

Research Paper

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Morphological and molecular characterization of *Encotyllabe vallei* Monticelli, 1907 (Monopisthocotylea, Monogenea) from the gilthead seabream *Sparus aurata* Linnaeus (Teleostei, Sparidae) from the southwestern Mediterranean and notes on host specificity of the genus *Encotyllabe* Diesing, 1850

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Abstract

Incomplete original descriptions, the unavailability or poor conditions of specimens and the lack of detailed redescrptions have caused the validity of several species of the genus *Encotyllabe* Diesing, 1850 to be questioned. To date, seven of the recognized species were described upon one or two specimens, hindering study of intraspecific variations. This was made worse by considering few morphoanatomical differences sufficient to erect new species. Among *Encotyllabe* spp. occurring in Mediterranean waters, *E. vallei* was first described from the gilt-head bream *Sparus aurata* (Sparidae) off Italy. Although beautifully illustrated for a paper from that century, morphometric data for *E. vallei* from the type-host *S. aurata* remain unavailable. Previous records of *E. vallei* provided either morphometrical or molecular data, and its validity was questioned. We provide a redescription of *E. vallei* based on newly collected specimens from the *S. aurata* from the southwestern Mediterranean (off Algeria) using integrative taxonomy. Analysis of *cox1* sequences of *E. vallei* from *S. aurata*, compared to sequences from other sparid hosts, mainly *Pagellus bogaraveo*, revealed a divergence not exceeding 2%, suggesting a stenoxenic specificity for this monogenean. Given that *P. bogaraveo* is the type-host for *Encotyllabe pagelli*, we were tempted to suggest a synonymy between *E. vallei* and *E. pagelli*. We refrained from doing so because *E. pagelli* was first described from the Atlantic coast off Brest, France. Morphological data for *Encotyllabe* from *P. bogaraveo* are warranted assessing the host specificity of *E. vallei* and whether there might be a species complex within individual sparid fish species.

Introduction

Capsalid monogeneans belonging to the genus *Encotyllabe* Diesing, 1850 are ectoparasitic Monopisthocotylea. They are commonly found on various parts of marine fish, such as the gills, body surface, mouth and pharyngeal toothpads of marine fish. Species of this genus are recognized by the elliptical ventral suckers surrounded by a wide membrane and a pedunculated aseptate haptor equipped with two pairs of substantial hooks and several marginal hooklets (Price 1939).

Encotyllabe spp. are known to inhabit a wide range of waters, encompassing temperate, subtropical and tropical regions (Lebedev 1967). They have a broad host spectrum (Table 1), infecting various teleost fish families (Sepúlveda et al. 2014). Currently, there are 24 species attributed to this genus (WoRMS 2023), however, the validity of several of these species has been questioned due to the incomplete and brief original descriptions (Taborda et al. 2023), the unavailability or poor conditions of some type specimens (Sepúlveda et al. 2014) and the lack of detailed redescrptions. Additionally, seven of the total recognized species were initially described based on only one or two specimens, which hinders the ability to understand intraspecific variability (Sepúlveda et al. 2014). Occasionally, minor morphoanatomical differences have been deemed sufficient grounds for the creation of new species (Khalil and Abdul-Salam 1988; Martorell 2004).

Only three *Encotyllabe* species occur in the Mediterranean: *Encotyllabe paronae* Monticelli, 1907, described from the East Atlantic peacock wrasse *Symphodus tinca* Linnaeus, 1758 (Labridae); *Encotyllabe nordmanni* Diesing, 1850, described from two Bramidae, the

Mediterranean pomfret *Brama mediterranea* (Bonnaterre, 1788) and the Atlantic pomfret *Brama brama* (Bonnaterre 1788) and a Pomacentridae *Chromis* (Linnaeus 1758). Two species are known from sparid fishes: *Encotyllabe pagelli* Van Beneden and Hesse, 1863, from the blackspot seabream *Pagellus centrodontus* (Delaroche 1809) collected off Brest, France, and *E. vallei* Monticelli, 1907, described from the gilt-head bream *Sparus aurata* Linnaeus, 1758 (Sparidae Rafinesque 1818) off Trieste, Italy. *Encotyllabe vallei* had been rarely mentioned, and apart from Radujkovic and Euzet (1989), the previous accounts provided scant or no morphometric data and lacked illustrations. Consequently, the brief original description has made it difficult to discern differences from other species (Taborda et al. 2023). As a result, assessing its similarities to and differences from other *Encotyllabe* species remains challenging due to the absence of morphometric data which led to its recent classification as *species inquirendae* (Taborda et al. 2023).

In this study, we provide an illustrated redescription of *E. vallei* based on newly collected specimens from the type-host *S. aurata* collected from the western Mediterranean, Algeria. We also provide *cox1* and 28S sequences and discuss the host specificity of *E. vallei*.

Material and methods

During the years 2020–2021, 115 specimens of *S. aurata* were collected from off Dellys (36°54' 48"N, 3°54' 51"E), Zemmouri El Bahri (36°48' 4.58"N, 3°34' 7.01"E), Cap Djinet (36°52' 37" N, 3°43' 23"E), Bouharoun (36°37' 24" N, 2°39' 17" E) and Cherchell (36° 36' 31" N, 2°11' 50" E) off the Algerian coast. Fish specimens were purchased dead from fishermen, transferred to the laboratory shortly after capture, identified using keys (Fischer et al. 1987; Kullander and Delling 2012) and examined fresh on the day of purchase.

Gills and pharyngeal tooth pads were detached, placed in individual Petri dishes containing saline solution following Lablack et al. (2022) and carefully examined for monogeneans under a stereomicroscope (Carl Zeiss Microimaging GmbH 37081 Göttingen, Germany). Monogeneans were removed from gills using fine dissection needles, heat-killed and preserved in molecular biology-grade ethanol and 70% ethanol for molecular and morphological analyses, respectively. We followed the terminology as defined by Combes (2003) to describe the host specificity of a parasite in relation to the relatedness of host species: Oioxenic is employed for parasites that exploit a single host species; the parasite is denoted as stenoxenic if it exploits a range of phylogenetically related species and euryxenic if it exploits a range of mutually unrelated species.

Morphological methods

For morphological study, monogeneans were stained with acetic carmine, dehydrated in graded ethanol series (70%, 95% and 100%), cleared in clove oil and mounted in Canada balsam using a Wild Heerbrugg stereo microscope. Voucher material was deposited at the Swedish Natural History Museum, Stockholm, Sweden, under registration numbers SMNH-208363–208373, SMNH-218760–218780. Drawings were made with the help of a Nikon Eclipse 80i microscope equipped with differential interference contrast (DIC) and a drawing tube (Department of Zoology, Swedish Museum of Natural History). Drawings were scanned and redrawn on a computer with Adobe Illustrator 2023. Measurements are in micrometers and indicated as the range followed by the mean.

Molecular methods

Three monogeneans were selected for DNA extraction. For three *S. aurata*, one monogenean was extracted. A tissue sample from the gill of the fish was taken, preserved in absolute ethanol and deposited as a voucher in the SMNH. For the monogenean, a small lateral part of the body above the haptor was separated with a scalpel following previous works on the barcoding of Monogenea (Ayadi et al. 2022; Azizi et al. 2021; Bouguerche et al. 2019a, 2019b; Bouguerche et al. 2019; Bouguerche et al. 2020; Bouguerche et al. 2021; Lablack et al. 2022) and submitted to molecular analysis, and the rest of the body was mounted on a slide as a voucher for drawing and deposition in a museum collection (Figure 1A). This enables the morphological assessment of sequenced monogeneans. Slides of monogeneans used for molecular work were deposited in the SMNH under registration numbers SMNH-208 363, SMNH-208 364 and SMNH-208 365.

Molecular barcoding of Monogenea

For *cox1*, total genomic DNA was isolated using a QIAmp DNA Micro Kit (Qiagen). The specific primers JB3 (=COIASmit1) (forward 50–TTTTTTGGGCATCCTGAGGTTTAT–30) and JB4.5 (=COI-ASmit2) (reverse 50–TAAAGAAAGAACATAATGAAAATG–30) were used to amplify a fragment of 402 bp of the *cox1* gene (Bowles et al. 1995; Littlewood et al. 1997). PCR reactions were performed in 20 µl of a mixture containing 1 ng of DNA, 1 CoralLoad PCR buffer, 3 mM MgCl₂, 0.25 mM dNTP, 0.15 µM of each primer and 0.5 units of Taq DNA polymerase (Qiagen). Thermocycles consisted of an initial denaturation step at 94°C for 2 min, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 40 s and extension at 72°C for 50 s. The final extension was conducted at 72°C for 5 min. PCR products were purified (Ampure XP Kit, Beckman Coulter) and sequenced in both directions on a 3730 I DNA Analyzer 96-capillary sequencer (Applied Biosystems, Foster City, CA, USA). We used CodonCode Aligner version 3.7.1 software (Codon Code Corporation, Dedham, MA, USA) to edit sequences and compare them to the GenBank database content with BLAST and deposited them in GenBank under accession numbers OR148271, OR148272, OR148273.

For 28S, DNA was extracted using a QIAamp DNA Micro Kit (Qiagen). A 28S rDNA fragment of 884 bp was amplified using the universal primers C10 (50–ACCCGCTGAATTTAAGCAT–30) and D2 (30–TCCGTGTTTCAAGACGG–50) (Hassouna et al. 1984). PCR reactions were performed in a final volume of 20 µL, containing: 1 ng of DNA, 16 CoralLoad PCR buffer, 3 mM MgCl₂, 66 mM of each dNTP, 0.15 mM of each primer and 0.5 units of Taq DNA polymerase (Qiagen). Thermocycles consisted of an initial denaturation step at 94°C for 1 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min. The final extension was conducted at 72°C for 7 min. PCR products were visualized on a 1.5% agarose gel, purified and directly sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems) at Eurofins Genomics. Sequences were edited and assembled using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA) and compared to the GenBank database content with BLAST. Sequences from three individual monogeneans were obtained and were found to be identical; they were deposited in GenBank under accession numbers OR149163, OR187608.

Table 1. The distribution of *Encotyllabe* spp. according to the systematics of host groups, reproduced from Lebedev (1967), updated. Note that *E. latridis* mentioned by Lebedev (1967) was omitted because it is currently included in *Mediavagina* Lawler and Hargis, 1968. *Encotyllabe masu* Ishii and Sawada, 1938; *E. monticelli* Perez Viguera, 1940; *E. pricei* Koratha, 1955 and *E. punctatai* Gupta et Krishna, 1980 as *species inquirendae* because these species were poorly described, based on only one or two specimens.

Parasite species	Family of type-host	Type-host	Reference	Other hosts
<i>Encotyllabe antofagastensis</i> Sepúlveda, González and Oliva, 2014	Haemulidae	<i>A. scapularis</i>	(Sepúlveda et al. 2014)	
<i>Encotyllabe bifurcatum</i> Taborda, Sepulveda, Luque, Escribano and Oliva, 2023	Sparidae	<i>P. pagrus</i>	(Taborda et al. 2023)	
<i>Encotyllabe caballeroi</i> Velasquez, 1977	Lethrinidae	<i>Lethrinus nebulosus</i>	(Williams and Beverley-Burton 1989)	<i>Lethrinus</i> spp., <i>L. nebulosus</i> (Lethrinidae), <i>Gymnocranius audleyi</i> (Lethrinidae), <i>Scolopsis monogramma</i> , <i>Scolopsis</i> sp. (Nemipteridae) (Egorova 2000; Justine et al. 2010; Rohde et al. 1994)
<i>Encotyllabe callaoensis</i> Tantalean, 1974	Sciaenidae	-	-	-
<i>Encotyllabe carangis</i> Pillai and Pillai, 1976 ¹	Carangidae	<i>Caranx</i> sp.	(Pillai 1968)	
<i>Encotyllabe caranxi</i> Lebedev, 1967	Carangidae			<i>Caranx lutescens</i> , <i>Caranx sexfasciatus</i> , <i>Caranx</i> sp., <i>Pseudocaranx dentex</i> (Carangidae) (Egorova 2000)
<i>Encotyllabe cheilodactyli</i> Sepúlveda, González and Oliva, 2014	Cheilodactylidae	<i>Cheilodactylus variegatus</i> (Cheilodactylidae)	(Sepúlveda et al. 2014)	
<i>Encotyllabe chironemi</i> Robinson, 1961	Cheilodactylidae	<i>Chirodactylus spectabilis</i>	(Robinson 1961)	
<i>Encotyllabe embiotocae</i> Noble, 1966	Embiotocidae	<i>Cymatogaster aggregata</i> (Noble, 1966)		<i>Amphistichus argenteus</i> (Noble 1966)
<i>Encotyllabe fotedari</i> Gupta and Krishna, 1975				
<i>Encotyllabe kuwaitensis</i> Khalil and Abdul-Salam, 1988	Carangidae	<i>Caranx</i> sp. (Khalil and Abdul-Salam, 1988)		<i>Plectorhinchus schotaf</i> (Haemulidae) (Kardousha et al. 2002) <i>Caranx sexfasciatus</i> (Egorova 2000)
<i>Encotyllabe lintoni</i> Monticelli, 1909	Sparidae	<i>Calamus calamus</i>	(Noble 1966)	
<i>Encotyllabe lutjani</i> Tripathi, 1959	Lutjanidae	<i>Lutjanus johnii</i>	(Tripathi 1959 (1957))	
<i>Encotyllabe monticelli</i> Perez Viguera, 1940	Sparidae	<i>Calamus bajonado</i>	(Noble 1966)	
<i>Encotyllabe nordmanni</i> Diesing, 1850	Bramidae	<i>Brama brama</i>	(Diesing 1850)	
<i>Encotyllabe pagelli</i> Van Beneden and Hesse, 1863	Sparidae	<i>Pagellus bogaraveo</i>	(Van Beneden and Hesse 1863)	<i>Pagrus pagrus</i> (referred to as 'common seabream') (Dawes 1947)
<i>Encotyllabe pagrosomi</i> MacCallum, 1917	Sparidae	<i>Pagrus auratus</i>	(MacCallum 1917)	<i>Caulolatilus</i> sp. (Malacanthidae) (Meserve 1938) <i>Rhencus macracanthus</i> (Günther 1864) (Yamaguti 1963) <i>Caulolatilus princeps</i> (Malacanthidae), <i>Chrysophrys auratus</i> (Sparidae), <i>Haemulon steindachneri</i> , <i>Orthopristis rubra</i> , <i>Rhencus macracanthus</i> (Haemulidae) (Taborda et al. 2023)
<i>Encotyllabe paronae</i> Monticelli, 1907	Labridae	<i>Symphodus tinca</i>	(Monticelli 1907)	
<i>Encotyllabe parvum</i> Taborda, Sepulveda, Luque, Escribano and Oliva, 2023	Haemulidae	<i>Orthopristis rubra</i>	(Taborda et al. 2023)	

(Continued)

Table 1. (Continued)

Parasite species	Family of type-host	Type-host	Reference	Other hosts
<i>Encotyllabe pricei</i> Koratha, 1955	Scorpaenidae	<i>Scorpaena plumieri</i>	(Yamaguti 1963)	
<i>Encotyllabe souzalimae</i> Carvalho and Luque, 2012	Trichiuridae	<i>Trichiurus lepturus</i>	(Carvalho and Luque 2012)	<i>Thyrsitops lepidoides</i> (Gempylidae) (Carvalho and Luque 2012)
<i>Encotyllabe spari</i> Yamaguti, 1934 ²	Sparidae	<i>Acanthopagrus schlegelii</i>	(Yamaguti 1934)	<i>Diagramma pictum</i> (Haemulidae) <i>Epinephelus akaara</i> (Serranidae) (Yamaguti 1934) <i>Haemulon sciurus</i> , <i>Orthopristis ruber</i> , <i>Anisotremus surinamensis</i> , <i>Conodon nobilis</i> (Haemulidae), <i>Pagrus pagrus</i> (Sparidae), <i>Menticirrhus americanus</i> , <i>Micropogonias furnieri</i> (Sciaenidae), <i>Dactylopterus volitans</i> (Dactylopteridae) (Taborda et al. 2023) <i>Carangoides bajad</i> (Carangidae) (Kardousha et al. 2002) <i>Sparus aurata</i> (Sparidae) (Mahmoud et al. 2014)
<i>Encotyllabe vallei</i> Monticelli, 1907	Sparidae			See Table 5
<i>Encotyllabe xiamenensis</i> Li, Yan and Wang, 2004	Sparidae	<i>Pagrosomus major</i>	(Taborda et al. 2023)	

¹ was originally described in an unpublished thesis and never published according to the rules of the International Code for Zoological Nomenclature; it is not considered valid (see Taborda et al. (2023)).

² Yamaguti (1934) noted that both *E. pagelli* and *E. pagrosomi* are parasitic in the sparid *Pagrus auratus* and probably more closely related to *E. spari*, but because the original papers of these species were inaccessible to him, he noted that his species should be looked upon as a provisional one.

Molecular analysis

Trees were constructed using our newly generated sequences and those of closely related taxa available in GenBank. For 28S, we used almost all closely related sequences available in GenBank (Table 2), except for those that were too short and/or did not align well. A sequence of a monocotyloid *Holocephalocotyle monstrosae* Derouiche, Neifar, Gey, Justine and Tazerouti, 2019 (Derouiche et al. 2019) was used as an outgroup. The 28S dataset included 50 nucleotide sequences. Both extremities of the sequences were trimmed to obtain a clean matrix. After estimating the best model with MEGA7 (Kumar et al. 2016), the tree was inferred using the maximum likelihood (ML) method based on the general time reversible model (GTR) with gamma distributed with invariant sites (G+I).

For *cox1*, the newly generated sequences of *E. vallei* were aligned with 25 species of *Encotyllabe* recovered after BLAST (Table 3). Two sequences of *Empruthotrema aoneken* Irigoitia, Braicovich, Rossin and Timi in Irigoitia, Braicovich, Rossin, Canel, Levy, Farber and Timi, 2019 (MN190708, MN190709 (Irigoitia et al. 2019)) were used as an outgroup. The trimmed matrix included 261 positions in the dataset. The tree was inferred using the ML method based on the Hasegawa–Kishino–Yano model (HKY) with gamma distributed (G).

All trees were constructed in MEGA7, with 500 replications for the ML trees. The neighbour-joining (NJ) method was also used for comparison in MEGA7, with 2000 bootstraps computed for *cox1* and 28S from the same datasets. Distances (p-distances and Kimura two parameter) were computed from the same datasets with MEGA7 (Kumar et al. 2016).

Results

Molecular characterization of monogeneans

For the 28S gene, molecular data were generated for three specimens of *E. vallei* collected from *S. aurata* off Algeria, Western Mediterranean (OR148271, OR148272, OR148273). The alignment of the new 28S sequence data with that of related taxa, primarily Encotyllabinae Monticelli, 1892, Benedeniinae Johnston and Capsalinae Baird, 1853, yielded 1288 characters. The final dataset used for phylogenetic analysis included 846 characters. The phylogenetic analysis using both ML and NJ methods produced phylograms with identical topologies (Figure 2). In these phylograms, each of the four capsalid subfamilies represented a distinct and well-supported clade. Within *Encotyllabe*, three subclades were identified: the first included *Encotyllabe* sp1. collected from *P. bogaraveo*, while the second included *E. vallei* from *S. aurata* and *D. vulgaris*. The third subclade within *Encotyllabe* included the following species: *Encotyllabe* sp. from *Pagrus pagrus*, *Encotyllabe* sp. from *Orthopristis ruber*, *E. caballeroi*, *Encotyllabe cheilodactyli*, *Encotyllabe chironemi*, *E. cf. spari* and *Encotyllabe antofagastensis*.

There were no intraspecific variations within our newly generated sequences of *E. vallei*. Additionally, all sequences of *E. vallei* from the Western Mediterranean (off Algeria) were identical, irrespective of host. Overall, the genetic distance between the newly generated sequences and those of Capsalidae ranged from 1% to 33%, while the divergence among *Encotyllabe* spp. ranged from 1% to 2%. The closest sequences to our newly generated sequences of *E. vallei* were those of *E. chironemi* from *Chironemus marmoratus* off Australia, *Encotyllabe cf. spari* from *O. ruber* off Brazil,

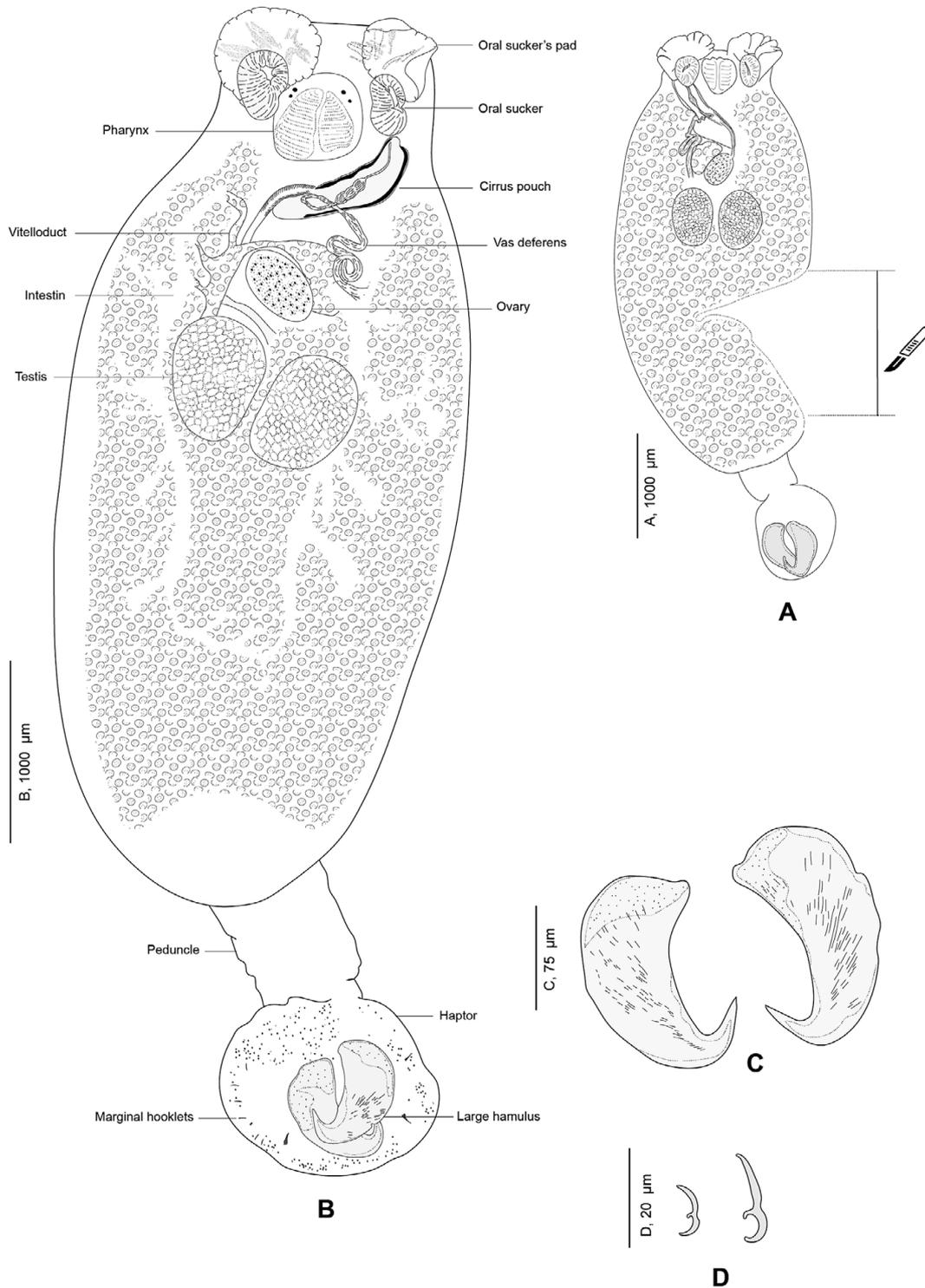


Figure 1. *Encotyllabe vallei* (Monticelli, 1907) ex *S. aurata*. A, Hologenophores SMNH-208 365 (GenBank OR148273); B, voucher, whole body, SMNH-208 366; C, Large hamulus; D, small hamulus.

Encotyllabe sp. from *P. pagrus*, and from *O. ruber* off Brazil, and *E. antofagastensis* from *Anisotremus scapularis* off Chile (1% inter-specific variation).

The newly generated *cox1* sequences (OR148271, OR148273 and OR148273, respectively) were 433, 416 and 432 bp long. Deleted ambiguously aligned characters resulted in a final dataset

of 261 characters for phylogenetic analysis. The ML and NJ analyses also have identical topologies and only the ML tree is shown (Figure 3). In the *cox1* dataset, the same clades (when *cox1* and 28S sequences are both available for a given species) were represented with much greater levels of difference between them. All sequences of *E. vallei* from Algeria clustered in a

Table 2. Sequences used in the molecular analysis of 28S sequences of monogeneans.

Parasite species	Host species	Origin	GenBank	Source
<i>Encotyllabe vallei*</i>	<i>Sparus aurata</i>	Algeria	OR149163	Present study
<i>Encotyllabe vallei*</i>	<i>Sparus aurata</i>	Algeria	OR187608	Present study
<i>Encotyllabe haemulii</i>	<i>Orthopristis ruber</i>	Brazil	MT968927	(Taborda <i>et al.</i> 2023)
<i>Encotyllabe yamagutii</i>	<i>Pagrus pagrus</i>	Brazil	MT968928	(Taborda <i>et al.</i> 2023)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679678	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679679	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679680	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679681	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679682	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679683	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679684	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp1.	<i>Pagellus bogaraveo</i>	Algeria	OL679685	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp2.	<i>Diplodus vulgaris</i>	Algeria	OL679687	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp2.	<i>Diplodus vulgaris</i>	Algeria	OL679688	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp2.	<i>Sparus aurata</i>	Algeria	OL679689	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe</i> sp2.	<i>Sparus aurata</i>	Algeria	OL679690	(Lablack <i>et al.</i> 2022)
<i>Encotyllabe antofagastensis</i>	<i>Anisotremus scapularis</i>	Chile	MT982166	(Taborda <i>et al.</i> 2023)
<i>Encotyllabe cheilodactyli</i>	<i>Cheilodactylus variegatus</i>	Chile	MT982167	(Taborda <i>et al.</i> 2023)
<i>Encotyllabe chironemi</i>	<i>Chironemus marmoratus</i>	Australia	AF382054	(Olson and Littlewood 2002)
<i>Encotyllabe cf. spari</i>	<i>Orthopristis ruber</i>	Brazil	KY553149	(Camargo <i>et al.</i> 2017)
<i>Encotyllabe caballeroi</i>	<i>Gymnocranius audleyi</i>	Australia	AF026112	(Mollaret <i>et al.</i> 1997)
<i>Allobenedenia dischizosepta</i>	<i>Acanthistius patachonicus</i>	Argentina	MH929436	
<i>Benedenia</i> sp.	<i>Plectropomus leopardus</i>	Japan	LC542978	(Ogawa <i>et al.</i> 2021)
<i>Benedenia sargocentron</i>	<i>Sargocentron spiniferum</i>	China	JN797597	Unpublished
<i>Benedenia lutjani</i>	<i>Lutjanus carponotatus</i>	Australia	AF026106	(Mollaret <i>et al.</i> 1997)
<i>Neobenedenia girellae</i>	<i>Trachinotus blochii</i>	South Korea	MT549677	(Nam <i>et al.</i> 2020)
<i>Neobenedenia melleni</i>	<i>Sphoeroides annulatus</i>	Mexico	MH843696	(Brazenor <i>et al.</i> 2018)
<i>Neobenedenia girellae</i>	<i>Rachycentron canadum</i>	Australia	MG193660	(Brazenor <i>et al.</i> 2018)
<i>Neobenedenia girellae</i>	<i>Plectropomus leopardus</i>	Australia	MG193661	(Brazenor <i>et al.</i> 2018)
<i>Neobenedenia girellae</i>	<i>Lates calcarifer</i>	Australia	MG193662	(Brazenor <i>et al.</i> 2018)
<i>Neobenedenia girellae</i>	<i>Epinephelus coioides</i>	Australia	MG193663	(Brazenor <i>et al.</i> 2018)
<i>Neobenedenia girellae</i>	<i>Sparus aurata</i>	Portugal	MW690095	(Tedesco <i>et al.</i> 2023)
<i>Neobenedenia</i> sp.	<i>Cheilodactylus variegatus</i>	Chile	MT982168	(Taborda <i>et al.</i> 2023)
<i>Neobenedenia</i> sp.	<i>Larimichthys polyactis</i>	South Korea	OM333244	Unpublished
<i>Neobenedenia</i> sp.	<i>Aplodactylus punctatus</i>	Chile	MK202438	(Sepúlveda and González 2019)
<i>Neobenedenia</i> sp.	<i>Anisotremus scapularis</i>	Chile	MK202439	(Sepúlveda and González 2019)
<i>Neobenedenia</i> sp.	<i>Pinguipes chilensis</i>	Chile	MK202441	(Sepúlveda and González 2019)
<i>Neobenedenia</i> sp.	<i>Pinguipes chilensis</i>	Chile	MK202442	(Sepúlveda and González 2019)
<i>Neobenedenia</i> sp.	<i>Cheilodactylus variegatus</i>	Chile	MK202444	(Sepúlveda and González 2019)
<i>Neobenedenia</i> sp.	<i>Paralabrax humeralis</i>	Chile	MK202447	(Sepúlveda and González 2019)
<i>Gracilobenedenia kuremibai</i>	<i>Plectorhynchus chaetodonoides</i>	Japan	LC602797	(Nitta 2021)
<i>Gracilobenedenia lutjani</i>	<i>Lutjanus fulviflamma</i>	Japan	LC602796	(Nitta 2021)
<i>Gracilobenedenia lutjani</i>	<i>Lutjanus vitta</i>	Japan	LC602795	(Nitta 2021)

(Continued)

Table 2. (Continued)

Parasite species	Host species	Origin	GenBank	Source
<i>Gracilobenedenia hichi</i>	<i>Priacanthus hamrur</i>	Japan	LC602798	(Nitta 2021)
<i>Nasicola klawei</i>	<i>Thunnus albacares</i>	USA	HQ721184	(Bullard et al. 2011)
<i>Nasicola klawei</i>	<i>Thunnus albacares</i>	USA	HQ721185	(Bullard et al. 2011)
<i>Nasicola klawei</i>	<i>Thunnus albacares</i>	USA	HQ721186	(Bullard et al. 2011)

*, new sequences.

Table 3. Sequences used in the molecular analysis of *cox1* sequences of monogeneans.

Parasite species	Host species	Origin	GenBank	Source
<i>Encotyllabe vallei</i> *	<i>Sparus aurata</i>	Algeria	OR148271	Present study
<i>Encotyllabe vallei</i> *	<i>Sparus aurata</i>	Algeria	OR148272	Present study
<i>Encotyllabe vallei</i> *	<i>Sparus aurata</i>	Algeria	OR148273	Present study
<i>Encotyllabe</i> sp.	<i>Orthopristis ruber</i>	Brazil	MW000907	Unpublished
<i>Encotyllabe</i> sp.	<i>Orthopristis ruber</i>	Brazil	MW000908	Unpublished
<i>Encotyllabe</i> sp.	<i>Orthopristis ruber</i>	Brazil	MW000909	Unpublished
<i>Encotyllabe</i> sp.	<i>Orthopristis ruber</i>	Brazil	MT967362	(Taborda et al. 2023)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675214	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675215	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675216	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675217	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675218	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675219	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675220	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675221	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675223	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Pagellus bogaraveo</i>	Algeria	OL675224	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Sparus aurata</i>	Algeria	OL675225	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Sparus aurata</i>	Algeria	OL675226	(Lablack et al. 2022)
<i>Encotyllabe</i> sp.	<i>Sparus aurata</i>	Algeria	OL675222	(Lablack et al. 2022)
<i>Encotyllabe</i> sp. 2	<i>Anisotremus scapularis</i>	Chile	JQ782840	(Sepúlveda and González 2019)
<i>Encotyllabe</i> sp. 2	<i>Anisotremus scapularis</i>	Chile	JQ782838	(Sepúlveda and González 2019)
<i>Encotyllabe</i> sp. 2	<i>Anisotremus scapularis</i>	Chile	JQ782836	(Sepúlveda and González 2019)
<i>Encotyllabe</i> sp. 2	<i>Anisotremus scapularis</i>	Chile	JQ782839	(Sepúlveda and González 2019)
<i>Encotyllabe</i> sp. 2	<i>Anisotremus scapularis</i>	Chile	JQ782837	(Sepúlveda and González 2019)
<i>Empruthotrema aoneken</i>	<i>Sympterygia bonapartii</i>	Argentina	MN190710	(Irigoitia et al. 2019)
<i>Empruthotrema aoneken</i>	<i>Sympterygia acuta</i>	Argentina	MN190709	(Irigoitia et al. 2019)
<i>Empruthotrema aoneken</i>	<i>Sympterygia acuta</i>	Argentina	<u>MN190708</u>	(Irigoitia et al. 2019)

*, new sequences. numbers SNHM 208 363-208 373, SMNH 218 760-218 780.

well-supported clade. Similarly, *Encotyllabe* sp. and *Encotyllabe* sp. 2 from *O. ruber* and *A. scapularis*, respectively, formed well-supported monophyla.

The replicate *cox1* sequences of *E. vallei* generated in the present study differed between them by 0% to 1% (intraspecific variations). All genotypes of *E. vallei* from Algeria from different hosts differed by 1% to 2% divergence in P-distances.

Morphology of *E. vallei* Monticelli, 1907 (Figures 1 and 4)

Type-host: *S. aurata* (Linnaeus), the gilt-head (sea) bream (Sparidae).

Additional hosts: *Dentex* sp., *D. dentex* (Linnaeus.), *Diplodus puntazzo* (Walbaum) and *Mullus surmuletus* Linnaeus.

Type-locality: Trieste, Italy (Monticelli, 1907).

Additional localities: Montenegro, Spain. Off Bouharoun, off Dellys (36°54' 48"N, 3°54' 51"E), Zemmouri El Bahri (36°48'



Figure 2. Maximum likelihood tree based on an analysis of 28S sequence data for Capsalidae. Bootstrap percentages (500 replicates) are indicated next to or below the branches (only values >70% are shown). There was a total of 846 positions in the final dataset. The NJ tree (p-distance method) had a similar topology and is not presented.

Table 4. Measurements of *E. vallei* from different hosts and localities.

Host	<i>Sparus aurata</i>		<i>Diplodus puntazzo</i>		<i>Diplodus annularis</i> , <i>Diplodus cervinus</i> , <i>Diplodus sargus</i> , <i>Diplodus vulgaris</i> , <i>Pagrus auriga</i>
Site on host	Gills, pharyngeal pads	Unknown	Gills	Gills	Gills
Locality	Algeria	Italy	Egypt	Montenegro	Spain
No. of specimens	33			2	10
Source	Present study	(Monticelli 1907)	(Mahmoud et al. 2014)	(Radujkovic and Euzet 1989)	(Martorell 2014)
Body length	1727 ± 267 (1106 – 228, n = 32)	3500	1060–2020	3500	764 (780–1560, n = 6)
Body width	693 ± 143 (447 – 1072, n = 32)	-	230–300	1100	344 (360–840, n = 5)
Haptor diameter	-	-	-	-	348 (300–680, n = 4)
Peduncle length	464 ± 100 (227–666, n = 32)	-	-	-	278 (260–500, n = 6)
Large hamulus length	141 (88 – 24, n = 18)	-	80–130	270	169 (136–248, n = 6)
Small hamulus length	20 (20 – 32, n = 19)	-	10–20	27	21 (20–32, n = 5)
Marginal hooklets length	-	-	10–20	12	11 (11–12, n = 4)
Oral suckers diameter	138 (82 – 192, n = 09)	-	-	-	140 (144–172, n = 4)
Pharynx length	137 (54 – 19, n = 26)	-	50–70*	-	51 (78–168, n = 3) *
Pharynx width	173 (98 – 253, n = 26)	-	-	-	-
Left testes length	228 ± 57 (128 – 35, n = 31)	-	20–110	-	66 (46–170, n = 11) *
Left testes width	188 ± 44 (98 – 29, n = 31)	-	-	-	-
Right testes length	224 ± 57 (108 – 31, n = 30)	-	40–150	-	-
Right testes width	173 ± 43 (94 – 25, n = 30)	-	-	-	-
Ovary length	109 (55 – 139, n = 29)	-	30–50	-	55 (42–142, n = 6)
Ovary width	132 (64 – 198, n = 29)	-	20–30	-	33 (20–108, n = 6)

* Diameter.

4.58°N, 3°34' 7.01"E), Cap Djinet (36°52' 37" N, 3°43' 23"E), Bouharoun (36°37' 24" N, 2°39' 17" E) and Chercell (36°36' 31" N, 2°11' 50" E), Algeria (this paper).

Site on host: pharyngeal tooth pads, gills.

Prevalence and intensity: ex *S. aurata* off Algeria: 34% (31 out of 90 fish).

Specimens from Algeria, from *S. aurata* (Figures 1 and 4): vouchers deposited in the collections of the Swedish Museum of Natural History, Stockholm (SMNH-208363–SMNH-208373; SMNH-218760–SMNH-218780).

Specimens with molecular information: entire specimen lacking only a small lateral part (below the haptor) parts of specimens mounted on slide, lateral excised part used for molecular analysis: specimens from Algeria, from *S. aurata*: SMNH-208 363 (GenBank accession numbers OR149163, OR148271), SMNH-208 364 (GenBank accession numbers OR187608, OR148272), SMNH-208 365 (GenBank accession numbers OR148273).

Redescription

Measurements and description based on 33 stained and mounted specimens (Table 4). Body stocky, 1727 ± 267 (1106–2280, n = 32)

long, 693 ± 143 (447–1072, n = 32) maximum width at posterior third of body proper (Figure 4B). Two prohaptor suckers muscular, 138 ± 33 (82–192) in diameter, oval, embedded in a lobed pad located in anterolateral margin of anterior region. Pharynx pyriform muscular. Two pairs of eyespots visible at level of pharynx. Intestinal ceca branched, with few lateral diverticula, mostly obscured by vitellarium, confluent posteriorly.

Haptor bell-shaped, without septa; peduncle 464 ± 100 (227–666, n = 32) in length. Haptor armed with a pair of large hamuli (Figure 4C), a pair of smaller hamuli (Figure 4D) and 14 marginal hooklets. Length of median haptor sclerites as follows: large hamulus, 141 ± 50 (88–246, n = 18) in length; small hamulus, 214 (84–289, n = 19) in length. Testes rounded, relatively large and unequal in size, located at midlevel of the body proper, length 228 ± 57 (128–356, n = 31); width 188 ± 44 (98–299, n = 31) for left testes and 224 ± 57 (108–319, n = 30) length, 173 ± 43 (94–258, n = 30) width for right testes (Figure 4). Vasa efferentia not observed. Vas deferens thick-walled, becoming convoluted alongside transverse vitelloducts, entering cirrus sac and enlarging into a fusiform internal seminal vesicle. Cirrus housed in cirrus sac, receiving posteriorly ejaculatory duct. Cirrus sac fusiform, muscular. Ovary anterior to tests, oval, giving rise to a long oviduct, length 109 (55–139, n = 29), 132 (64–198, n = 29) width. Ovo-vitelline duct and

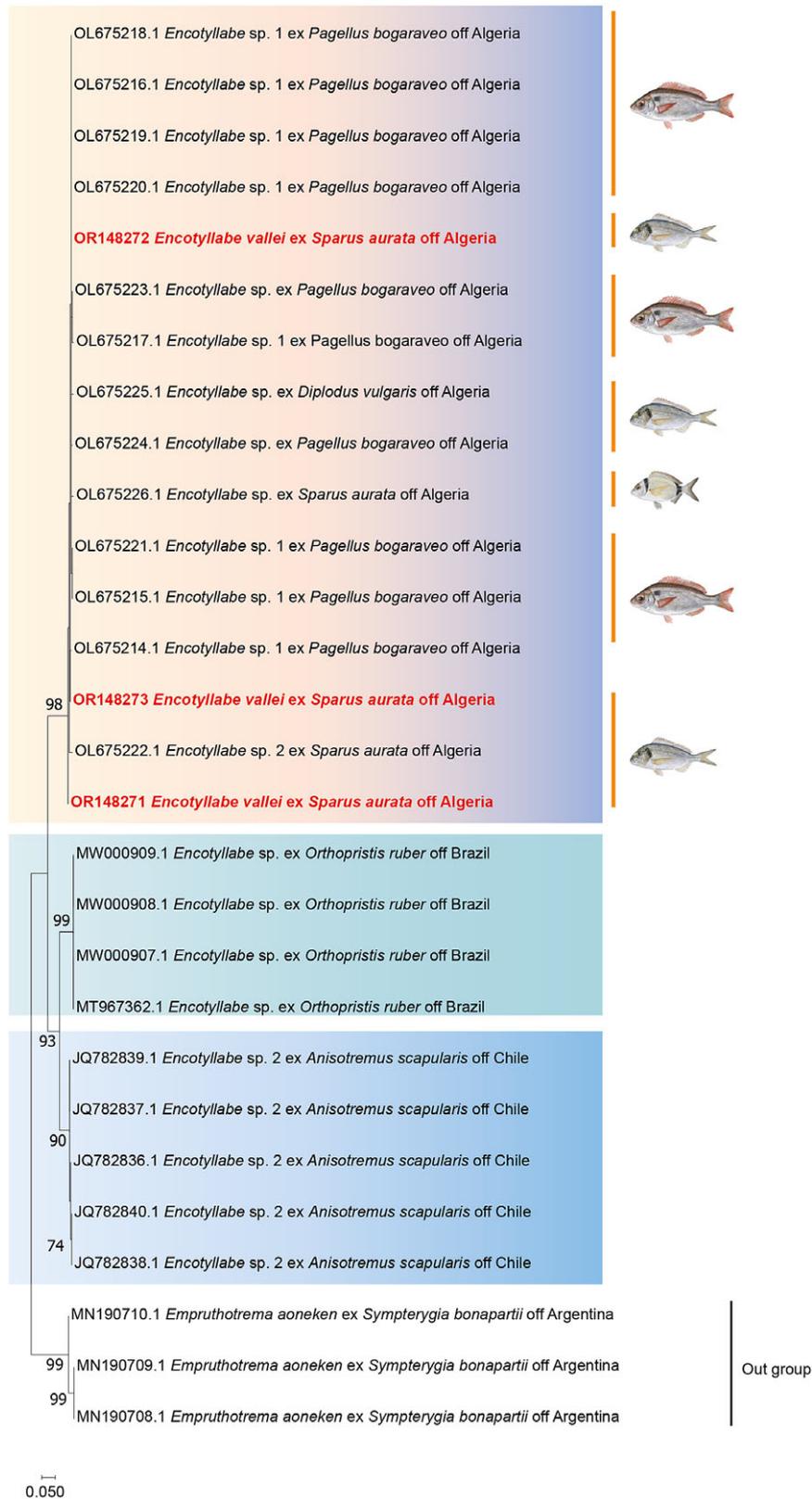


Figure 3 . Maximum likelihood tree based on an analysis of *cox1* sequence data for *Encotyllabe* spp. Bootstrap percentages (500 replicates) are indicated next to or below the branches (only values > 70% are shown). There was a total of 261 positions in the final dataset. The NJ tree (p-distance method) had a similar topology and was not shown.

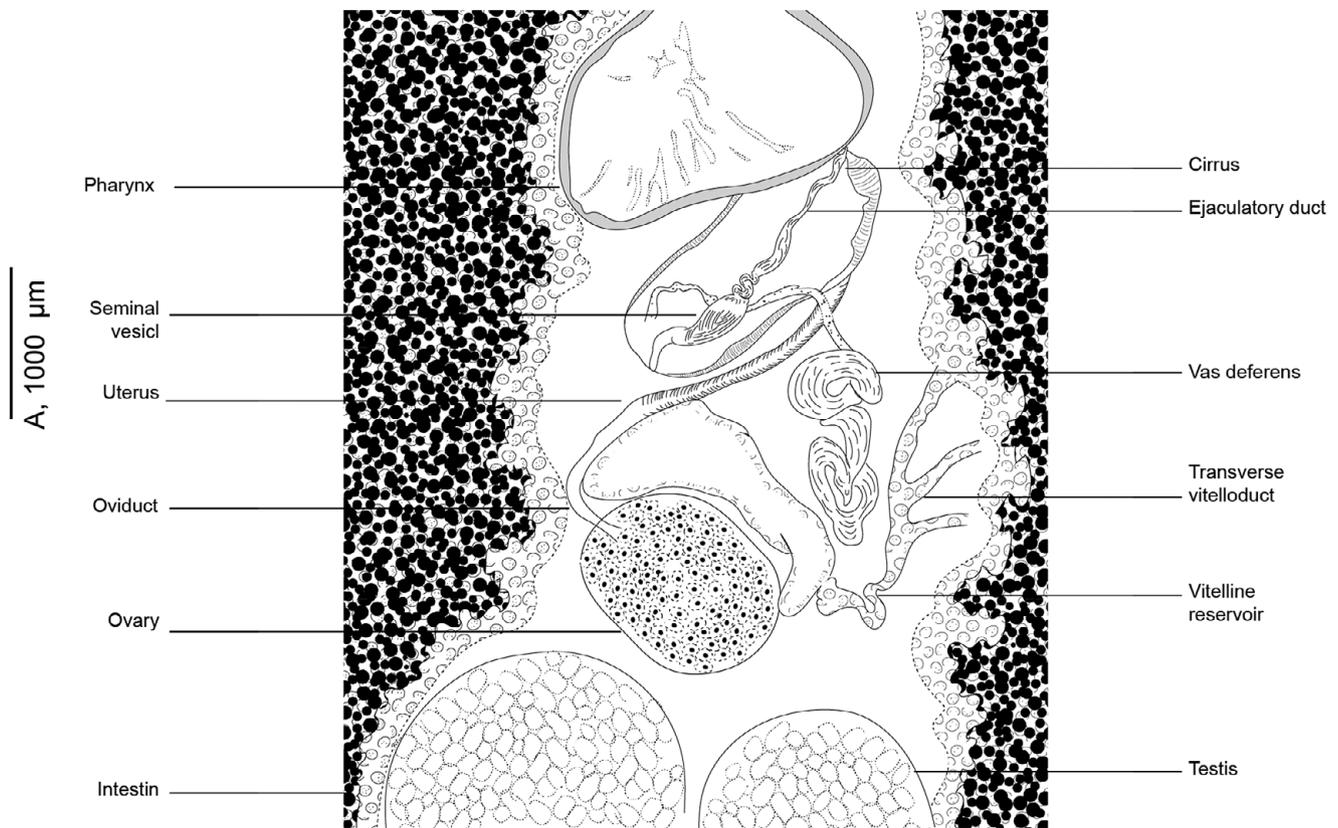


Figure 4. *Encotyllabe vallei* (Monticelli, 1907) ex *S. aurata*, Detail of the reproductive organs in the region of the vagina (SMNH-208 367).

oötype not observed. Vitelline reservoir large. Vitellarium muscular, extending from level of ejaculatory duct to posterior end of body proper. Eggs not observed.

Discussion

To date, the genus *Encotyllabe* comprises 24 species, all of which are parasites of marine fish belonging to various families. They are found in a wide range of aquatic environments, including temperate, subtropical and tropical waters (Lebedev 1967; WoRMS 2023). Among these species, *E. vallei* is common in sparid fish species as indicated in Table 5. First described from *S. aurata*, it was reported on sparids mainly of the genus *Diplodus* (Martorell 2004). There is also a single mention of its occurrence on the common dentex *D. dentex* (Linnaeus, 1758) (Euzet et al. 1993), *Dentex* sp. (Palombi, 1949) and on the redbanded seabream *Pagrus auriga* Valenciennes, 1843 (Martorell 2004).

Furthermore, there have been records of *E. vallei* being on the striped red mullet, *M. surmuletus* Linnaeus, collected off the coast of Spain. However, the authors of this report suggested an accidental parasitism given that both the sparid type-host (*S. aurata*) and the mullet host (*M. surmuletus*) share the same habitat. Alternatively, it could potentially indicate the existence of a distinct species (Ferrer-Castello 2015). Most interestingly, in the figure's caption, Ferrer Castelló (2015) additionally referred to four individuals of *Encotyllabe* found on the annular sea bream *Diplodus annularis* (Linnaeus, 1758) but did not discuss it further. Curiously, in

previous investigations of the transmission of monogeneans in farmed fish, the authors noted the absence of transmission of *E. vallei* from *S. aurata* to *D. labrax* (Fernandez-Jover et al. 2010; Papoutsoglou 2016).

Mahmoud et al. (2014) attributed the capsalid Monogenea collected on *S. aurata* off Egypt, which is the usual host and the type-host of *E. vallei* to *E. spari* (Mahmoud et al. 2014). However, a closer examination and comparison of the photograph of their specimens with the illustration of *E. spari* provided by Yamaguti (1934) reveals slight differences in the position of tests and shape of large hamuli (see Figure 1 in Mahmoud et al. (2014) versus Figure 7 provided by Yamaguti (1934)). Additionally, *E. spari* was first described from three different hosts: the blackhead seabream *Acanthopagrus schlegelii* (Bleeker.) (jun. syn. *Sparus macrocephalus* (Basilewsky)), the trout sweetlips *Plectorhinchus pictus* (Tortonese) and Hong Kong grouper *Epinephelus akaara* (Temminck and Schlegel) from a distinct locality off Japan. It is highly likely that the capsalids reported by Mahmoud et al. (2014) are *E. vallei*.

We note that some authors referred to the 'absence' of a detailed redescription of *E. vallei* (Sepúlveda et al. 2014; Taborda et al. 2023). However, it should be noted that Radujkovic and Euzet (1989) did indeed provide a detailed and beautifully illustrated redescription of *E. vallei*, even though their specimens were collected from Montenegro and not from the type-host. Furthermore, it was sometimes mentioned that Monticelli reported *E. vallei* on *S. aurata* and *Dentex* sp. (Radujkovic and Euzet 1989; Zakia 2019). However, it is important to clarify that the original description of *E. vallei* was based on specimens collected from *S. aurata*, and the first mention

Table 5. Hosts and localities of *E. vallei* Monticelli, 1907. All localities are from the Mediterranean.

Host, locality	References
<i>Sparus aurata</i>	
Italy	(Monticelli 1907), (Palombi 1949)
Spain	(Fernandez-Jover et al. 2010)
Egypt	(Mahmoud et al. 2014) ¹
Algeria (gills)	(Kaouachi et al. 2012; Zakia 2019), present study
<i>Dentex</i> sp.	
Unknown locality	(Palombi 1949)
<i>Dentex dentex</i>	
Mediterranean	(Euzet et al. 1993)
<i>Diplodus puntazzo</i>, gill filaments	
Montenegro	(Radujkovic and Euzet 1989)
Spain	(Sánchez-García et al. 2015)
Algeria	(Kaouachi et al. 2012; Meftah 2012)
<i>Diplodus annularis</i>, <i>Diplodus cervinus</i>, <i>Pagrus auriga</i>	
Spain	(Martorell 2004)
<i>Diplodus sargus</i>	
Spain	(Martorell 2004)
Algeria (gill filaments)	(Kaouachi et al. 2012; Meftah 2012)
<i>Diplodus vulgaris</i>	
Spain	(Martorell 2004)
Algeria (gill filaments)	(Kaouachi et al. 2012; Meftah 2012)
<i>Mullus surmeletus</i>, pharyngeal plates	
Spain, Mediterranean	(Ferrer-Castello 2015)

¹ Reported as *E. spari*.

Table 6. Intraspecific and interspecific variations of the *cox1* gene within species of *Encotyllabe*

Species	Sequences divergence in P-distances (%)	Source
Intraspecific variations		
<i>Encotyllabe haemuli</i>	0	(Taborda et al. 2023)
<i>Encotyllabe yamagutii</i>	0	(Taborda et al. 2023)
<i>Encotyllabe antofagastensis</i>	0.2–0.8	(Sepúlveda et al. 2014)
<i>Encotyllabe cheilodactyli</i>	0.2–1.4%	(Sepúlveda et al. 2014)
Interspecific variations		
<i>Encotyllabe haemuli</i> vs. <i>Encotyllabe antofagastensis</i>	7	(Taborda et al. 2023)
<i>Encotyllabe haemuli</i> vs. <i>Encotyllabe cheilodactyli</i>	9.2	(Taborda et al. 2023)
<i>Encotyllabe cheilodactyli</i> vs. <i>Encotyllabe antofagastensis</i>	10.4–11.4	(Sepúlveda et al. 2014)

on *Dentex* sp. was made by Palombi (1949). The illustrations and morphometric data, along with the molecular data generated herein, will provide satisfactory and reliable material for comparison and for understanding the patterns of host specificity of this monogenean.

The first 28S rDNA and *cox1* sequences for species of *Encotyllabe* in the Mediterranean and of *E. vallei* were provided by Lablack et al. (2022), who conducted an extensive and valuable data

collection of monogeneans from the southwestern Mediterranean. However, some issues arise when analyzing all records of *Encotyllabe* from sparids from Algeria:

In their study, Lablack et al. (2022) proposed the existence of two putative species within the genus *Encotyllabe* based on genetic divergence in the *cox1* gene. The first putative species, referred to as *Encotyllabe* sp. 1, was found on *Pagellus bogaraveo*, while the second putative species, *Encotyllabe* sp. 2, was found

concurrently on *D. vulgaris* and *S. aurata*. They observed a range of genetic divergence within these species that fell between 0% and 1.9%.

Lablack et al. (2022) considered this divergence to be 'slightly higher' than the upper limit of the intraspecific ranges observed in other *Encotyllabe* species, specifically referring to *E. antofagastensis* and *E. cheilodactyli*.

In light of the available data and taking into consideration the molecular data generated so far, it can be deduced that *Encotyllabe* sp. 1 is oioxenic,¹ meaning it is highly specific and primarily associated with *P. bogaraveo*. On the other hand, *Encotyllabe* sp. 2 appears to be stenoxenic, indicating it exhibits a narrower host range, primarily infecting *D. vulgaris* and *S. aurata*.

A similar situation had been demonstrated for microcotylids from sparids: *Microcotyle visa* Bouguerche, Gey, Justine and Tazerouti, 2019 and *M. isyebi* Bouguerche, Gey, Justine and Tazerouti, 2019 are oioxenic to their hosts, *Pagrus caeruleostictus* (Valenciennes.) and *Boops boops* (Linnaeus.) (Bouguerche et al. 2019a, 2019b; Villora-Montero et al. 2020); *Microcotyle whittingtoni* Villora-Montero, Pérez-del-Olmo, Georgieva, Raga and Montero, 2020 is oioxenic to *D. dentex* (Villora-Montero et al. 2020), while the enigmatic *M. erythrini* Van Beneden et Hesse, 1863 is rather stenoxenic, occurring on *Pagellus erythrinus* (Linnaeus) and on *P. pagrus* (Linnaeus) (Villora-Montero et al. 2020). Hence, we could assume that *Encotyllabe* sp. 1 is oioxenic while *Encotyllabe* sp. 2 is stenoxenic, but a more significant concern arises when trying to distinguish between *Encotyllabe* sp. 1 and *Encotyllabe* sp. 2. (1.1–1.9%) is well below the threshold for Monogenea and invertebrates, and higher divergences were used to separate *Encotyllabe* species (see Table 6). In the present analysis, the divergence of *E. vallei* from *S. aurata* ranged between 0–2 % which suggests that a single species is present in *S. aurata*. Similarly, the divergence between *Encotyllabe* from *S. aurata*, *D. vulgaris* and *P. bogaraveo* does not exceed 2%, below the interspecific divergence for *cox1* known for Monogenea. We thus suggest that *E. vallei* is the sole species infesting the previously mentioned host.

It is important to note that stenoxenic specificity is not unusual in Monogenea and has been previously demonstrated using molecular barcoding (Bouguerche et al. 2019a, Lablack et al. 2022; Villora-Montero et al. 2020). For instance, the microcotylid *M. erythrini* was described from the sparid *Pagellus erythrinus* off Brest (Brittany, Atlantic Ocean) than recorded from three other hosts (*Pagellus acarne*, *B. boops* and *D. dentex*) in several localities in the Mediterranean (Bouguerche et al. 2019b). Despite the record of *M. erythrini sensu lato* from the record from *B. boops* being demonstrated to be a distinct species, *M. erythrini sensu stricto* had been shown to be exceptionally a stenoxenic microcotylid, occurring on *P. erythrinus* and *P. pagrus* (demonstrated by *cox1* barcoding) (Villora-Montero et al. 2020). *Encotyllabe vallei* presents another 'enigmatic' monogenean species, displaying a host specificity pattern distinct from that of its congeners.

In our current study, it is noteworthy that the molecular tree based on 28S sequences illustrates the monophyly of three subfamilies of capsalids: the Encotyllabinae, Benedeniinae and Capsalinae

(Figure 2). Perkins et al. (2009), in a molecular study based on different markers, retrieved only the Capsalinae as monophyletic. Similarly, Gastineau et al. (2023) retrieved a monophyly of the Capsalinae, with representatives of all four genera of the subfamily (*Capsala* Bosc, 1811; *Capsaloides* Price, 1936; *Tristoma* Cuvier, 1817 and *Nasicola* Yamaguti, 1968) united together in a well-supported monophyletic clade. Results for other subfamilies were less in accordance with traditional taxonomy (Gastineau et al. 2023). Hence, future phylogenetic investigations, using more extensive datasets, will certainly offer a more comprehensive insight into the evolutionary history of capsalid monogeneans.

The distribution of *Encotyllabe* spp. according to the systematics of host groups is given in Table 1, which summarizes updated data originally compiled by Lebedev (1967). From the available data, a discernible pattern emerges in the geographical distribution of *Encotyllabe* species: *Encotyllabe lintoni* Monticelli, 1909; *Encotyllabe monticelli* Perez Viguera, 1940 and *Encotyllabe pricei* Koratha, 1955 occur in North America. *E. pagrosomi* MacCallum, 1917 and *Encotyllabe embiotocae* Noble, 1966 occur in Pacific waters; *E. chironemi* is restricted to the coast of New Zealand and in the seas of Japan; *Encotyllabe caranxi* Lebedev, 1967 described from New Zealand-Australian fish; *E. pagelli* is known from the coast of Belgium and Ireland; *E. spari* Yamaguti, 1934; *E. lutiani* Tripathi, 1959 and *E. masu* Ishii and Sawada, 1938 is found off the coast of India. The Mediterranean species are *E. vallei*, *E. paronae* and *E. nordmanni*. The validity of all the Mediterranean species in addition to *E. pagelli* was questioned (Taborda et al. 2023). Another striking aspect when analyzing these data is that nearly all *Encotyllabe* spp. were described from a single host species or closely related host species, as previously noted by Taborda et al. (2023), suggesting host specificity. However, the findings presented in our study challenge the notion of strict host specificity for *Encotyllabe*, particularly for *E. vallei* indicating that it primarily associates with a narrower but still specific range of host species. A similar conclusion was reached for *Encotyllabe* species from Haemulidae hosts, as such *Encotyllabe haemuli* and *E. antofagastensis* which formed a well-supported clade (see Taborda et al. (2023)).

Determining the taxonomic status of *Encotyllabe* spp. presents a challenging task, as traditional taxonomy relies on a variety of morphological characteristics, including 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 (Robinson 1961; Sepúlveda et al. 2014; Taborda et al. 2023). However, it is important to note that the presence of an evaginated or protrusible pharynx, as suggested by Martorell (2004), adds a layer of complexity to this taxonomic classification. Considering this, it may be advisable to exclude the size of the pharynx as a definitive criterion for taxonomic differentiation.

Because *P. bogaraveo* is the type-host of *E. pagelli* (Van Beneden and Hesse 1863), we were at first tempted to suggest a synonymy between *E. vallei* and *E. pagelli*. However, we refrained from doing so as *E. pagelli* was first described from the Atlantic coast off Brest, France. In the absence of available morphological data for *Encotyllabe* from *P. bogaraveo*, it remains challenging to definitively determine the host specificity of *E. vallei*. There is the possibility that a species complex exists within individual sparid fish species, as has been observed in some other *Encotyllabe* species. The acquisition of morphometric and morphological data from specimens collected from *P. bogaraveo* would be instrumental in shedding light on the host specificity patterns of this capsalid and clarifying its taxonomic status within the genus.

¹To describe the host specificity of a parasite considering the relatedness of host species, Combes (2003) employed the following terms: If the parasite attacks a single host species, it is denoted as oioxenic or monoxenic; if it attacks only a range of phylogenetically related species, it is termed stenoxenic and, if it attacks a range of mutually unrelated species, it is termed euryxenic.

Conflict of interest declaration. The authors declare that they have no competing interests.

Author contributions. Fatima-Zohra Zedam: conceptualisation; data curation; investigation; methodology; project administration; validation; writing – original draft. Chahinez Bouguerche: data curation; funding acquisition; investigation; methodology; project administration; validation; writing – original draft, review and editing. Mohammed Ahamed: conceptualisation; data curation; investigation; methodology; project administration; validation. Fadila Tazerouti: project administration. All authors read and approved the final manuscript.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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