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

Keywords: Monogenea, Encotyllabe haemuli n. sp., Encotyllabe yamagutii n. sp., Orthopristis ruber, Pagrus pagrus, Southwestern Atlantic, Host specificity

DOI: https://doi.org/10.21203/rs.3.rs-82727/v1

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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 (LSUrRNA 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. vallei 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 Cheilodactyliidae) 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 redescriptions 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. souzalimae 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), Menticirrus 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 Thysitops 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'-TTTTTTGGGATCCTGAGGTTTAT-3') and reverse primer ASmit2 (5'-TAAAGAAAAGACATAATGAAAATG-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 MgCl2 (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 H2O 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 (\textit{LSU}rRNA + \textit{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 \textit{LSU}rRNA and TIM1+I for \textit{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 \textit{Encotyllabe} species were calculated using MEGA v6 [39] (Table 2). Two species of \textit{Neobenedenia} (Capsalidae) were used as outgroups (Table 1).

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

### Results

**Class Monogenea** (van Beneden, 1858)

**Family Capsalidae** Baird, 1853

**Genus Encotyllabe** Diesing, 1850

\textit{Encotyllabe yamagutii} n. sp.

**Type-host:** \textit{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 xxxxx – 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 (\textit{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 xxxx – 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.

[M]easurements 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 haptoral 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. yamaguti* 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. yamaguti* (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 seriolae*, 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. cheilodactyl*, *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 *Scolopsis monogramma* and *Scolopis* sp. (Nemipteridae) from 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 *Thrysitops 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 sciuirus* and *Orthopristis ruber*, one species of Sparidae, *Pagrus pagrus* (Sparidae); two species of Sciaenidae (*Menticirrhus americanus*, *Micropogonias furnier*); 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. cheilodactyl*) , MT982168 (*Neobenedenia* sp.1) (LSU rRNA gene)

**Competing interests:**

The authors declare that they have no competing interests

**Funding:**

Grant Grant 5303 “Programa Semilleros de Investigación” DGI, Universidad de Antofagasta, funded this study. The Millennium Institute of Oceanography (IMO-Chile) grant, ICM-ANID, ICN12_019-IMO

**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.
Acknowledgements:

We thank Tomáš Scholz (Institute of Parasitology, České Budějovice) for helpful suggestions to an early draft of the manuscript. NLT was supported by a student fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil).

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: Coleccion Helmintologica 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

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**Acknowledgements:** We thank Tomáš Scholz (Institute of Parasitology, České Budějovice) for helpful suggestions to an early draft of the manuscript. NLT was supported by a student fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil).
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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 | cox1 | LSU rRNA | Reference |
|--------|------|--------|---------|------|----------|-----------|
| E. haemuli n. sp. | Orthopristis ruber | Haemulidae | Brazil | MT967362 | MT968927 | This study |
| E. yamagutii n. sp. | Pagrus pagrus | Sparidae | Brazil | - | MT968928 | This study |
| E. antofagastensis | Anisotremus scapularis | Haemulidae | Chile | JQ782838 | MT982166 | [3] / This study |
| E. caballeroi | Gymnocranius audleyi | Lethrinidae | Australia | - | AF026112 | [52] |
| E. caranxi | Pseudocaranx dentex | Carangidae | Australia | - | FJ971990 | [53] |
| E. cheilodactyli | Cheilodactylus variegatus | Cheilodactylidae | Chile | JQ782842 | MT982167 | [3] / This study |
| E. chironemi | Chironemus marmoratus | Chironemidae | Australia | - | AF382054 | [54] |
| Neobenedenia sp. 1 | Cheilodactylus variegatus | Cheilodactylidae | Chile | JQ782846 | MT982168 | [41] / This study |
| Neobenedenia sp. 2 | Paralabrax humeralis | 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 Encotyllabe haemuli n. sp.| -   | -   | -   | -   | 7 (18.8) | 9.2 (24.8) |
| 2 Encotyllabe yamagutii n. sp.| 0.9 (7) | -   | -   | -   | -     | -     |
| 3 E. caballeroi             | 1.3 (10) | 1.4 (11) | -   | -   | -     | -     |
| 4 E. caranxi                | 1.6 (6) | 1.9 (7) | 2.5 (9) | -   | -     | -     |
| 5 E. antofagastensis        | 0   | 0.9 (7) | 1.3 (10) | 1.6 (6) | 9.5 (25.6) |
| 6 E. cheilodactyli          | 0.6 (5) | 1.1 (9) | 1.3 (10) | 1.6 (6) | 0.6 (5) |
| 7 E. chironemi              | 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 |
|--------------|-----------------------------------|-------------|-------------------------|-----------|
| *E. antofagastensis* | *Anisotremus scapularis*           | Haemulidae  | Chile                   | 3         |
| *E. caballeroi* | *Gymnocranius audleyi*             | Lethrinidae | Australia               | 5         |
| *E. caballeroi* | *Lethrinus miniatus (‡)*           | Lethrinidae | Australia; New Caledonia | 5, 46, 47 |
| *E. caballeroi* | *Lethrinus nebulosus*              | Lethrinidae | Philippines             | 5         |
| *E. caballeroi* | *Scolopsis monogramma*             | Nemipteridae | Australia               | 5         |
| *E. caballeroi* | *Scolopsis sp.*                    | Nemipteridae | Vietnam                | 5         |
| *E. caranxi*   | *Caranx lutescens*                 | Carangidae  | Australia               | 5         |
| *E. caranxi*   | *Caranx sexfasciatus*              | Carangidae  | Mediterrano             | 5         |
| *E. caranxi*   | *Caranx sp.*                       | Carangidae  | Australia               | 5         |
| *E. caranxi*   | *Pseudocaranx dentex*              | Carangidae  | Australia               | 5         |
| *E. cheilodactyli* | *Cheilodactylus variegatus*        | Cheilodactylidae | Chile               | 3         |
| *E. embiotocae* | *Amphistichus argenteus*           | Embiotocidae | California USA          | 5         |
| *E. embiotocae* | *Cymatogaster aggergata*           | Embiotocidae | California USA          | 5         |
| *E. haemuli*   | *Orthopristis ruber*               | Haemulidae  | Brazil                  | This study|
| *E. kuwaitensis* | *Caranx sexfasciatus*             | Carangidae  | Mediterrano             | 5         |
| *E. kuwaitensis* | *Caranx sp.*                      | Carangidae  | Arabian Gulf            | 5         |
| *E. kuwaitensis* | *Plectorhinchus shotaf*            | Haemulidae  | Arabian gulf            | 47        |
| *E. lutjani*   | *Lutjanus johni*                   | Lutjanidae  | India                   | 5         |
| *E. pagrosomi* | *Caulolatilus princeps*            | Malacanthidae | Peru                 | 48        |
| *E. pagrosomi* | *Caulolatilus sp.*                | Malacanthidae | Galapagos Islands      | 5         |
| *E. pagrosomi* | *Chrysophrys auratus*              | Sparidae    | Australia               | 5         |
| *E. pagrosomi* | *Haemulon steindachneri*           | Haemulidae  | Venezuela               | 49        |
| *E. pagrosomi* | *Orthopristis ruber*               | Haemulidae  | Venezuela               | 49        |
| *E. pagrosomi* | *Pagrosomus auratus*               | Sparidae    | Australia               | 5         |
| *E. pagrosomi* | *Pomadasys macracanthus*           | Haemulidae  | Mexico                  | 5         |
| *E. souzalimae* | *Thysitops lepidopoides*           | Gempylidae  | Brazil                  | 24        |
| *E. souzalimae* | *Trichiurus lepturus*              | Trichiuridae | Brazil                 | 7         |
| *E. spari*     | *Acanthopagrus bifasciatus*        | Sparidae    | Arabian Gulf            | 47        |
| E. spari | Anisotremus surinamensis | Haemulidae | Brazil | 25 |
| E. spari | Argyrops spimniofer | Sparidae | Arabian Gulf | 47 |
| E. spari | Carangoides bajad | Carangidae | Arabian Gulf | 47 |
| E. spari | Conodon nobilis | Haemulidae | Brazil | 25 |
| E. spari | Epinephelus akaara | Serranidae | Japan | 5 |
| E. spari | Gymnocranius griseus | Lethrinidae | Vietnam | 5 |
| E. spari | Haemulon sciurus | Haemulidae | Brazil | 5 |
| E. spari | Lethrinus nebulosus | Lethrinidae | Japan | 5 |
| E. spari | Menticirrhus americanus | Sciaenidae | Brazil | 20 |
| E. spari | Micropogonias furnieri | Sciaenidae | Brazil | 25 |
| E. spari | Nemipterus virgatus | Nemipteridae | Japan | 5 |
| E. spari | Orthopristis ruber | Haemulidae | Brazil | 22 |
| E. spari | Pagrus major | Sparidae | Japan | 5 |
| E. spari | Pagrus pagrus | Sparidae | Brazil | 26 |
| E. spari | Parapristipoma trilineatus | Haemulidae | Japan | 5 |
| E. spari | Plectorhinchus cinctus | Haemulidae | Arabian Gulf | 6 |
| E. spari | Plectorhinchus pictus | Haemulidae | Arabian Gulf | 5,6 |
| E. spari | Plectorhinchus schotaf | Haemulidae | Arabian Gulf | 6 |
| E. spari | Plectorhinchus sp. | Haemulidae | Vietnam | 5 |
| E. spari | Plectorhinchus spp. | Haemulidae | Arabian Gulf | 47 |
| E. spari | Sebastes inermis | Sebastidae | Japan | 5 |
| E. spari | Sparus aurata | Sparidae | Mediterranean Sea | 51 |
| E. spari | Sparus macrocephalus | Sparidae | Japan | 5 |
| E. spari | Umbrina canosai | Sciaenidae | Argentina | 50 |
| E. spari | Upeneus tragula | Mullidae | Japan | 5 |
| E. xiamenesis | Pagrosomus major | Sparidae | Taiwan | 44 |
| E. yamagutii | Pagrus pagrus | Sparidae | Brazil | This study |

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