<i>E. spari</i>	<i>Sebastes inermis</i>	Sebastidae	Japan	5
<i>E. spari</i>	<i>Sparus aurata</i>	Sparidae	Mediterranean Sea	51
<i>E. spari</i>	<i>Sparus macrocephalus</i>	Sparidae	Japan	5
<i>E. spari</i>	<i>Umbrina canosai</i>	Sciaenidae	Argentina	50
<i>E. spari</i>	<i>Upeneus tragula</i>	Mullidae	Japan	5
<i>E. xiamenesis</i>	<i>Pagrosomus major</i>	Sparidae	Taiwan	44
<i>E. yamagutii</i>	<i>Pagrus pagrus</i>	Sparidae	Brazil	This study

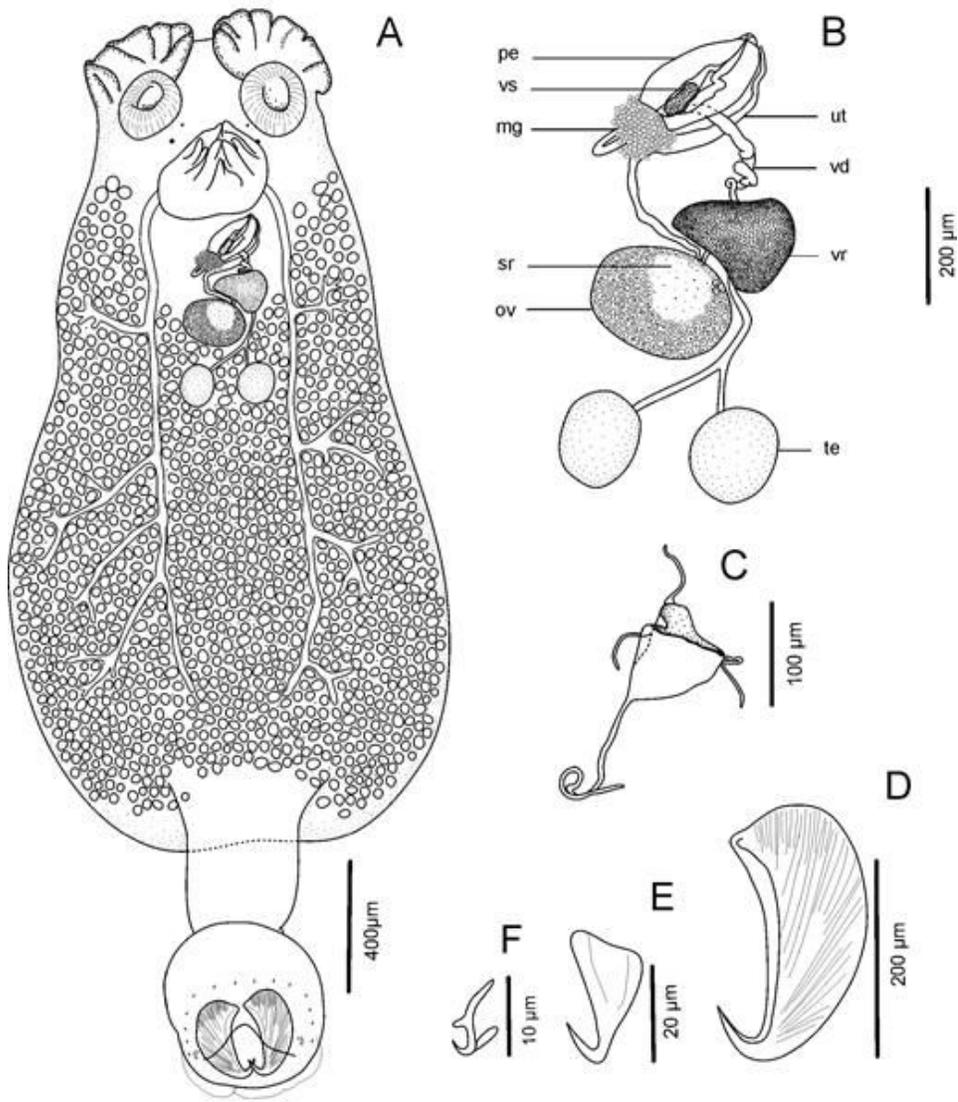
\**Lethrinus chrysostomus* reported as host for *E. caballeroi* is a synonymous for *Lethrinus miniatus*.

## Figures



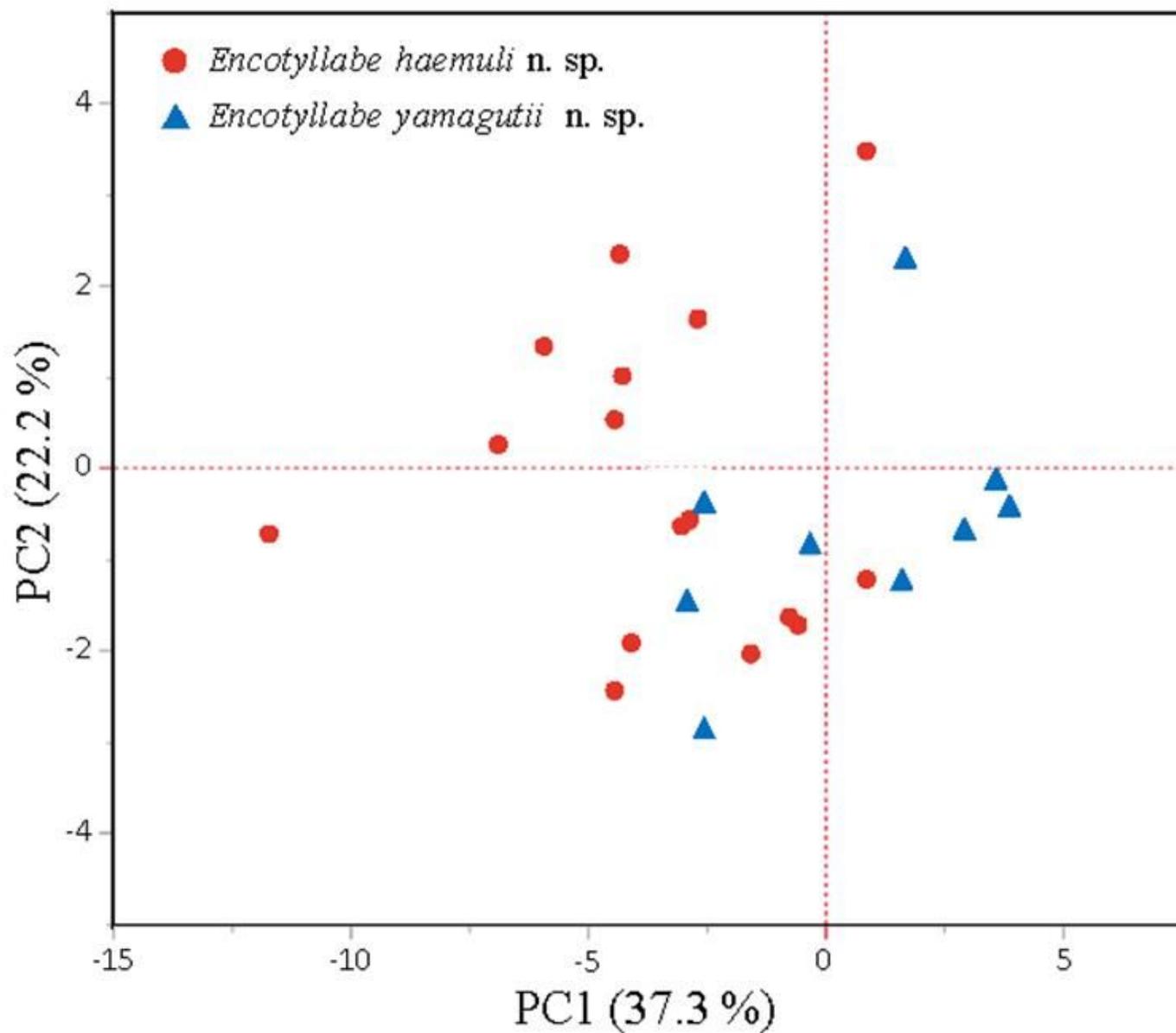
**Figure 1**

*Encotyllabe yamagutii* n. sp. ex *Pagrus pagrus*. A Ventral view of the holotype. B Reproductive system. C Marginal hooklets. D Small hamuli. E Large hamuli. Abbreviations: pe, penis; vs, seminal vesicle; mg, Mehlis' gland; ut, uterus; sr, seminal receptacle; ov, ovary; te, testes; vr, vitelline reservoir; vd, vas deferens.



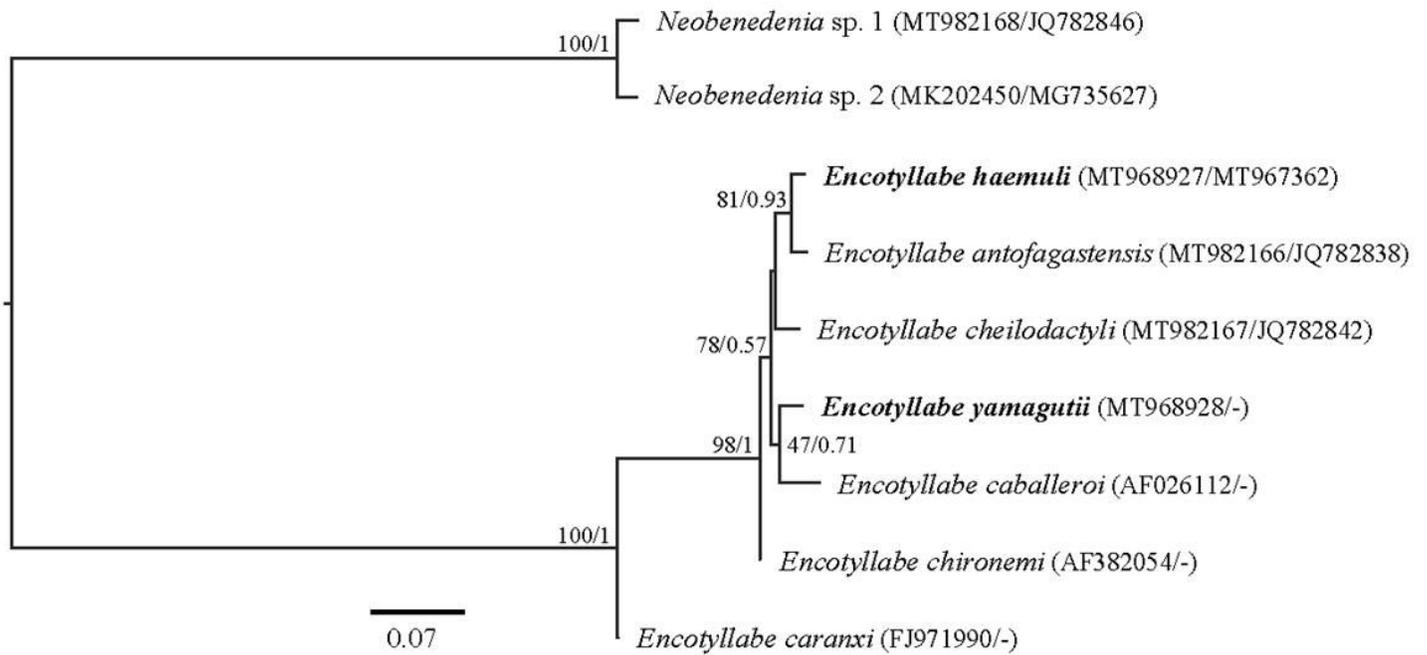
**Figure 2**

*Encotyllabe haemuli* n. sp. ex *Orthopristis ruber*. A Ventral view of the holotype. B Reproductive system. C egg. D Marginal hooklets. E Small Hamulus. F Large hamulus. Abbreviations: pe, penis; vs seminal vesicle; mg, Mehlis' gland; sr, seminal receptacle; ov, ovary; te, testes; vr, vitelline reservoir; vd, vas deferens; ut, uterus.



**Figure 3**

Principal components analysis based on proportional morphometric measurements standardized by total length from the new species of *Encotyllabe* from Brazil. Red circles: *E. haemuli* ex *Orthopristis ruber*; Blue triangle *E. yamagutii* n. sp. ex *Pagrus pagrus*.



**Figure 4**

Phylogenetic tree based on concatenated LSU rRNA and *cox1* genes for *Encotyllabe* spp. inferred by Maximum Likelihood (ML) and Bayesian inference (BI). Numbers along branches indicate the bootstrap values obtained from the posterior probability support of 1000 replicates for the Maximum Likelihood analysis and posterior probability of Bayesian Inference (ML/PP).