APOROCOTYLE MARIACHRISTINAE N. SP., AND A. YMAKARA VILLALBA & FERNÁNDEZ, 1986 (DIGENEA: APOROCOTYLIIDAE) OF THE PINK CUSK-EEL, GENYPTERUS BLACODES (OPHIDIIFORMES: OPHIDIIDAE) FROM PATAGONIA, ARGENTINA

HERNÁNDEZ-ORTS J.S.*, ALAMA-BERMEJO G.**, CARRILLO J.M.***, GARCÍA N.A.****, CRESPO E.A.****, RAGA J.A.* & MONTERO F.E.*

Summary:
Aporocotyle mariachristinae n. sp. and A. ymakara Villalba & Fernández, 1986 were collected from the bulbus arteriosus and ventral aorta of pink cusk-eels, Genypterus blacodes (Forster, 1801) from Patagonia, Argentina. A. mariachristinae n. sp. can be distinguished from all the species of Aporocotyle by the asymmetrical extension of posterior caeca (right posterior caecum longer, terminating at the area between mid-level of ovary and posterior body end; left posterior caecum shorter, terminating at the area between mid-level of cirrus sac and posterior to reproductive organs), the distribution of spines along the ventro-lateral body margins and the number of testes. The new species clearly differs from A. ymakara, from the same host species, in the esophagus / body length ratio, the absence of distal loops at caeca, the anterior caeca / posterior caeca length ratio, and the number of testes. Additionally, in A. ymakara the left posterior caecum may be longer than right posterior caecum, while in the new species left posterior caecum is always shorter. The specimen of A. ymakara collected from Argentina is also described. We also provide observations of the distribution of spines in different species of Aporocotyle, including new specimens of A. argentinensis Smith, 1969 from Merluccius hubbsi Marini, 1933. Molecular sequence data obtained from partial 18S and 28S rDNA regions were compared between the new species and other two species of Aporocotyle (A. argentinensis and A. spinosicanalis Williams, 1958). This is a new locality record for A. ymakara, extending the known geographical distribution for this species from Chile to Argentina, and the first report of two species of Aporocotyle in the same host species and locality.

Key words: Aporocotylidae, Aporocotyle, A. mariachristinae n. sp., A. ymakara, Genypterus blacodes, Ophidiidae, Patagonia, Argentina, rDNA sequences.

INTRODUCTION
Aporocotyle Odhner, 1900 is the type genus of the family Aporocotylidae (Bullard et al., 2009). Species of Aporocotyle infect the heart, bulbus arteriosus, ventral aorta and branchial vessels, and other blood vessels of fishes of five teleost orders: Gadiformes, Ophidiiformes, Perciformes, Pleuronectiformes and Scorpaeniformes (Yamaguti, 1934, 1970; Williams, 1958; Smith, 1967, 1969; Ichihara, 1970; Holmes, 1971; Parukhin & Tkachuk, 1980; Thulin,
A total of 52 specimens of *G. blacodes* measuring from 24.7 to 92.4 cm in total length, were collected between 2007 and 2010 from two zones of the Argentine shelf: north (42° 45' S – 42° 59' S, 61° 09' W – 62° 58' W) and central Patagonia (47° 00' S – 47° 19' S, 61° 59' W – 64° 25' W). Fish were collected by commercial bottom trawling vessels and kept fresh in ice (n = 8) or deep frozen at -20 °C (n = 44). The heart and bulbus arteriosus of fresh and thawed fish were removed, placed in seawater and examined under a stereomicroscope. A total of 18 blood flukes were found and fixed in 70 % ethanol (n = 13) or alum carmine (n = 2), dehydrated in ethanol, cleared in dimethyl phthalate, and mounted in Canada balsam. Specimens were examined using a compound microscope equipped with bright field and differential interference contrast optics. Morphometric measurements were taken from drawings made with the aid of a drawing tube. Measurements are reported in micrometers and presented as the mean followed in parentheses by the range and the number of structures measured. The same procedure was followed for the specimens collected from the Argentinean hakes (4 worms stained with iron acetylcarmine). Blood flukes were identified as *A. argentinensis* according to Smith (1969), and were used for morphological comparison with the specimens collected from *G. blacodes*.

**MORPHOLOGICAL DESCRIPTION**

15 blood flukes from 13 fish, fixed in 70 % ethanol (11 parasites from fresh fish and 4 from thawed fish), were used for morphological study, stained with iron acetylcarmine (n = 13) or alum carmine (n = 2), dehydrated in ethanol, cleared in dimethyl phthalate, and mounted in Canada balsam. Specimens were examined using a compound microscope equipped with bright field and differential interference contrast optics. Morphometric measurements were taken from drawings made with the aid of a drawing tube. Measurements are reported in micrometers and presented as the mean followed in parentheses by the range and the number of structures measured. The same procedure was followed for the specimens collected from the Argentinean hakes (4 worms stained with iron acetylcarmine). Blood flukes were identified as *A. argentinensis* according to Smith (1969), and were used for morphological comparison with the specimens collected from *G. blacodes*.

For morphological comparison with the species studied type specimens of the following *Aporocotyle* spp., deposited in the Zoology Museum of the University of Concepción (Concepción, Chile (ZMUC)) and the US National Parasite Collection (Beltsville, Maryland, USA (USNPC)), were examined and measured: *A. keli* – one paratype (ZMCU No. 7811); *A. kuri* – one paratype (ZMCU No. 7808); *A. wilhelmi* Villalba & Fernández, 1986 – three paratypes (USNPC No. 79462: MT24-19K, MT24-19L, and MT24-19N); and *A. ymakara* – five paratypes (ZMUC No. 7759 and USNPC No. 79463: MT24-20A, MT24-20B, MT24-20G, and MT24-20H). Additionally, 3 vouchers of *A. spinosicanalis* Williams, 1958, collected from the heart of *Merluccius merluccius* L. from the Western Mediterranean from the private collection of Dr. F. E. Montero, were studied. Measurements from previously described species were obtained from specimens or original descriptions (manuscripts or drawings), depending on the availability (see Table I).

Ecological terms follow Bush et al. (1997) and Rózsán (2000). The prevalence, mean abundance and mean intensity are presented as the number followed in parentheses by the 95 % confidence intervals (CI). The 95 % CI for prevalence was set with Sterne's exact method (Reiczigel, 2003), while the 95 % CIs for the mean abundance and mean intensity were estimated with 20,000 bootstrap replications with the statistical software Quantitative Parasitology v. 3.0 (Reiczigel & Rózsán, 2005).

Museums in which type specimens have been deposited, are indicated as follows: Natural History Museum, Paris, France (NHM Paris); ZMUC, Concepción, Chile. Voucher of *A. ymakara* has been deposited in NHM...
| Species          | References          | Host                     | Locality     | BL   | Lateral body depression | Spine distribution | Ratio ES/BL | Maximum extension of PC | AC/PC Ratio | LPC/BL Ratio | No. tests | Cirrus sac (length × width) | Ovary (length × width) |
|------------------|---------------------|--------------------------|--------------|------|--------------------------|-------------------|-------------|--------------------------|-------------|--------------|-----------|--------------------------------|------------------------|
| A. argentinensis | Smith, 1969         | Merluccius hubbsi        | Southwest Atlantic | 4.5  | (4.1-4.7)                | No               | AVLM       | 1:3.2                    | 1:2.8**     | 1:1.4**      | 41-45     | 520 (440-600) × 130 (110-150) | 250 (230-270)         |
| A. australis     | Fernández & Durán, 1985 | Merluccius australis     | Southeast Pacific   | 6.7  | (5.1-8.0)                | No               | AVLM       | 1:3.4                    | 1:1.3-5     | 1:1.4**      | 56-71     | 450 (350-530) × 130 (110-150) | 170 (160-200)         |
| A. garcii        | Tantaleán & Martínez, 1990 | Genypterus sp.          | Southeast Pacific   | 3.2  | (3.1-3.3)                | No               | LM         | 1:4.4                    | 1:1.4       | 1:1.9**      | 96-100    | 280 (250-300) × 60 (50-70)  | 160 (150-170)         |
| A. garci*        | Kamegai et al., 1990 | Hoplobrotula armata     | Northwest Pacific   | 3.7-6.6 | No               | LM         | 1:9.9-1.4 | 1:2.8-5.1                | 1:1.7**     | 60-140       | 570-940   | 80-150 × 380-720                    |                       |
| A. veli         | Villalta & Fernández, 1986b | Genypterus chilenensis  | Southeast Pacific   | 3.8  | (3.0-4.7)                | Yes              | LM         | 1:3.5-4.1                | 1:2.2**     | 1:1.8**      | 35-38     | 360 (290-420) × 160 (120-190)      | 180 (160-200)         |
| A. kuri         | Villalta & Fernández, 1986b | Genypterus maculatus    | Southeast Pacific   | 2.4  | (1.5-3.2)                | Yes              | LM         | 1:2.9-3.8                | 1:1.8**     | 1:2.4**      | 28-32     | 250 (200-240) × 96 (60-120)       | 120 (70-150)          |
| A. macfarkinsi  | Holmes, 1971        | Sebastes caurinus       | Northeast Pacific   | 3.3-5.2 | No               | LM         | 1:3.5** | 1:4.6                   | 1:1.5**     | 37-63        | 191 (130-260) × 110 (70-165)   | 236 (142-340)         |
| A. margolisi     | Smith, 1967         | Merluccius productus    | Northeast Pacific   | 4.5  | (3.8-5.0)                | No               | AVLM       | 1:3.6                    | 1:2.6**     | 1:1.5**      | 35-46     | 525 × 131** × 177 (150-200)      |                       |
| A. rotoheriae   | Panukhin, 1985       | Notobranchus rosii     | Indian Ocean        | 10.5 | No               | B.E.C      | 1:11.2    | 1:1.8                   | 1:1.2**     | 135          | 476 × 142** | 360 × 400                        |                       |
| A. orientalis   | Yamaguti, 1954       | Cottocinclus sp.        | Japan Sea           | 5.2-7.6 | No               | B.E.C      | 1:6.2    | 1:1.7                   | 1:1.2**     | 137-171      | 560 × 125  | 270 × 370                         |                       |
| A. pacifica     | Yamaguti, 1970       | Buvettia pretiosus      | North Pacific       | 5.9  | No               | B.E.C      | 1:7.8    | 1:1.8                   | 1:1.2**     | 100          | 350 × 100  | 250 × 110                        |                       |
| A. simplicia    | Thulin, 1990         | Platichthys flexus      | Baltic Sea          | 3.4-9.4 | No               | LM         | 1:3.6    | 1:1.7                   | 1:1.3**     | 110-203      | 577 × 115** | 259 × 422**                      |                       |
| A. smithi       | Panukhin & Tkachuk, 1980 | Genypterus capensis    | Indian Ocean        | 4.0  | No               | B.E.C      | 1:4.8    | 1:3.5                   | 1:1.7**     | 36           | 340 × 130** | 280 × 340**                      |                       |
| A. spinosicanalis | Williams, 1958      | Merluccius merluccius   | Northeast Atlantic  | 4.0-6.4 | No               | AVLM       | 1:2.7    | 1:2.1                   | 1:1.6**     | 25-35         | –         | 400 × 210                         |                       |
| A. thraagae     | Ichihara, 1970       | Theragra chalcogramma  | Japan Sea           | 6.6-7.7 | No               | AVLM       | 1:6.0-7.7 | 1:8.3                   | 1:1.1**     | 98-125       | 380-390   | 380-390 × 180-240                   | 200-360 × 520-610     |
| A. wibertmi     | Villalta & Fernández, 1986b | Merluccius gayi        | Southeast Pacific   | 3.6  | (2.6-5.1)                | No               | AVLM       | 1:2.6-3.6                | 1:1.6-3.0   | 1:1.5**      | 33-40     | 350 (250-460) × 120 (90-170)      | 120 (87-160)          |
| A. yndakara     | Villalta & Fernández, 1986b | Genypterus blacodes    | Southeast Pacific   | 2.1  | (1.58-2.74)              | No               | LM         | 1:2.1-2.6                | 1:1.1-1.3**  | 1:2.5-3.3** | 18-21     | 220 (212-228) × 61 (49.79)**     | 119 (93-142) × 143 (114-168)** |
| A. yndakara     | Present study        | G. blacodes            | Southwest Atlantic  | 1.7  | No               | LM         | 1:2.6      | 1:1.8                   | 1:2.5          | 18           | 265 × 70   | 121 × 141                        |                       |
| A. mariachristinae n. sp. | Present study         | G. blacodes             | Southwest Atlantic  | 2.7  | (1.9-3.4)                | No               | LM         | 1:2.7-3.9                | 1:2.6-4.7   | 1:1.5-2.3    | 37-39     | 280 (140-400) × 70 (40-80)       | 175 (133-257)         |

Body length in millimeters, other measurements in micrometers. BL: body length; ES: esophagus; AVLM: anterior ventral body and lateral margins; LM: lateral margins; B.E.C: body entirely covered; AC: anterior caeca; PC: posterior caeca; LPC: long posterior caecum; * data from A. garci* collected in H. armata are also included as it is the only Aporocotyle species from Genypterus spp. described in other host species; ** measured from the figure in the species descriptions; *** measured from type material.

Table I. – Taxonomic data from the species of the genus Aporocotyle in type hosts.
Parasite. Vouchers of *A. argentinensis* have also been deposited in NHM Paris (HEL260 and HEL261) and ZMUC (N°37106 and N°37107).

**MOLECULAR ANALYSIS**

For DNA extraction, the anterior half of each blood fluke specimen fixed in 100 % ethanol from *G. blacodes* was dissolowed in 300 μL of TNEs urea (10 mM Tris-HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 0.5 % sodium dodecyl sulphate, 4 M urea). The posterior half of each fluke was processed as previously described for morphological analysis, in order to confirm their specific status. The samples were digested with 100 μg/mL proteinase K overnight at 55 ºC. DNA was extracted using a conventional phenol–chloroform protocol following Alama-Bermejo *et al.* (2011). The extracted DNA was resuspended in 30 μL of RNAse/DNase free water and left to dissolve overnight in the fridge. Polymerase chain reactions (PCR) were performed with a programmable thermal cycler (Techne, TC-512, GMI) in a final volume of 30 μL containing 0.5 U of Thermoprime Plus DNA polymerase and 3 μL of the related 10X buffer with 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μM of each primer and approximately 100 ng of template DNA.

Partial 18S rDNA and 28S rDNA were amplified from the genomic ribosomal DNA (rDNA) gene. The primers used in the PCR reaction were the forward primer WormA (5′-GCGAATGGCTCATTAAATCAG-3′) and the reverse primer WormB (5′-CTTGTTACGACTTTTACTTCC-3′) (Littlewood & Olson, 2001) for 18S rDNA; the forward primer U178 (5′-GCACCCGCTAAYT-TAAG-3′) and the reverse primer WormA (5′-GCGAATGGCTCATTAAATCAG-3′) and WormB (5′-CTTGTTACGACTTTTACTTCC-3′) (Lockyer *et al.*, 2003) for 28S rDNA.

The PCR protocol was conducted as follows: denaturation (95 ºC for 3 min) followed by 40 cycles of amplification. Cycling consisted of 95 ºC for 30 s, 55 ºC as annealing temperature for 30 s and 72 ºC for 2 min. Final extension was allowed at 72 ºC for 7 min. After checking for the presence of PCR amplicons in a 1 % agarose gel in sodium acetate buffer, the PCR products were purified for sequencing using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare UK Ltd.). PCR primers and additional primers Lin3 (5′-GCGGTATTTCCACCTCCA-3′) (Lin *et al.*, 1999) and LSU1200R (5′-GCTAGTCACATTCTTCCG-3′) (Lockyer *et al.*, 2003) were used for sequencing of the 18S and 28S rDNA fragments respectively. Cycle sequencing was conducted in a 48 capillary ABI 3730 sequencer (Applied Biosystems) using the Big Dye Terminator v 3.1 Ready Sequencing Kit (Applied Biosystems) according to the manufacturer’s instructions. Sequences obtained were submitted to the Basic Local Alignment Search Tool (BLAST) on GenBank™ to identify the closest relatives published to date. The same protocols were used to obtain the partial 28S rDNA sequence of the whole specimen of *A. argentinensis* from *M. hubbsi* fixed in 100 % ethanol.

**RESULTS**

*Aporocotyle mariachristinae* N. sp. (Figs 1-6, 11)

Type host: pink cusk-eel, *Genypterus blacodes* (Forster, 1801) (Ophidiiformes: Ophidiidae).

Host size: total length, 30.6-92.4 cm.

Site in host: bulbos arteriosus and ventral aorta.

Type locality: north Patagonia (42º 45’ S – 42º 59’ S, 61º 09’ W – 62º 58’ W); and central Patagonia (42º 2’ S – 47º 19’ S, 61º 59’ W – 64º 25’ W), Argentina.

Type specimens: holotype (NHM Paris HEL255), three paratypes (NHM Paris HEL256, HEL257 and HEL258), three paratypes (ZMUC N°37103, N°37104 and N°37105).

Infection parameters: prevalence, 25.0 % (14.9-38.3); abundance, 33.0 (17.0-63.0); intensity, 1.31 (1.0-1.9).

Sequence data: GenBank Accession number JX094801 (*Aporocotyle mariachristinae* partial 18S rDNA sequence, ex. *Genypterus blacodes*); JX094802 (*Aporocotyle mariachristinae* partial 28S rDNA sequence, ex. *Genypterus blacodes*).

Etymology: the species is named in memory of Maria Cristina Orts Pino, mother of the first author.

- **Description**

  Based on 14 mounted specimens. Aporocotylidae, *Aporocotyle* Odhner, 1900. Body lanceolate, pointed anterior to posterior end (Figs 1, 11), 2,730 (1,910-3,380, n = 12) long and 450 (220-670, n = 12) wide. Maximum body width approximately at mid-level. Tegument bearing clusters of approximately 2/3 distance between body margin to caecum, never joining at sagittal axis (Fig. 11), but rea-

  - **Dimensions**

    - **Head**
      - Oral sucker absent. Mouth ventral, extending dorsally. Spines approximately 20 long (Fig. 5).
      - Spines less abundant at posterior half body, occupying approximately 2/3 distance between body margin to caecum (Figs 4 & 11).
    - **Body Shape**
      - Body lanceolate, pointed anterior to posterior end (Fig. 11). Spines approximately 20 long (Fig. 6).
      - Ventrally, spines are distributed along entire body length, never joining at sagittal axis (Fig. 11), but reaching closer at anterior half body, occupying approximately 2/3 distance between body margin to caecum (Figs 4 & 11).
      - Spines less abundant at posterior half body (Figs 5 & 11). Oral sucker absent. Mouth ventral, leading to a buccal capsule 55 (47-63, n = 4) long and 24 (18-31, n = 4) wide. External caecum 653 (571-1,024, n = 8) long or 31 % (26-37 %) of body length, surrounded by glands approximately from its midlevel (anterior to level of anterior caeca distal end) to caeca bifurcation. Intestine X- or H-shaped (Figs 1 & 11). Anterior caeca almost equal in length. Anterior caeca/body length ratio: 1:6.5 (1:4.8-7.6). Right anterior caecum 435 (342-
598, n = 8); left anterior caecum 418 (289-646, n = 8). Posterior caeca unequal: right posterior caecum always longer than left posterior caecum. Right posterior caecum 1,462 (1,061-1,877, n = 9), about 54 % (44-67 %) of body length, ending from mid-level of ovary to near posterior body end (at mid-level of ovary (n = 3) (Fig. 11), posterior to reproductive organs (n = 9) (Fig. 1), or near to posterior body end (n = 2)). Left posterior caecum 1,345 (974-1,772, n = 9), ending from mid-level of cirrus sac to posterior to reproductive organs (at mid-level of cirrus sac (n = 6), at proximal female genitalia level (n = 5) (Figs 1, 11), or posterior to reproductive organs (n = 3)). Anterior caeca/right posterior caecum length ratio 1:3.5 (1:2.7-5.1).
Testes irregularly-shaped (Fig. 1), 37-39 (n = 4) in number, 70 (40-125, n = 49) long axis and 81 (24-118, n = 49) wide axis. Testes intercaecal, between caecal bifurcation and anterior to reproductive organs. Vas deferens extending from posterior border of testicular field. Cirrus sac claviform, 271 (144-396, n = 7) long and 70 (41-84, n = 10) wide, with thin muscular wall, directing sinistrally, ending at genital atrium (Fig. 2). Seminal vesicle elongate, slightly sinuous, in middle of cirrus sac, surrounded by prostatic cells (Fig. 2). Cirrus not observed. Genital atrium and genital pore sinistral, dorsal to left posterior caecum, at about 75 % (64-84 %) of body length from anterior body end. Ovary central or slightly dextral (Figs 1, 11), sub-ellipsoidal, in posterior 1/8 of body, 175 (133-237, n = 10) long and 160 (89-215, n = 10) wide (Fig. 2). Oviduct running posteriorly from posterior margin of ovary and turning sinistrally (Fig. 2). Oötype surrounded by Mehlis’ gland, connecting to uterine seminal receptacle. Laurers’ canal absent. Uterus intercaecal, extending from uterine seminal receptacle and coiling from ovary to level of posterior testes, curving dorso-sinistrally (Fig. 2). Metraterm muscular 174 (117-238, n = 4) long, parallel to cirrus sac (Fig. 2). Vitelline fields branched, extending anteriorly from nerve comissure to posterior end of right posterior caecum on dextral side, and anterior to genital atrium on sinistral side. Vitelline reservoir posterior to ovary. Vitellocord connects to oviduct at sagittal axis (Fig. 2). Eggs thin-shelled, irregular to ellipsoidal shaped 35 (30-43, n = 25) long and 34 (25-43, n = 25) wide.

Nerve comissure ventral, approximately at first quarter of esophagus (Fig. 1). Nerve cords extending anteriorly from approximately 1/8 of the esophagus; indistinct posteriorly. Excretory pore not observed.

- Comparison

Among the 16 known species of Aporocotyle, *A. mariachristinae* is distinguished from the other species by its unique asymmetric arrangement of posterior caeca. This is the only species known of the genus with the right posterior caecum ending at the area between mid-level of ovary and posterior body end, and the left posterior caecum, always shorter, ending at the area between mid-level of cirrus sac and posterior to reproductive organs, never ending near posterior body end. Posterior caeca reach near to the posterior body end in most of the species of *Aporocotyle*, except for those in fishes of the Ophidiiformes (*Genypterus* spp. and *H. armata*, see Table I) in which posterior caeca never reach to ovary (Parukhin & Tkachuk, 1980; Villalba & Fernández, 1986b; Tantaleán & Martínez, 1990; Kamegai et al., 2002). *A. mariachristinae* would be the first species in an ophidiiform fish in which at least one posterior caecum extends beyond the anterior ovary end. The new species clearly differs from *A. ymakara*, the other species described from *G. blacodes*, by the higher number of testes (37-39 instead of 18-21), the absence of distal curves at caeca (see

Figs 3-6. – *Aporocotyle mariachristinae* n. sp. from *Genypterus blacodes*: 3, cluster of spines; 4, distribution of clusters of spines along ventro-lateral body margins near the anterior left caecum, ventral view; 5, distribution of clusters of spines at ventral posterior body end; 6, tegumental spines.

Scale bars: Figs 3 and 4: 100 μm; Figs 5 and 6: 10 μm.
Figs 7-9. – *Aporocotyle ymakara* from *Genypterus blacodes* from Patagonia, Argentina: 7, whole worm, voucher specimen, ventral view; 8, tegumental spine; 9, reproductive organs, ventral view.

Scale bars: Fig. 7: 500 μm; Fig. 8: 10 μm; Fig. 9: 100 μm. Abbreviations: vd, vas deferens; sv, seminal vesicle; m, metraterm; pc, prostatic cells; ut, uterus; ga, genital atrium; c, cirrus; ue, uterine egg; ov, ovary; cs, cirrus sac; vr, vitelline reservoir; usr, uterine seminal receptacle; od, oviduct; ōō, ootype; mg, Mehlis’ gland.
Figs 10-12. – Extension of caeca and diagrammatic representation of distribution of clusters of spines along the entire body length in three species of Aporocotyle, ventral view: 10, *A. argentinensis*, voucher; 11, *A. mariachristinae* n. sp., paratype; 12, *A. ymakara*, paratype (ZMUC No. 7759).

Scale bar: 1,000 μm.
Fig. 1 in Villalba & Fernández, 1986b), the different esophagus/body length ratio (1:2.7-3.9 instead of 1:2.1-2.6), and the different anterior caeca/posterior caeca length ratio (1:2.6-4.7 instead of 1:1.1-1.3). Additionally, in A. ymakara, the left posterior caecum can be equal or longer than the right posterior caecum (see Villalba & Fernández, 1986b), while in the new species is always shorter. The most similar species is A. keli, described from G. chilensis from the Southeast Pacific, with similar size and number of testes. This species differs from A. mariachristinae by the presence of a “muscular fold at posterior body region at level of genital atrium” (Villalba & Fernández, 1986b, see discussion for comments on this trait) together the different host and the different posterior caeca arrangement.

Aporocotyle mariachristinae also differ from A. argentinensis, A. australis, A. margolisi, A. nototheniae, A. orientalis, A. pacifica, A. smithi, A. spinosicanalis, A. theragrae, and A. wilhelmi, in which the clusters of spines can join ventrally at sagittal axis (Fig. 10). Other specific differences with the other Aporocotyle species are reported in Table I.

- Molecular characterization

Partial 18S rDNA sequences were obtained from A. mariachristinae (1,726 bp) and sequence submission to the BLAST server showed that the 18S rDNA closest sequence matches were A. spinosicanalis (GenBank Accession Number JX094803; Query coverage 100 %; Maximum identity 97 %). Partial 28S rDNA sequences from A. mariachristinae (1,554 bp) submitted to the BLAST server showed that the 28S rDNA closest sequences matches were also A. spinosicanalis (GenBank Accession Number AJ222177; Query coverage 81 %; Maximum identity 94 %).

For molecular comparison, partial 28S rDNA sequence was also obtained from A. argentinensis (1,540 bp) (GenBank Accession number JX094803; A. argentinensis partial 28S rDNA sequence, ex. M. hubbsi). The alignment of the partial 28S rDNA sequences of A. mariachristinae and A. argentinensis with the only sequence of the genus available in the database, A. spinosicanalis, showed the following interspecific sequence similarities over an alignment of 1,651 bp: A. mariachristinae/A. argentinensis, 94.3 %; A. mariachristinae/A. spinosicanalis, 93.3 %; A. argentinensis/ A. spinosicanalis, 96.8 %.

APOROCOTYLE YMAKARA
VILLALBA & FERNÁNDEZ, 1986 (Figs 7-9)

Type locality: Arauco Gulf (37º 00’ S – 73º 20’ W), Chile.
New locality: central Patagonia (42º 2’ S, 47º 19’ S – 61º 59’ W, 64º 25’ W), Argentina.

Voucher specimen: NHM Paris (HEL259).

Infection parameters: prevalence, 1.9 (0.1-10.2); abundance, 2.0 (0.0-6.0); intensity, 1.0.

- Description of the specimen from Patagonia, Argentina

Measurements from the single specimen collected in the present study. Aporocotylidae, Aporocotyle Odhner, 1900, A. ymakara Villalba & Fernández, 1986. Body lanceolated (Fig. 7), 1,698 long and 364 wide. Maximum body width approximately at mid-level. Terms of bearing clusters of approximately 20 spines each, in ventro-lateral fields, slightly extending dorsally. Spines approximately 18 long (Fig. 8). Ventrally, spines are distributed along entire body length, never joining at sagittal axis, but reaching closer at anterior half body, occupying, approximately 2/3 distance between body margin to caecum (see similar distribution of paratype in Fig. 12). Spines less abundant at posterior half body. Mouth subterminal. Buccal capsule not observed. Esophagus 659 long (39 % of body length), surrounded by glands at its posterior half (posterior to level of anterior caeca distal end) until caeca bifurcation. Intestine H-shaped. Right anterior caecum slightly longer than the left anterior caecum. Anterior caeca/body length ratio 1:5.3. Right anterior caecum 333; left anterior caecum 310. Posterior caeca unequal: right posterior caecum longer than left posterior caecum. Right posterior caecum 674 (about 40 % of body length), ending approximately at cirrus sac level (Fig. 7). Left posterior caecum 508, ending before the posterior end of testes field (Fig. 7). Anterior caeca/right posterior caecum length ratio 1:2.1.

Testes irregularly-shaped (Fig. 7), 18 in number, 36 (23-64, n = 18) long axis and 65 (39-81, n = 18) wide axis. Testes intercaecal, between caecal bifurcation and anterior to reproductive organs. Vas deferens extending from posterior border of testicular field. Cirrus sac claviform, 263 long and 70 wide, with a thick muscular wall, directing sinistrally, ending at genital atrium (Fig. 9). Seminal vesicle elongate, slightly sinuous, in middle of cirrus sac, surrounded by prostatic cells (Fig. 9). Cirrus short, 15 long (Fig. 9). Genital pore dorso-sinistrally, at 78 % of body length from anterior body end. Ovary central, slightly dextral, sub-ellipsoid, in posterior 1/8 of body, 121 long and 144 wide (Fig. 9). Oötype running posteriorly from posterior margin of ovary, sinistrally oriented (Fig. 9). Oötype surrounded by Mehlis’ gland, connecting to uterine seminal receptacle. Laurers’ canal absent. Uterus extending from uterine seminal receptacle and coiling from posterior...
ovary end to level of anterior cirrus sac end, curving
dorso-sinistrally (Fig. 9). Metraterm muscle 79 long,
parallel to cirrus sac (Fig. 9). Vitelline follicles small,
compact. Vitelline fields branched, extending, on
dextral side, from nerve commissure to posterior end of
right posterior caecum, and, on sinistral side, slightly
beyond posterior end of left posterior caecum. Vitelline
reservoir posterior to ovary (Fig. 9). Vitelloduct after
vitelline reservoir short, connecting dorsally to oviduct.
Eggs thin-shelled, irregular to ellipsoidal shaped 16
(13-19, n = 2) long and 20 (17-23, n = 2) wide.
Nerve commissure ventral, approximately at first
quarter of esophagus (Fig. 7). Nerve cords indistinct.
Excretory pore not observed.

• Remarks

The morphological traits of the specimen collected
from Argentina coincide with those described by
Villalba & Fernández (1986b) for A. ymakara from
Chile: body size, esophagus/body length ratio, number
of testes, and distribution of spines along the body
(see Table I). The extension of the caeca showed
a slight variability in specimens of both localities.
Right posterior caecum ends before cirrus sac (three
paratypes), or at cirrus sac level (two paratypes and
the specimen from Argentina). Left posterior caecum
ends at last testis level in all specimens (Chilean and
Argentinean). Villalba & Fernández (1986b) reported
that the distal end of caeca of some specimens was
curved, however this curvature was not observed in
the paratypes or in the Argentine specimen analysed
in present study. The ratio between anterior caeca
length/posterior caecum length of A. ymakara was
1:2.8-3.5 according to Villalba & Fernández (1986b),
however revision of paratypes from Chile revealed that
the ratio was 1:1.1-1.3.

DISCUSSION

The most used morphological traits to differen-
tiate species of the genus Aporocotyle are body
shape, distribution of spines along the body,
esophagus/body length ratio, anterior caeca/posterior
caecum length ratio, number of testes, shape of cirrus
sac, shape of ovary, presence of a “muscular fold”
at level of genital atrium, and extension of posterior
caecum (Smith, 1967, 1969; Holmes, 1971; Parukhin &
Tkachuk, 1980; Villalba & Fernández 1986b).

The distribution of spines along the body has been
commonly used to separate species of Aporocotyle (see
Smith, 1967, 1969; Ichihara, 1970; Holmes, 1971). From
the species examined in this study, two different pat-
terns of distribution of spines have been observed: (i)
in A. argentinensis, A. spinosicanalis, and A. wilhelmi,
tyle described from fishes of the orders Gadiformes, Perciformes, Pleuronectiformes and Scorpaeniformes which have posterior caeca near ending at the posterior body end (Fig. 10) (Yamaguti, 1934, 1970; Williams, 1958; Smith, 1967, 1969; Ichihara, 1970; Holmes, 1971; Thulin, 1980; Fernández & Durán, 1985; Parukhin, 1985; Villalba & Fernández, 1986a); and (ii) species of *Aporocotyle* described from ophidiid fishes, with posterior caeca never ending near the posterior body end and never reaching to the level of the ovary (Fig. 12) (Parukhin & Tkachuk, 1980; Villalba & Fernández, 1986b; Tantaleán & Martínez, 1990). According to this classification, *A. mariachristinae* could be considered to belong to a third intermediate group with at least one posterior caecum reaching to the area from mid-level of ovary to posterior body end (Fig. 11). Unfortunately, the existence of different phylogenetic groups of *Aporocotyle* species could not be confirmed in present study with molecular analyses as only three sequences exist (two of them provided in this study). We encourage authors to provide more molecular data on aporocotyloid genera to explore phylogenetic relationships.

Currently, there are six species of *Aporocotyle* described from four species of fishes from the genus *Genypterus* (see Parukhin & Tkachuk, 1980; Villalba & Fernández, 1986b; Tantaleán & Martínez, 1990; present study). To our knowledge, this is the only fish genus in which two different species of *Aporocotyle* infect the same host species. Moreover, *A. mariachristinae* and *A. ymakara* could not be the only two *Aporocotyle* species infecting the same host species: although the specific identity of the definitive host for *A. garciai* is unknown (reported as *Genypterus* sp. in Tantaleán & Martínez, 1990), *G. chilensis* or *G. maculatus* must be the type host as they are the only species of *Genypterus* distributed along the Peruvian coast (Nielsen et al., 1999), and both fish species harbour other *Aporocotyle* species (*A. keli* and *A. kuri* respectively, see Table I). More than one blood fluke species of the same genus in the same host have been previously reported: *i.e.*, *Cardicola* Short, 1953 (see Nolan & Cribb, 2006) and *Paradeontacalyx* McIntosh, 1934 (see Ogawa & Egusa, 1986; Repullés-Albelda et al., 2008).

**ACKNOWLEDGEMENTS**

The authors are grateful to L. Nouis, B. Berón-Vera, M. I. Aversa and S. Leonardi for their technical assistance. Thanks are also given to Prefectura Naval Argentina and ALPESCA S.A. for allowing us to collect our material on the hake trawlers. Thanks are also given to G. Muñoz of the School of Molecular and Microbial Sciences, University of Queensland, and L. Sánchez Pérez of the Departamento de Protozoolgia of the Museo de Historia Natural of Peru for their assistance. This study was supported by the following projects: BBVA project n. BIOCON 04, CGL2007-63221 and AGL2010-20892 from the Spanish Government, PROMETEO 2011-040 of the Valencian Government and the Czech Science Foundation (No. P505/12/G112). J.S.H.O. benefited a PhD student grant from the National Council on Science and Technology (CONACYT) of the Mexican Government (scholarship number 197266). G.A.B. benefited a PhD student grant (BFPI/2007/289) from the Conselleria d’Educació of the Generalitat Valenciana (Valencia, Spain). J.M.C. was funded by the Coastal Impact Assistance Program through a grant award (M10AF20151) by the State of Mississippi, Mississippi Dept. of Marine Resources and Dept. of Interior. Institutional support was given by Centro Nacional Patagónico (CONICET, Argentina) and the Institute of Parasitology (RVO:60077344).

**REFERENCES**

Alama-Bermejo G., Montero F.E., Raga J.A. & Holzer A.S. *Skoulekia meningialis* n. gen., n. sp. (Digenea: Aporocotylidae Odhner, 1912) a parasite surrounding the brain of the Mediterranean common two-banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) (Teleostei-Sparidae): description, molecular phylogeny, habitat and pathology. *Parasitology International*, 2011, 60, 34-44.

Bullard S.A., Jensen K. & Overstreet R.M. Historical account of the two family-group names in use for the single accepted family comprising the “fish blood flukes”. *Acta Parasitologica*, 2009, 54, 78-84.

Bush A.D., Lafferty K.D., Lotz J.M. & Shostak A.W. Parasitology meets ecology on its own terms: Margolis et al. revised. *Journal of Parasitology*, 1997, 84, 575-583.

Fernández J. & Durán L. *Aporocotyle australis* n. sp. (Digenea: Sanguinicolidae), parásito de *Merluccius australis* (Hutton 1872) en Chile y su relación con la filogenia de *Aporocotyle* Odhner, 1900 en *Merluccius* spp. *Revista Chilena de Historia Natural*, 1985, 58, 121-126.

Holmes J.C. Two new sanguinicolid blood flukes (Digenea) from scropaenid rockfishes (Perciformes) of the Pacific coast of North America. *Journal of Parasitology*, 1971, 57, 209-216.

Ichihara A. A new blood fluke *Aporocotyle theragrae* n. sp. (Digenean trematode: Aporocotylidae) from a marine fish, *Theragra chalcogramma*. *The Research Bulletin of the Meguro Parasitological Museum*, 1970, 3, 1-4.

Kamegai S., Machida M. & Kuramoto M. Two blood flukes from deep-sea fishes of Suruga Bay, Japan. *Bulletin of the National Science Museum, Tokyo. Series A*, 2002, 28, 29-34.

Lin D., Hanson L.A. & Pote L.M. Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxospora) identifies the actinosporean stage from an oligochaete host. *Journal of Eukaryotic Microbiology*, 1999, 46, 66-68.

**GENYPTERUS BLACODES** (Geoffroy Saint-Hilaire, 1817) (Teleostei-
LITTLEWOOD D.T.J. & OLSON P.D. Small subunit rDNA and the phylum Platyhelminthes: signal, noise, conflict and compromise, in: Interrelationships of the Platyhelminthes. Littlewood D.T.J and Bray R.A. (eds), Taylor and Francis, London, 2001, 262-278.

LOCKYER A.E., OLSON P.D & LITTLEWOOD D.T.J. Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biological Journal of the Linnean Society, 2003, 78, 155-171.

NIELSEN J.G., COHEN D.M., MARKLE D.F. & ROBINS C.R. FAO Species catalogue. Volume 18. Ophidiiform Fishes of the World (Order Ophidiiformes). Food and Agriculture Organization of the United Nations, Rome, Italy, 1999.

NOLAN M.J. & CRIBB T.H. Cardicola Short, 1953 and Bray n. gen. (Digenea: Sanguinicolidae) from five families of tropical Indo-Pacific fishes. Zootaxa, 2006, 1265, 1-80.

OGAWA K. & EGUSA E. Two new species of Paradeontacylix McIntosh, 1934 (Trematoda: Sanguinicolidae) from the vascular system of a cultured marine fish Seriola purpursens. Fish Pathology, 1986, 21, 15-19.

PARUKHIN A. & TKACHUK L. New species of trematodes from fish in the Indian Ocean. Nauchnye Doklady Vysshie Shkoly. Biologicheskie Nauki, 1980, 6, 41-44.

PARUKHIN A. New trematode species from commercial fish of the Indian Ocean. Nauchnye Doklady Vysshie Shkoly. Biologicheskie Nauki, 1985, 8, 29-34.

REICZIGEL J. Confidence intervals for the binomial parameter: some new considerations. Statistics in Medicine, 2003, 22, 611-621.

REICZIGEL J. & RÓZSA L. Quantitative Parasitology 3.0. Budapest, 2005.

REPUÑES-ALBEELDA A., MONTERO F.E., HOLZER A.S., OGAWA K., HUTSON K.S. & RAGA J.A. Speciation of the Paradeontacylix spp. (Sanguinicolidae) of Seriola dumerili. Two new species of the genus Paradeontacylix from the Mediterranean. Parasitology International, 2008, 57, 405-414.

RÓZSA L., REICZIGEL J. & MAJOROS G. Quantifying parasites in samples of hosts. Journal of Parasitology, 2000, 86, 228-232.

SMITH J.W. Aporocotyle margolisi n. sp. (Digenea: Aporocotylidae) from Merluccius productus. Journal of the Fisheries Research Board of Canada, 1967, 24, 1763-1773.

SMITH J.W. On Aporocotyle argentinensis n. sp. (Digenea: Sanguinicolidae) from Merluccius hubbsi, and the phylogeny of Aporocotyle Odhner, 1900 in Hake. Journal of Helminthology, 1969, 43, 371-382.

TANTALEÁN M. & MARTÍNEZ R. Aporocotyle garciai n. sp. (Digenea: Sanguinicolidae), parásito de Genypterus sp. de la costa peruana. Parásitología al día, 1990, 14, 67-69.

THULIN J. A redescription of the fish blood-fluke Aporocotyle simplex Odhner, 1900 (Digenea, Sanguinicolidae) with comments on its biology. Sarsia, 1980, 65, 35-48.

VILLALBA C. & FERNÁNDEZ J. Dos nuevas especies de trematodos parásitos de peces marinos en Chile. Parasitología al día, 1986a, 10, 45-51.

VILLALBA C. & FERNÁNDEZ J. Tres nuevas especies de Aporocotyle Odhner, 1900 (Digenea: Sanguinicolidae) parasitas de Genypterus spp. en Chile (Pisces: Ophidiidae). Revista de Biología Marina, 1986b, 22, 125-139.

WILLIAMS H.H. The anatomy of Aporocotyle spinosicanalis sp. nov. (Trematoda: Digenea) from Merluccius merluccius (L). Annals and Magazine of Natural History, 1958, 13, 291-297.

YAMAGUTI S. Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes. I. Japanese Journal of Zoology, 1934, 5, 249-541.

YAMAGUTI S. Digenetic trematodes of Hawaiian fishes. Keigaku Publishing Co., Tokyo, 1970.

Received on March 21st, 2012
Accepted on June 16th, 2012