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Speciation of the *Paradeontacylix* spp. (Sanguinicolidae) of *Seriola dumerili*. Two new species of the genus *Paradeontacylix* from the Mediterranean ☆

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ABSTRACT

Two new species of teleost blood fluke belonging to the sanguinicolid genus *Paradeontacylix* are described from the greater amberjack, *Seriola dumerili*, i.e. *Paradeontacylix ibericus* n. sp. from the Iberian Peninsula and *Paradeontacylix balearicus* n. sp. from the Balearic Islands. *P. ibericus* n. sp. and *P. balearicus* n. sp. show morphological similarities with *Paradeontacylix kampachi* and *Paradeontacylix grandispinus* respectively, which occur in mixed infection in *S. dumerili* from Japan. Multivariate analysis of morphometrical data provided statistical evidence for the separation of four species. However, component by component analysis did not show statistically significant differences between *P. balearicus* and *P. grandispinus*. Molecular data based on rITS2 and mCO1 gene sequences also supported the separation into four species. Morphological and molecular data were used to examine phylogenetic relationships between *Paradeontacylix* species from *S. dumerili* and other species in the genus. The results coincided in revealing two main branches with *P. kampachi*+*P. ibericus* and (((*P. grandispinus*+*P. balearicus*) *Paradeontacylix sanguinicoloides*) *Paradeontacylix godfreyi*). *Paradeontacylix odhneri*, for which little data are available, was located basal in a separate branch. This is the only species of *Paradeontacylix* which parasitizes a non-carangid host which might probably explain the separation from the other species. Paired similarities between the Japanese and the Mediterranean species, despite the large geographic distance, could be explained by the speciation of parasite geminate lines before host separation by tectonic events. Consequently, geographic and historical isolation support the morphological and genetic differences leading to the evolution of the new species described here.

1. Introduction

The amberjacks are species of the genus *Seriola* (Carangidae, Perciformes) which have a long tradition in aquaculture and fisheries, especially in Japan [1]. One of the most valuable species for consumption worldwide is greater amberjack *Seriola dumerili* (Risso, 1810). In the Mediterranean area, it has been experimentally cultivated in Italy, Spain, Malta and Greece [1–3]. Attempts involving more intensive culture have been unsuccessful due to high juvenile mortality. One of the most significant causes of loss of 0+ and 1+ *S. dumerili* are parasitic infections caused by blood flukes [4,5] belonging to the genus *Paradeontacylix* (Sanguinicolidae, Digenea).

Mainly, pathologies are related to egg release in the circulatory system. The accumulation of eggs in gills and the lost of branchial epithelium caused by the hatching and leaving of miracidia provokes abnormal blood circulation, haemorrhages, hypoxia and cause fish mortalities. Severe sanguinicolid infections episodes have been reported in some places around the world as far as Majorca in Mediterranean and Japan in Pacific Ocean [4,5].

In Japan, mortalities have been associated with the sanguinicolid *Paradeontacylix grandispinus* Ogawa et Egusa, 1986 occurring in mixed infections with *Paradeontacylix kampachi* Ogawa et Egusa, 1986 [5]. The first experimental cultures of *S. dumerili* in the western Mediterranean were carried out in Andratx (Majorca, Balearic Islands) and in Puerto de Mazarrón (Murcia, Iberian Peninsula). Although sanguinicolidosis was reported in both localities, juvenile fish mortality was only found in Majorca being so high that the culture was discontinued [4]. The blood flukes from the Mediterranean are very similar to those from

☆ Note: Nucleotide sequences data reported in this paper are available in the EMBL, GenBank and DDJB data bases under the accession numbers AM489593–AM489607.

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Japan: those from Murcia were provisionally identified as *P. kampachi* [3,6] whereas the parasites from Majorca were described as *P. grandispinus*-like [7]. Type of culture facilities was also different in both localities: sea-cages in Majorca and tanks in Murcia. Invertebrate intermediaries in sea-cages together with the high density of host in culture conditions could increase the infection abundance levels [8]. Determining the identity of the involved parasite is necessary in order to detect other possible related factors as different species virulence.

This study deals with the morphological and genetic analysis of Mediterranean blood flukes from *S. dumerili* in order to determine the identity of the species of *Paradeontacylix* in Balearic Islands and in the Iberian Peninsula and their relation to the known species of the genus.

2. Materials and methods

2.1. Sample collection

Ninety *S. dumerili* were obtained from two sites off the island of Majorca (Balearic Islands, Spain): between Cala Figuera and Cabrera (South, 39°8′–21′N/2°42′–3°02′E) and between Pollença and L'Alcúdia (North, 39°52′–40°07′N/3°20′–2°53′E). Fifteen fish were obtained from Santa Pola (Iberian Peninsula, Spain, 38°06′–17′N/0°16′–34′W). Herein, the blood flukes from Majorca will be referred to as "Balearic" and those collected from Santa Pola, as "Iberian". Five samples of 15 *S. dumerili* were obtained every 2 months between April 2005 and March 2006 (except in the fishing restriction period, between July and September), from local fish markets in Majorca. One sample of 15 individuals was obtained from Santa Pola in February 2006. The circulatory system and organs of strong blood supply were scrutinized for the presence of blood flukes and eggs including the: sinus venosus, atrium, ventricle, bulbus arteriosus, aorta dorsalis, vena caudalis, gill arches, kidney, spleen and liver. Thoracic and pelvic girdles and the associated muscular tissue were also examined, following the suggestion of Montero et al. *et al.* [6] that blood flukes are frequently found in these microhabitats. The organs were washed with saline, pressed between Petri dishes and observed under the stereomicroscope at 10 magnifications. The presence of fluke eggs was recorded and adult blood flukes were collected and fixed in 70% ethanol for morphological analysis and in 100% ethanol for molecular analysis. Ecological terms according to Bush et al. *et al.* [9].

2.2. Morphological analysis

Worms were stained with iron acetocarmine [10], dehydrated and mounted with Canada balsam. In order to compare the Mediterranean *Paradeontacylix* spp. with morphologically similar species 10 paratypes of *P. grandispinus* (K. Ogawa's collection) and 4 paratypes of *P. kampachi* (K. Ogawa's collection) from Kochi and Kagoshima Prefectures, Japan were analysed. In addition, 10 specimens of *Paradeontacylix* sp. from experimental cultures of *S. dumerili* at the Spanish Institute of Oceanography (SIO) in Puerto de Mazarrón (Murcia, peninsular Spain) were examined. These specimens had been examined previously [7,6] and were obtained from the parasite collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology (ICBIBE, University of Valencia, Spain). Drawings and measurements of adult specimens and eggs were made with the aid of a drawing tube and digital images using Image Tools for Windows 3.00. Co. 1995–2002, UTHSCSA. Measurements are given as the mean in μm, with the range in parentheses.

2.3. Statistical analysis

The morphometrical information obtained was analysed by discriminant analysis and principal component analysis using SPSS® 12.0 (SPSS, Inc., Norusis, 2002). Fourteen morphometric characters were obtained to describe individuals: perimeter/2, maximum body width, posterior marginal spine length, oesophagus length, length of anterior and posterior caeca, ganglion width, length of testicular zone, ovary length and width, post-testicular space, uterus length and wide, length of seminal receptacle and oviduct length. PCA was performed with the discriminant variables resulting from the discriminant analysis. Metrical data was log-transformed and corrected to reduce the differences caused by isometric growth: data of each individual were divided by the geometric average of all variables measured [11]. In order to verify that the PCA assumption of homogeneity of variance distribution was correct, and considering that the analysis had been made with multiple groups, the difference between the variances of the study groups, *P. kampachi*, *P grandispinus*, *Paradeontacylix ibericus* and *Paradeontacylix balearicus*, was tested [12] using the statistical software Philips CPC* (Phillips, P., 1994–7. CPC — common principal component analysis program. University of Texas at Arlington, Texas). Tested hypotheses were: 1, equality of variance; 2, homogeneity of variance; 3, variances with four, three, two or one common components; 4, random distribution of the variance. The eigenvectors resulting from the latter analysis were used to perform a common principal component analysis (CPC); eigenvectors were transformed in components and graphically represented with SPSS 12.0. The relaxation degree of the *P. grandispinus* paratypes was different but all the type specimens were included in the analysis. Thereafter, the average measurements of each species were statistically compared and the significance of the differences between the species by each component was tested [13].

2.4. Phylogenetic analysis based on morphological data

Morphological data of all the *Paradeontacylix* species [*P. kampachi*; *P. sanguinicoloides* McIntosh, 1934; *P. godfreyi* Hutson et Whittington, 2006; *P. grandispinus*; *P. sinensis* Liu, 1997; and *P. odhneri* (Layman, 1930)] [14–18] and the Mediterranean morphotypes were analysed under the criterion of maximum parsimony following the methodology of Wiley et al. *et al.* [19]. The revision of the original descriptions of all known *Paradeontacylix* species showed that *P. sinensis* does not agree with the diagnostic character for the genus *Paradeontacylix*. The main differences are: only one large testis in *P. sinensis*, which reaches near the lateral body margins in contrast to multiple testes between the caeca (*Paradeontacylix*); and genital pores close together in *P. sinensis* whereas they are separated in *Paradeontacylix*. This species should be assigned to a different genus and was consequently excluded from the analysis.

Phylogenetic analyses using PAUP* version 4.0b10 (Swofford, 2001. PAUP 4 — phylogenetic analysis using parsimony. Version 4. Sinauer Associates, Sunderland, Massachusetts) were conducted using a heuristic search algorithm, characters unordered and the ACCTRAN option. Character polarization was done using *Aporocotyle spinosicanalis* Williams, 1958, as outgroup [20]. The outgroup was selected because the type species of the genus *Aporocotyle*, *A. simplex*, was described as a junior synonym of the *Paradeontacylix* genus [21].

Table 1
Morphological data matrix of *Paradeontacylix* species and *Aporocotyle spinosicanalis* as an outgroup

Sanguinicolid species	Morphological characters											Host
	1	2	3	4	5	6	7	8	9	10	11	
Aporocotyle spinosicanalis	0	0	0	0	0	0	0	0	0	0	0	*Merluccius merluccius*
P. kampachi[a]	1	2	1	1	0	0	1	1	0	0	1	*Seriola dumerili*
P. ibericus[a]	1	2	1	1	0	0	1	1	0	0	0	*Seriola dumerili*
P. grandispinus	1	1	0	0	1	1	1	1	0	0	2	*Seriola dumerili*
P. balearicus	1	1	0	0	1	1	1	1	0	0	2	*Seriola dumerili*
P. godfreyi	1	0	0	2	1	1	0	0	0	1	0	*Seriola lalandi*
P. sanguinicoloides	1	1	0	1	1	1	1	0	0	0	2	*Seriola lalandi*
P. odhneri	0	0	0	1	0	0	1	0	1	0	1	*Takifugu porphyreus*

Includes host identity.
[a] Note that *P. grandispinus* and the *P. balearicus* share the same codification.

However *A. spinosicanlis* was the only sequenced species of the genus. Clade support was determined via 1000 bootstrap replicates. Character argumentation: see Table 1.

Eleven characters, 8 binary and 3 multistate characters with a total of 25 character states were used in the analysis. 0 represents the plesiomorphic condition and 1 or 2 an apomorphic one. Categories of quantitative variables were established with one third of the range as step, except for the number of tegumental spines as the range was very small and categories were established with one half of the range as step. 1) Relative position of ovary and uterus: 0, ovary posterior; 1, ovary anterior. 2) Number of testes rows: 0, irregular (no rows); 1, 1 row; 2, 2 rows. 3) Arrangement of testes: 0, contiguous; 1, separated. 4) Number of testes: 0, until 35; 1, between 35 and 70; 2, >70. 5) Ovary shape: 0, "tear-shaped"; 1, "heart-shaped". 6) Large posterior tegumental spines: 0, absent; 1, present. 7) Number of marginal tegumental spines per row: 0, >15; 1, <15. 8) Caeca shape: 0, "H-shaped"; 1, "X-shaped". 9) Vitelline fields extend posterior to ovary: 0, no; 1, yes. 10) Testes posterior to caeca: 0, absent; 1, present. 11) Maximum body size: 0, between 3500 and 7000; 1, >7000; 2, <3500. *P. grandispinus* and *P. balearicus* share all these characters. Consequently, they were joined as a single taxon for the analysis.

2.5. Phylogenetic analysis, based on molecular data

DNA was extracted from two individual worms each from the Iberian and the Balearic *Paradeontacylix* morphotypes from *S. dumerili*; from one specimen each of *P. kampachi* and two specimens of *P. grandispinus* from *S. dumerili* from Japan (Ushine, Kagoshima Prefecture, 31°33′N/130°43′E, June 2006), and from two individuals of *P. godfreyi* from *Seriola lalandi*, from Australia (Port Augusta, South Australia, 32° 42′S/137° 46′E, August 2006).

Fixed specimens were transferred to 300 µl TNES urea (10 mM Tris–HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetra-acetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS), 4 M urea) and DNA was extracted using a phenol-chloroform protocol as described previously [22]. DNA of two ribosomal gene regions, i.e. the partial 28S and the complete internal transcribed spacer 2 (ITS2), as well as of the mitochondrial cytochrome oxidase 1 (COI) gene was amplified using the primers summarised in Table 2. The forward primer 3S was used for all *Paradeontacylix* spp. except for *P. godfreyi*, for which GA1 was used. Each 30 µl PCR reaction contained 1.5 U of Thermoprime Plus DNA polymerase and 10× buffer containing 1.5 mM $MgCl_2$ (ABgene), 0.2 mM of each dNTP, 15 pmol of each primer and 50–80 ng of template DNA. Denaturation of DNA (95 °C for 2 min) was followed by 35 cycles of amplification (95 °C for 50 s, annealing temperature for 50 s and 72 °C for 50 s) and terminated by a 4-minute extension (72 °C). Annealing temperature was 56 °C for all reactions but the ones amplifying COI, for which it was set to 50 °C. The PCR products obtained were purified for sequencing using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Cycle sequencing of the DNA fragments was conducted on a 48 capillary ABI 3730 sequencer (Applied Biosystems) using the BIG Dye Terminator v3.1 Ready Sequencing Kit (Applied Biosystems, USA). Forward and reverse strands were sequenced in all cases. Nucleotide sequence data reported in this paper are available in GenBank™, EMBL and DDBJ databases under the accession numbers AM489593–AM489607.

The obtained sequences were aligned using Clustal X [23] using a gap opening penalty of 10.00, a gap extension penalty of 5.00 and the DNA weight matrix of the International Union of Biochemistry (IUB). The resulting alignments of 28S and ITS2 rDNA sequences as well as of the COI sequences were joined in a combined dataset using BioEdit 4.8.9 [24] and were exported in NEXUS format. Tree inference was carried out by a likelihood-based Bayesian tree sampling procedure (BI) and under the maximum parsimony optimality criterion (MP). BI was conducted using MrBayes v 3.0 [25] with parameters corresponding to the general time-reversible model GTR+I+Γ [26], which was estimated as the best substitution model for the dataset by using Modeltest version 3.7 [27]. BI used the following parameters: nst=6, rates=invgamma, ngammacat=4. The MCMC was allowed to run for 1,000,000 generations sampling every 100th tree. The first 50,000 generations were later discarded as the burn-in period. The maximum parsimony (MP) analysis was conducted with PAUP*, version 4.0b10 using a heuristic search with tree bisection-reconnection (TBR) branch swapping, random addition of taxa (10 replications) and the ACCTRAN option. Gaps were treated as missing data. Clade support was assessed with bootstrapping of 1000 replicates.

3. Results

Eggs and adults of *Paradeontacylix* were frequently found at all Spanish sampling localities. In fish from Majorca eggs were detected all year round with a mean prevalence of 51.3%. The adult mean prevalence throughout the year was 10.2%. *S. dumerili* sampled from Santa Pola (February 2006) showed a prevalence of 53.3% for eggs and 20.0% for adults. Eggs found were located exclusively in the heart and gills. The position of the eggs inside the gills was also different between localities: the eggs from Majorca were located in both the afferent and efferent vessels, as well as in the secondary lamellae, whereas in Santa Pola they were only detected in the afferent vessels of the primary gill lamellae. The comparison of specimens from the Iberian Peninsula sites (Santa Pola and Puerto de Mazarrón) revealed no morphological differences. The Iberian and Balearic blood blood-fluke adults were markedly different but similar to *P. kampachi* and *P. grandispinus* respectively, however according to their morphological and molecular peculiarities two new species are proposed.

3.1. P. balearicus n. sp. (Fig. 1A,B,C)

3.1.1. Description

Based on 7 whole mounted, gravid specimens; metrical data from all the 7 adult worms. Body smooth, elongate, dorsoventrally flattened, lancet-shaped, approximately 10 times as large as wide. Total length 1579 (1318–1901); maximum width 170 (112–219). Lateral margins armed ventrally with elongate marginal tegumental spines distally

Table 2
Primers used for the amplification and sequencing of ribosomal and mitochondrial genes of *Paradeontacylix* spp.

Gene/region	Primer	Primer sequence	Direction	Application	Reference
Ribosomal					
28S	U178	5′-GCACCCGCTGAAYTTAAG-3′	Forward	PCR+seq	Lockyer et al., 2003
28S	L1642	5′-CCAGCGCCATCCATTTTCA-3′	Reverse	PCR+seq	Lockyer et al., 2003
28S	LSU1200R	5′-GCATAGTTCACCATCTTTCGG-3′	Reverse	Seq	Lockyer et al., 2003
ITS2	3S	5′-GGTAACGGTGGATCACGTGGCTAGTG-3′	Forward	PCR+seq	Bowles et al., 1993
ITS2	ITS2.2	5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′	Reverse	PCR+seq	Cribb et al., 1998
ITS2	GA1	5′-AGAACATCGACATCTTGAAC-3′	Forward	PCR+seq	Anderson and Baker, 1998
Mitochondrial					
COI	JB3	5′-TTTTTTGGGCATCCTGAGGTTTAT-3′	Forward	PCR+seq	Bowles et al., 1993
COI	JB4.5	5′-TAAAGAAAGAACATAATGAAAATG-3′	Reverse	PCR+seq	Bowles et al., 1993

hooked, distributed in 341 (321–361) rows surrounding entire body margin. Each row with 8–12 spines, decreasing in number towards posterior end until it reaches 2–3. Large posterior tegumental spines 23 (22–24) long and 3 (2–4) wide (Fig. 1C) gradually decreasing in length towards anterior end. Body marginal tegumental spines 2.5 (2–5) in length and 1 (0.5–1.5) in width. Nerve-cord laterally extended 1516 (1278–1872) long, 18 (14–22) maximum wide, 29 (20–35) from body margin in mid-body, connecting in posterior end of body. Ganglion arranged perpendicular to mid-line of body connecting dorsolateral nerve-cords, 89 (66–118) or 5–6% of body length from anterior end of body, 56 (48–69) across width of worm 33 (41–27) in diameter.

Mouth anteroventral subterminal. Oesophagus 403 (241–546) long or 15–34% of body length, 15 (11–20) in maximum width. Oesophagus ventral to nerve ganglion, medial, winding smoothly and extending posteriorly along mid-line for 1/3–1/4 of body length, complete extension surrounded by gland-cells, most abundant in area 204 (160–230) long or 42–66% of oesophagus total length, up to approximately. Intestinal caeca X-shaped, caecal intersection 573 (523–638) or 34–39% from anterior end of body. Anterior caeca 50 (36–62) long or 3% of body length, 19 (14–24) wide; posterior caeca 522 (437–645) long or 33–34% of body length, 15 (13–17) wide. Posterior caeca located laterally along testes zone and ending blindly anterior to ovary.

Testes 22 (20–26), rounded or ellipsoidal, in a single row between posterior caeca. Testicular zone 496 (403–586) in length, occupying 34% of total length, 102 (87–117) wide or 53–78% of body width, 4.6–5.0 longer than wide. Post-testicular space 515 (381–613) long or 30–32% of body length. Vasa efferentia not observed. Vas deferens, 750 (619–886) long, 37 (27–72) wide, originating in anterior testes, extending sinistral, dorsally to ovary, leading to seminal vesicle enclosed in cirrus sac. Seminal vesicle 106 (86–124) long, 35 (23–48) wide. Cirrus 25 (11–40) long or 13–32% of seminal vesicle length. Male genital pore dorsal, 272 (204–339) or 15–18% of body length from posterior extremity of body, 15 (7–21) from sinistral body margin and 149 (97–193) from dextral body margin.

Ovary heart-shaped, distally elongate, 111 (95–142) long or approximately 7% of body length, 123 (105–158) wide or 70–90% of body width, 1.1–1.3 wider than long. Located immediately posterior to testis, between nerve-cords, anterior to seminal vesicle and ventral to vas

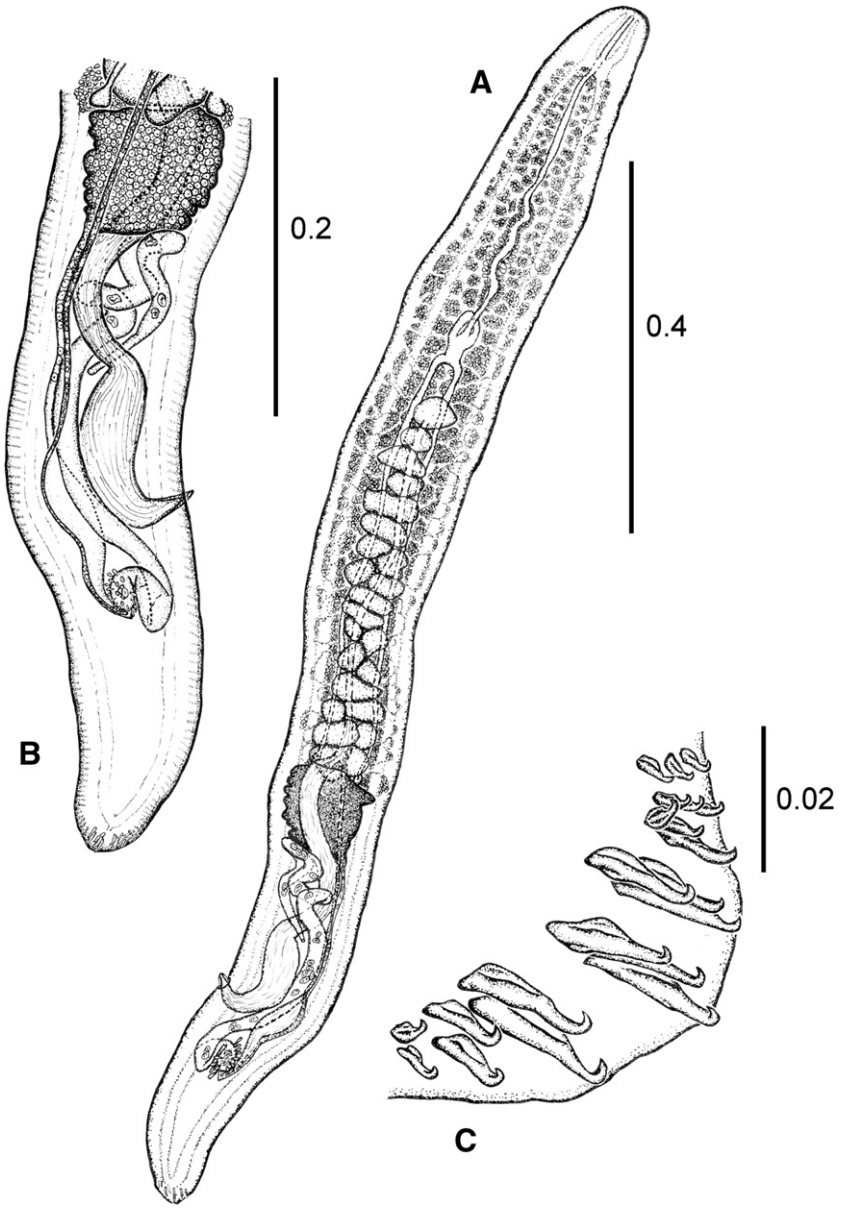

Fig. 1. *Paradeontacylix balearicus* sp. n. A. Holotype, whole mount, dorsal view. B. Posterior end, ventral view showing post-ovarian region. C. Large posterior tegumental spines, ventral view. Scale bars in millimetres.

deferens. Oviduct originates at dextral posterior convex part of ovary. Oviduct 170 (103–251) long, 8 (6–11) wide. Posterior portion of oviduct forms seminal receptacle 148 (120–192) long, 28 (22–41) wide. Seminal receptacle narrowing progressively and joining vitelline duct before forming oötype. Oötype 32 (15–49) long and 21 (11–27) wide, surrounded by Mehlis' gland. Vitelline follicles extending along body from posterior margin of nerve commissure to ovary, with much lower density close to lateral margins. Vitelline duct in anterior portion of testes area running ventrally until reaching seminal receptacle and oötype. Uterus folded 467 (348–586) long and 29 (24–38) wide, extending along post-ovarian zone, short portion descending posterior to oötype connecting to ascending portion slightly dextral to body mid-line ending with ventral curvatures near the ovary, terminating with the descending portion 88 (71–119) long or 20% of uterus length, 12 (9–19) wide. Female genital pore opening dorsally at 307 (229–368) of posterior body margin, 96 (69–119) of sinistral body margin and 49 (33–86) of dextral body margin. Uterine eggs amorphous, spherical to ellipsoidal, measuring 8–14 long and 4–11 wide or 16–37% of uterus maximum width (Fig. 1B).

3.1.2. Taxonomic summary

Type-host: *Seriola dumerili* (Risso, 1810) (Carangidae).

Site: Afferent gill vessels and heart.

Type-locality: Cabrera — Cala Figuera (South, 39°8′–21′N/2°42′–3°02′E), southern Majorca, Balearic Islands.

Other locality: L'Alcúdia — Pollença (North, 39°52′–40°07′N/3°20′–2°53′E), northern Majorca, Balearic Islands.

Type specimens: Holotype MPM Coll. No. 18871-A5123, 1 Paratype MPM Coll. No. 18871-A5124; 1 Paratype NHM 2008.5.9.1. GenBank accession numbers: AM489594 (28S rRNA), AM489599 (ITS2), AM489604 (COI).

Collectors: A. Repullés (University of Valencia) and F.E. Montero (Autonomous University of Barcelona).

Infection details: Number of infected fish=43 (eggs)/8 (adults); prevalence 51.3% (eggs); mean intensity 1 (adults); infected host size 32.50–42.50 cm TL (32.00–44.50 cm TL, *n*=90).

Etymology: The species name refers to the host origin, the Balearic Islands.

3.1.3. Remarks

Paradeontacylix McIntosh, 1934 (*sensu* Smith, 1907). *P. balearicus* n. sp. displays all the diagnostic characteristics of the genus *Paradeontacylix*. It is differentiated from the other species of the genus by the combination of: short length (the shortest species ranging from 1318 to 1901), no more than 26 testes arranged in one row, ovary heart-

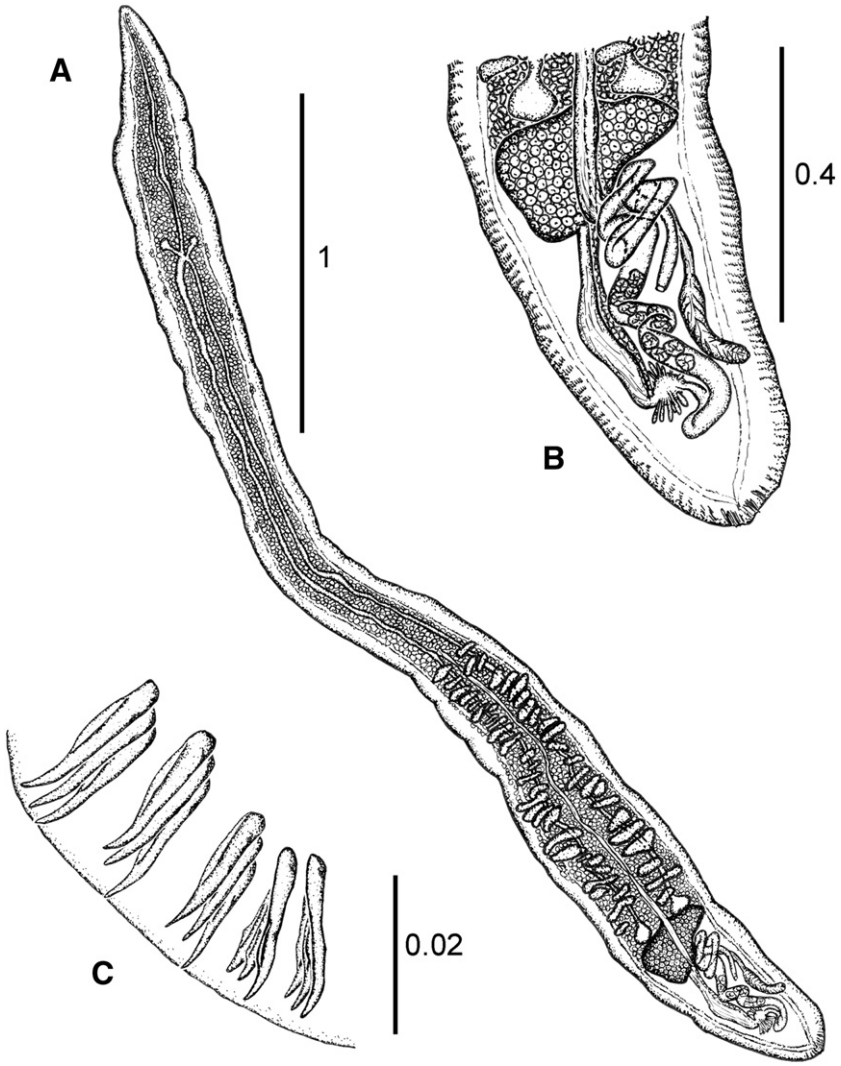

Fig. 2. *Paradeontacylix ibericus* sp. n. A. Holotype whole mount. B. Posterior end, showing post-ovarian region. C. Large posterior tegumental spines. Ventral views. Scale bars in millimetres.

shaped, uterus post-ovarian, female genital pore slightly dextral near mid-line, from 8 to 12 spines per row and large posterior tegumental spines. *P. balearicus* most closely resembles *P. grandispinus*, as both species share a similar number and arrangement of testes, 19 to 32 testes in *P. grandispinus* and 20 to 26 testes in *P. balearicus* arranged in one row; also share very large elongate marginal posterior tegumental spines, 21 to 26 in *P. grandispinus* and 22 to 24 in *P. balearicus*. However, *P. balearicus* differs from *P. grandispinus* in that its dimensions and proportions are halved. Furthermore, the marginal posterior tegumental spines are relatively longer: posterior spine length/total body length: 1/60–1/80 in *P. balearicus*; 1/93–1/128 in *P. grandispinus*. The discriminant analysis and the host distribution are also disparate.

3.2. *P. ibericus n. sp.* (Fig. 2A,B,C)

3.2.1. Description

Based on 13 whole mounted, gravid specimens from Santa Pola ($n=3$) and from Puerto Mazarrón ($n=10$); metrical data from 10 adult worms. Body smooth, elongate, dorsoventrally flattened, lancet-shaped, approximately 14 times as long as wide. Total length, 4090 (2480–5700); maximum width 340 (290–460). Lateral margins armed ventrally with elongate marginal tegumental spines distally hooked, distributed in 678 (577–746) rows, which surround entire body margin except anterior extremity. Each row with 12–14 spines, decreasing in number towards the posterior end until reach 3–5. Large posterior tegumental spines 13 (8–18) long and 3 (2–4) wide (Fig. 2C) gradually decreasing towards the anterior end. Body marginal tegumental spines 5 (4–6) long and 1 (0.5–1.5) wide. Nerve-cord laterally extended 4039 (2438–5652) long, 15 (13–19) maximum wide, 43 (31–52) from body margin in mid-body, connecting in posterior end of body. Ganglion arranged perpendicular to mid-line of body connecting dorsolateral nerve-cords, 130 (114–152) or 2–5% of body length from anterior end of body, 61 (54–67) across width of worm 33 (29–43) in diameter.

Mouth anteroventral subterminal. Oesophagus 700 (580–1140) long, 14–28% of body length, 15 (10–18) in maximum width. Oesophagus ventral to nerve ganglion, winding smoothly and extending posteriorly along mid-line for 1/5–1/6 of body length, surrounded the complete extension by gland-cells, most abundant in area 368 (179–510) long or 30–44% of oesophagus total length, up to approximately. Intestinal caeca X-shaped, caecal interseccion intersection 854 (800–952) or 17–32% from anterior end of body. Anterior caeca limbs 90 (40–130) long or 2% of body length, 18 (14–24) wide; and posterior caeca limbs, 2483 (2143–2914) long or 51–86% of body length, 15 (13–19) wide; ending blindly anterior to ovary. Posterior caeca extended laterally along testes zone and ending blindly anterior to ovary.

Testes 46 (34–65), ellipsoidal, arranged in two rows, distributed along and ventral to distal third of posterior caeca. Testicular zone 905 (550–1280) in length, occupying 22% of total body length, 298 (227–382) wide or 78–83% of body width, 2.4–3.4 longer than wide. Post-testicular space 577 (382–724) long or 13–15% of body length. Vasa efferentia not observed. Vas deferens, 893 (643–1383) long, 31 (24–37) wide, originating in anterior testes, extending medial, dorsally to ovary, leading to seminal vesicle enclosed in cirrus sac. Seminal vesicle 133 (108–153) long, 26 (19–34) wide. Cirrus 47 (26–64) long or 24–41% of seminal vesicle length. Male genital pore dorsal, 323 (211–401) or 7–9% of body length from posterior extremity of body, 39 (25–64) from sinistral body margin, 236 (180–289) from dextral body margin.

Ovary kidney-shaped, laterally widened, 130 (70–190) long or approximately 3% of body length, 190 (140–270) wide or 48–58% of body width, 1.4–2.0 wider than long. Located immediately posterior to testis, between nerve-cords, anterior to seminal vesicle and ventral to vas deferens. Oviduct begins at the dextral posterior part of ovary. Oviduct 220 (150–360) long, 11 (7–18) wide. Wider posterior portion of oviduct forming seminal receptacle, measuring 150 (70–220) long, 30 (20–38) wide. Seminal receptacle narrowing progressively and joining vitelline duct before forming oötype. Receptacle narrowing progressively joining and terminating in oötype together with vitelline duct. Oötype 57 (48–69) long and 36 (31–43) wide surrounded by the Mehlis' gland. Vitelline follicles filling space from anterior body margin to anterior margin of ovary, extending to body margin laterally. Vitelline duct originates in anterior portion of testes, running ventrally until seminal receptacle and oötype. Uterus folded 751 (547–905) long and 31 (24–43) wide, extending in the post-ovarian zone, short portion descending posterior to oötype connecting to ascending medial with dorsal slight curvatures and anteriorly, near the ovary, ventral curvatures, terminating with the descending portion 100 (83–127) long or 15% of uterus length, 15 (10–24) wide. Female genital pore opening dorsally at 321 (223–417) of posterior body margin, 130 (95–164) of sinistral body margin and 198 (114–261) of dextral body margin. Uterine eggs amorphous, spherical to ellipsoidal, measuring 23–31 long and 12–30 wide or 72–96% of uterus maximum width (Fig. 2B).

3.2.2. Taxonomic summary

Type-host: *Seriola dumerili* (Risso, 1810) (Carangidae).

Site: Heart, gill and girdles.

Type-locality: Santa Pola (38°06′–17′N/0°16′–34′W), Iberian Peninsula, Mediterranean sea.

Other locality: Puerto de Mazarrón (37°29′–34′N/1°9′–15′W).

Type specimens: Holotype MPM Coll. No. 18872-A5125, 2 Paratypes MPM Coll. No. 18872-A5126 from Puerto de Mazarrón; 2 Paratypes NHM 2008.5.9.2 from Puerto de Mazarrón. GenBank accession numbers: AM489593 (28S rRNA), AM489598 (ITS2), AM489603 (COI).

Collectors: A. Repullés (University of Valencia) and F.E. Montero, (Autonomous University of Barcelona).

Infection details: Santa Pola: Number of infected fish=8 (eggs)/3 (adults); prevalence 57% (eggs); mean intensity (adults) 1; host sizes 36.50–42.00 cm TL ($n=15$). Puerto de Mazarrón (additional

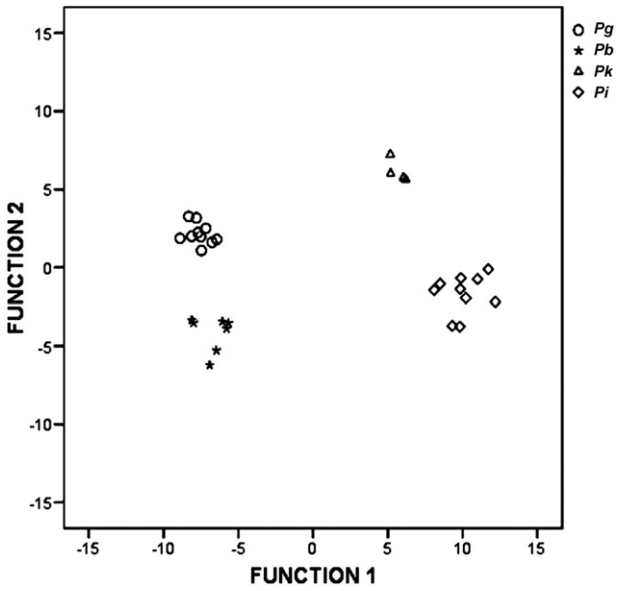

Fig. 3. Scatterplot of the discriminant scores performed on 14 morphometrical variables of 31 *Paradeontacylix* spp. specimens collected from *Seriola dumerili* (*Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*).

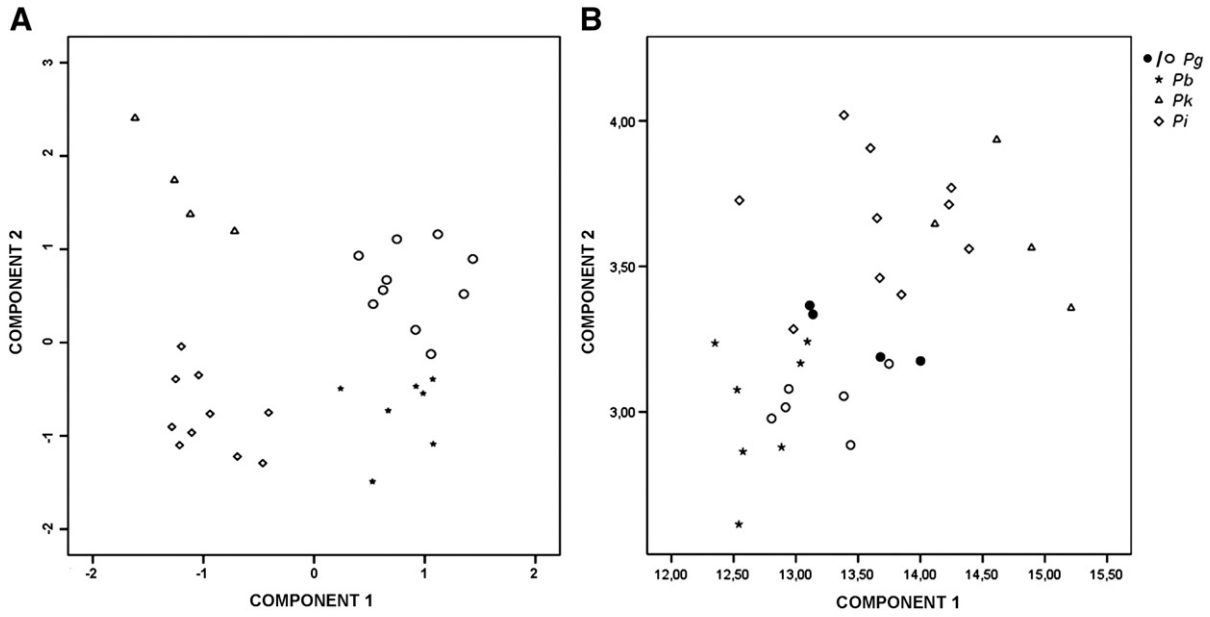

Fig. 4. Scatterplot of the 2 first principal components performed on 7 morphometrical and discriminant variables of 31 *Paradeontacylix* spp. specimens collected from *Seriola dumerili*. *Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*. A. Principal components analysis. B. Common principal components analysis. Non-contracted *P. grandispinus* are represented with solid circles.

data in [6,7]): number of infected fish = 94 (eggs)/43 (adults); prevalence 96.9% (in months when infection was detectable); mean intensity (adults) 3 (1–78); infected host sizes 21.00–43.00 cm TL (16.50–46.00 cm TL, $n = 137$).

Etymology: The species name refers to the host origin, the coasts of the Iberian Peninsula, emphasizing the separation from the species from the Balearic Islands.

3.2.3. Remarks

Paradeontacylix (McIntosh, 1934) (*sensu* Smith, 1907). *P. ibericus* n. sp. exhibits the characters of *Paradeontacylix* genus. It is distinguished from the other species of the genus by the combination of total length from 2480 to 5700, testes arranged in two rows and ovary kidney kidney-shaped, uterus post-ovarian, marginal tegumental spines distributed in 678 rows (577–746), and short posterior tegumental spines, from 8 to 18. The morphology is almost identical to that of *P. kampachi*, described from *S. dumerili* in Japan: short posterior tegumental spines 8 to 18; two rows with separated testes; and a teardrop-shaped ovary. The number of tegumental spines per row in *P. ibericus* (12–14) is the same as in *P. kampachi* specimens, which was found different from the original description (7–10 spines). *P. ibericus* can be distinguished from *P. kampachi* in the smaller body length 2480 to 5700 in *P. ibericus* and 4680 to 8100 in *P. kampachi* and relatively longer posterior marginal tegumental spines: posterior spines length/total body length 1/310–1/316 in *P. ibericus* vs. 1/360–1/480 in *P. kampachi*. Other noticeable differential characteristics of the new species are the higher total number of spine rows, 577 to 746 in *P. ibericus* and 510 to 590 in *P. kampachi*; and the narrower ovary 140 to 270 in *P. ibericus* and 305 to 530 in *P. kampachi* (see also the discriminant analysis in the statistical results). The host geographic distribution of *P. ibericus* and *P. kampachi* is dissimilar.

3.3. Statistical analyses

The four species, *Pg*, *Pb*, *Pk*, *Pi* appear clearly separated in the scatterplot of the discriminant analysis. The selected discriminant variables were: perimeter/2, maximum width, posterior marginal spine length, length of testicular zone, oviduct length, ovary width and uterus width. Function 1 separated the groups (*Pg*+*Pb*) and (*Pk*+*Pi*) and the variables that contributed more significantly to this function were perimeter/2, oesophagus length and posterior marginal spine length. Function 2 separated the groups (*Pg*+*Pi*), *Pb* and *Pk* based on the ovary width, ganglion length and posterior marginal spine length (Fig. 3). PCA allowed the differentiation of two clearly defined groups with 2 subgroups each: *P. kampachi* (*Pk*) and *P. ibericus* (*Pi*) and; *P. grandispinus* (*Pg*) and *P. balearicus* (*Pb*) (Fig. 4A). Variance distribution within the groups was no homogeneous although 4 common components emerged between the variances of the four data groups (*p*-values between 0.065 and 0.160 higher than 0.050). CPC analysis showed that *Pk* and *Pi* overlapped only slightly while *Pg* and *Pb* exhibited more common ground (Fig. 4B). However, in the scatterplot of CPC results the separation of *Pg* and *Pb* was improved markedly when we only focus on the more relaxed paratypes of *Pg* marked as solid circles in the representation.

The Levène test for variance of the 4 common components indicated that *p*-values of the first three (0.596, 0.561, 0.050) were high enough to obtain a reliable evaluation. However, the *p*-value of the fourth component was lower than 0.05, and was not taken into account. Table 3 shows the *p*-values for the pairwise comparisons of the species for each component. Three differentiated subgroups for component 1 were established using ANOVA: *Pg* and *Pb*; *Pg* and *Pi*; and *Pk*. Component 2 separates 2 subgroups, *Pg*–*Pb* and *Pk*–*Pi*. Notably, the *p*-value for the combination *Pg*–*Pb* in component 1 is a little higher than 0.05.

Table 3
ANOVA *p*-values for the pairwise comparison of *Paradeontacylix* species in *Seriola dumerili* for components 1 and 2

Pair of species	Component 1	Component 2
Pg–*Pb*	0.057	0.691
Pg–*Pk*	0.000*	0.002*
Pg–*Pi*	0.362	0.000*
Pb–*Pi*	0.001*	0.000*
Pb–*Pk*	0.000*	0.000*
Pk–*Pi*	0.003*	0.997

Significant values are indicated with an asterisk. *Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*.

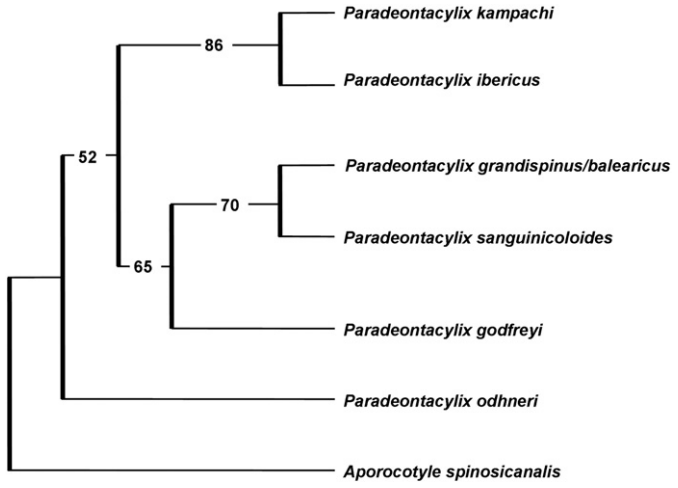

Fig. 5. Phylogenetic tree based on morphological data treated as unordered (11 characters; TL=17, CI=0.82, HI=0.18, RI=0.77, RC=0.63). *Aporocotyle spinosicanalis* as outgroup. Numbers represent bootstrap values of branches (1000 replications).

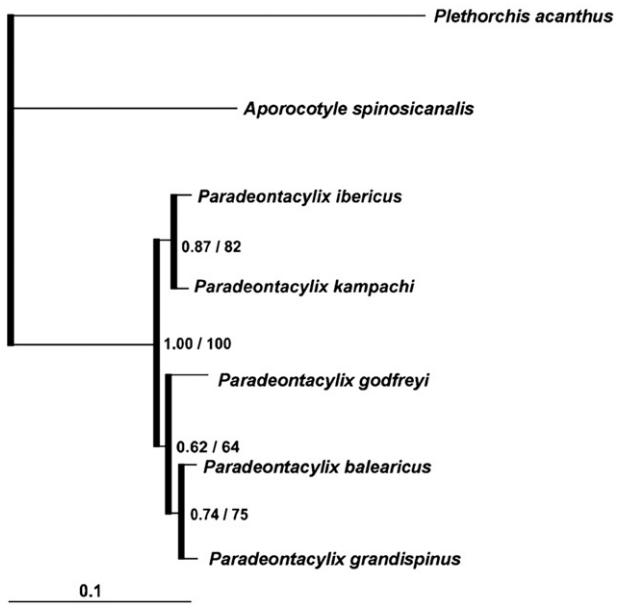

Fig. 6. Phylogenetic tree based on partial 28S, partial 5.8S, complete ITS2 ribosomal, and COI mt sequences, using *Aporocotyle spinosicanalis* (AY222177) and *Plethorchis acanthus* (AY222178 + AY465875) as outgroup. Numbers at the nodes show: above, clade posterior probability, i.e. the proportion of the trees sampled containing that branch (BI), and below, percent of bootstrap support of 1000 replicates for the MP analysis.

3.4. Phylogenetic results from morphological data

The cladistic analysis resulted in a single phylogenetic tree with a consistency index of 0.81 (Fig. 5). This tree shows 2 clades: (*Pk*+*Pi*) and (((*Pg*/*Pb*)+*P. sanguinicoloides*)+*P. godfreyi*). *P. odhneri* was located basal to all these species in a separate branch. Bootstrap levels for the branch (*Pk*+*Pi*) are high so these 2 species are clearly related. Bootstrap levels for the branch relating (*Pg*/*Pb*) and *P. sanguinicoloides* is 70% pointing to a relationship between these 3 species. The remaining branches presented bootstrap values lower than 70%; these are shown for a comparison with molecular results.

3.5. Phylogenetic results from molecular data

With the exception of *P. kampachi*, all genes from all *Paradeontacylix* spp. used for molecular analysis were sequenced from two specimens each. The replicate sequences obtained for each species were found to be 100% identical.

Partial 28S DNA sequences of 1613 bp (GenBank accession numbers AM489593–97) complete ITS2 sequences (including partial 5.8S sequence) of 540 bp (AM489598–02), and complete mt COI sequences of 420 bp (AM489503–07) were obtained for all species. The sequence difference matrices (Table 4) show that the percent sequence difference increases from 28S over ITS2 to COI. 28S rDNA sequences show little variation up to only 0.9% between the *Paradeontacylix* species. The ITS2 and COI sequences allowed better species discrimination with sequence differences between 2.5–12.5% (ITS2) and 6.3–16.0% (COI) between different species. Low sequence differences between all genes were found between *P. grandispinus* and *P. balearicus* (0.2%/2.5%/6.3%), and to a slightly smaller extent between *P. kampachi* and *P. ibericus* (0.2%/4.7%/7%). However, ITS2 sequence differences of *P. kampachi* with *P. grandispinus* and with *P. balearicus* (4.1% and 4.3%) are slightly lower than between *P. kampachi* and *P. ibericus*. Based on the ITS2 and COI data, *P. godfreyi* showed the highest percentage of sequence difference with all other species, with values as high as 12.5% (ITS2) and 16.0% (COI).

Table 4
Sequence differences (percentage) of the aligned 28S and ITS2 ribosomal as well as the mitochondrial COI sequences obtained for *Paradeontacylix* spp. Smallest sequence divergence marked in bold and grey

	P.iber	*P.kamp*	*P.bale*	*P.grand*	*P.godf*
Partial ribosomal 28S sequences (1613 bp)					
P.iber	0	**0.2**	0.9	0.9	0.6
P.kamp		0	0.8	0.9	0.5
P.bale			0	**0.2**	0.7
P.grand				0	0.7
P.godf					0
Ribosomal ITS2 sequences (including partial 8.5S; 540 bp)					
P.iber	0	**4.7**	6.0	6.9	10.5
P.kamp		0	**4.3**	**4.1**	12.5
P.bale			0	**2.5**	10.0
P.grand				0	11.8
P.godf					0
Mitochondrial COI sequences (420 bp)					
P.iber	0	**7.0**	11.5	11.7	16.0
P.kamp		0	11.5	11.5	15.1
P.bale			0	**6.3**	13.7
P.grand				0	13.9
P.godf					0

The ITS2 sequence of the *Paradeontacylix* spp. contains an area of at least 10 bases of repetitive GT sequences. At the beginning of this region, an expansion segment of 20 bases occurs (mainly AT repetitions) in *P. godfreyi* and *P. kampachi.* Furthermore, *P. godfreyi* shows a unique addition of 30 repetitive AT/GT sequences following the GT rich region, which differentiates it from all other *Paradeontacylix* spp.

The result of the phylogenetic analysis using all sequence data obtained shows that the *Paradeontacylix* species split into two well supported clades, separating (*P. ibericus*+*P. kampachi*) from (*P. godfreyi*+(*P. balearicus*+*P. grandispinus*)) (Fig. 6).

4. Discussion

4.1. Identity of the Mediterranean Paradeontacylix species

In the current study, two new species of *Paradeontacylix* are described, *P. balearicus* from the Balearic Islands and *P. ibericus* from the Iberian Peninsula. Each species could be distinguished morphologically from the other species of the genus by size or shape. However, *P. grandispinus* and *P. balearicus* differences are not statistically significant.

The results of morphologically based matrices depend on the method of character coding and any set of features favoured by one author may yield a widely different range of phylogenetic estimates depending on the coding strategy employed (see review by [28]). To redress the conflict and increase resolution molecular data have been sought as an independent estimate of phylogeny and were thus used in this study to support the interpretation of the morphological data. Thereby, it was decided to use a combination of more conservative and more variable gene regions, as this has been shown to improve phylogenetic estimates considerably (e.g. [29]).

Molecular analysis of five *Paradeontacylix* spp. showed that 28S rDNA sequences were not informative with regard to intrageneric phylogeny as they show only 0.2–0.9% sequence divergence between species. The 28S rDNA has many divergent domains or expansion segments and is useful for the phylogenetic estimation of general evolutionary events into the Cenozoic [30]. The ITS2 region of the rDNA gene shows a much higher degree of divergence between taxa and has been successfully used to distinguish digeneans on a species level (see review by [31]). Thus, as expected, more divergent sequences were obtained from the ITS2 region (2.5–11.2%). Morgan and Blair [32] showed that mt DNA evolves more quickly than ITS, and is even more useful for distinguishing among closely related species. This can be confirmed in the current study as mt COI sequences differed by 6.3–16.0%. The levels of interspecific sequence variation is typically ≤1% for ITS and a fraction of a percent up to 2% for mtDNA (see review by [33]). It is thus suggested that, molecularly, the Mediterranean species of *Paradeontacylix* are clearly distinct from the Japanese ones. However, all gene regions showed high sequence identities between *P. ibericus*/*P. kampachi* and *P. balearicus*/*P. grandispinus*. The ITS2 rDNA of *Paradeontacylix* spp. was found to have a fast evolving, highly variable region with repetitive inserts, resulting in a similar sequence divergence between the Mediterranean and the Japanese species. Thus, with regard to *Paradeontacylix* spp., the mt COI sequences seem to be more suitable for phylogenetic analyses. [34,35] used the ITS2 region for phylogenetic analysis of various sanguinicolid genera, i.e. *Cardicola*, *Braya*, *Phthinomita* and *Ankistromeces*. Their sequences lack the *Paradeontacylix* spp. specific expansion segment of base repeats and thus seem to be suitable for phylogenetic comparison. Nolan and Cribb [34,35] considered sequence divergences as low as 0.3%, i.e. a single base e.g. for *Cardicola lafii* and *Cardicola parilus* or for *Phthinomita littlewoodi* and *Phthinomita jonesi* as sufficient for species separation. Although morphological differences were present between the *Cardicola* spp., the two *Phthinomita* species were morphologically almost identical. However, the single base was consistently different in 25 specimens sequenced for *P. jonesi* and 8 for *P. littlewoodi*, including from different sympatric hosts, supporting the interpretation and different species designation of Nolan and Cribb [35]. According to [35] the sequence divergence between species of different genera shows variable degrees, however, as *Cardicola* and *Paradeontacylix* are molecularly very closely related, the degree of divergence should be comparable within the two genera. It is thus concluded that the difference of 2.5% between *P. ibericus*/*P. kampachi* and 4.7% between *P. balearicus*/*P. grandispinus* justifies the distinct species status of the Mediterranean forms, especially as this is supported by some morphological differences.

Habitat of the two new species was found to be different. Eggs of *P. balearicus* were present in the afferent and efferent gill vessels, as well as in the secondary lamellae. In contrast, *P. ibericus* eggs were only found in the afferent vessels. The distribution of eggs in the former species increases their dispersion, which could be related to the higher pathogenicity previously observed in fish infected with *P. balearicus* in comparison to fish infected with *P. ibericus* [4,7]. No adult *P. balearicus* were found in girdles of Balearic fish whereas this was the site of maximum adult infection in fish from Puerto de Mazarrón [6]. The distribution of eggs and adults of *P. ibericus* was similar to that of *P. kampachi*, as for *P. balearicus* and *P. grandispinus* [36,6, present study].

4.2. Isolation between the Paradeontacylix spp. populations

The Balearic Islands are separated from the Iberian Peninsula by approximately 300 km and 1900 m of maximum depth. This study shows that, despite inhabiting the same host, the two species of *Paradeontacylix* remain isolated. There are no reports of mixed infections of *S. dumerili* by the two species in either of the two geographical localities [4,7,6, present study]. However, Ogawa and Egusa [16] found mixed infections of *P. kampachi* and *P. grandispinus* in *S. dumerili* off Japan.

Although adult greater amberjacks are pelagic and migratory [37] and could potentially move between the peninsular coastal waters and the islands, juvenile *S. dumerili* have a coastal habitat, restricted mobility and are usually associated with floating objects. Previous studies on Mediterranean *S. dumerili* have not reported the presence of *Paradeontacylix* sp. in adult fish [7]. It appears that in the Mediterranean *Paradeontacylix* spp. only infect juvenile amberjacks. Although the close proximity of the localities would imply merging of the Balearic and Iberian host populations, present data suggest they remain isolated during fish youth.

4.3. Phylogeny of the genus, a global study

The phylogenetic study of *Paradeontacylix* spp. worldwide is preliminary as molecular data of *Paradeontacylix* spp. is limited. However, as the morphological clustering is strongly supported by the molecular data available, the information obtained allows some conclusions: The tree is divided in two main branches, separated from *P. odhneri*, one allocating *P. kampachi* and *P. ibericus* and the other *P. grandispinus*, *P. balearicus*, *P. sanguinicoloides* and *P. godfreyi*. All *Paradeontacylix* spp. infect fish of the genus *Seriola* (see Table 1) except *P. odhneri* parasite of *Takifugu porphyreus*, (Teleostei: Tetraodontiformes). This is the species most similar to the outgroup, *A. spinosicanalis*. However, the description of *P. odhneri* is brief [14] and there are no deposited specimens available.

P. sanguinicoloides, *P. godfreyi*, *P. grandispinus* and *P. balearicus* share several characters that differentiate these species from the *P. kampachi*/*P ibericus* group. Interestingly, *P. sanguinicoloides* and *P. godfreyi* parasitize *S. lalandi*, whereas *P. grandispinus* and *P. balearicus* parasitize *S. dumerili*. It is suggested that the speciation of the geminate species of the two parasite groups could have occurred after the speciation of *S. lalandi* and *S. dumerili*.

Concerning *Paradeontacylix* spp. from *S. dumerili*, the trees show clustering of *P. kampachi*/*P. ibericus* in a different clade than *P. grandispinus*/*P. balearicus*, associating Japanese species with Mediterranean ones in both groups. Considering the isolation observed between parasite populations of very close localities in the western Mediterranean, a greater degree of isolation would be expected between the populations in regions as distant as Japan and the Mediterranean. In addition, the distribution of *S. dumerili* is circumglobal, but restricted to warm and temperate zones. Only an insignificant interchange between the populations could have recently existed *via* the Suez Channel and, even less probably, via the Panama Channel. A likely explanation for the paired morphological and genetic similarities between the current species could be that the ancestral species of *P. kampachi*/*P. ibericus* and *P. grandispinus*/*P. balearicus* groupings existed before the separation of host populations. The isolation of the Mediterranean and Japanese host populations could have taken place during middle Miocene, 14 or 15 million years ago, when the Tethys sea divided, separating the Paratethys (Indian Ocean) and the Mediterranean [38]. Similarly, individuals of *P. sanguinicoloides* have been recovered from *S. lalandi* from two very isolated areas, i.e. southern Australia (Pacific, Southern and Indian Oceans) [18,39] and Florida (Atlantic Ocean) [15]. It would be necessary to investigate the molecular relationships between *P. sanguinicoloides* from the two localities since morphological differences between sanguinicolid species are not always apparent.

The worldwide diversity of *Paradeontacylix* species in *Seriola* spp. contrasts with the recently reported high cosmopolitan similarity of the parasite faunas in tunas (*Thunnus* spp.), although both genera include large pelagic and migratory fish species. [40] reported the same blood-fluke species, *Cardicola forsteri*, in *T. thynnus* from the Mediterranean and in *T. maccoyii* from Australia. Interestingly, the *Thunnus* spp. parasites were collected from adult migratory fish and the *Paradeontacylix* spp. from *S. dumerili* seem only to parasitize the sedentary juvenile amberjacks. Sedentarism could have contributed to the isolation of hosts. The presently described Mediterranean species show small morphological and more prominent genetic differentiation along possibly 14–15 million years of geographical separation from the Japanese species.

Note Added in Proof

The authors would like to report the existence of 2 nomina nuda, synonyms of the 2 species published in the present paper, and previously reported in Repullés-Albelda, A., Montero, F.E., Holzer, A.S., Cuadrado, M. and Raga, J.A. (2007) Differences in the pathogenicity of four *Paradeontacylix* spp. (Trematoda, Sanguinicolidae) from cultured greater amberjack (*Seriola dumerili*). 13th international conference of the EAFP, 24th–28th September, Viterbo Italy.

/Paradeontacylix balearicus/ Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008.

Syn:

/Paradeontacylix balearicus/ Repullés-Albelda, Montero, Holzer, Cuadrado et Raga, 2007. nomen nudum

/Paradeontacylix ibericus/ Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008.

Syn:

/Paradeontacylix ibericus/ Repullés-Albelda, Montero, Holzer, Cuadrado et Raga, 2007. nomen nudum

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