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A NEW SPECIES OF *ASCOCOTYLE* (TREMATODA: HETEROPHYIDAE) FROM THE SOUTH AMERICAN SEA LION, *OTARIA FLAVESCENS*, OFF PATAGONIA, ARGENTINA

Jesús Servando Hernández-Orts, Francisco Esteban Montero, Enrique Alberto Crespo*, Néstor Aníbal García*, Juan Antonio Raga, and Francisco Javier Aznar

Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, 46980 Valencia, Spain. *e-mail: jesus.s.hernandez@uv.es*

ABSTRACT: We describe a new heterophyid species, *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp., based on specimens collected from the intestines of the South American sea lion *Otaria flavescens* from Patagonia (Argentina). *Ascocotyle* (*A.*) *patagoniensis* n. sp. is distinguished from the other species of the subgenus by the number of circumoral spines, which are arranged in 2 rows of 18 to 23. The new species also differs from the other species in having a gonotyl without papillae. The specimens exhibited the widest seminal receptacle described for a species of this subgenus. Species of the subgenus *Ascocotyle* usually infect fish-eating birds or mammals in freshwater or brackish habitats. *Ascocotyle* (*A.*) *patagoniensis* n. sp. is the first species of the subgenus described from a marine mammal. However, no metacercariae of *Ascocotyle* spp. were found in 542 marine teleosts from 20 species collected in the same locality. The life cycle of the marine species from the *Ascocotyle*-complex infecting pinnipeds remains elusive.

During a survey of intestinal parasites of the South American sea lion *Otaria flavescens* (Shaw, 1890) (Carnivora: Otariidae) from the Argentine Patagonian coast, a number of heterophyid digenean specimens were collected. The worms appeared to represent a new species of the *Ascocotyle*-complex sensu Sogandares-Bernal and Lumsden (1963) that we describe herein. As it is the first species of *Ascocotyle* (Ascocotyle) Looss, 1899, infecting a marine mammal (see below), a parasitological survey of fish species from the study area was also conducted in an attempt to identify potential intermediate hosts for this species.

MATERIALS AND METHODS

Fifty-six South American sea lions (30 males and 26 females) were collected from 2000 to 2009 in northern Patagonia (40°43′–43°20′S, 63°04′–65°07′W); animals were found as by-catch in fisheries or stranded on the coast. At necropsy, intestines were removed and kept frozen at −20 C. After thawing, intestines were opened and the contents washed with tap water through sieves of either 0.2- or 0.5-mm mesh size. Intestinal contents were later examined using a stereomicroscope; a large number of minute digeneans (ca. 0.6 × 0.2 mm) were found entangled in the mucous contents of 2 host specimens. Given the small size of the parasite, we believe that a significant number of specimens could have been lost during sieving.

The flukes were fixed and preserved in 70% ethanol. A total of 629 specimens was stained with iron acetocarmine (n = 184) or alum carmine (n = 445), dehydrated through an ethanol series, cleared in clove oil, and mounted in Canada balsam. Specimens were examined with a compound microscope using bright field and differential interference contrast optics. Measurements were taken from drawings made with the aid of a drawing tube. Measurements are in micrometers and are shown as the mean followed by standard deviation (SD), with the range in parentheses and the number of measured specimens or structures.

Specimens from the type series are deposited in the Natural History Museum (NHMUK), London, U.K., the United States National Parasite Collection (USNPC), Beltsville, Maryland, and the National Museum of Natural Sciences (MNCN), Madrid, Spain; voucher specimens are deposited in the Collection of the Marine Zoology Unit (MZU), the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

A total of 542 individual fish from 20 species was examined for helminths including metacercariae (Table I). Fishes were collected from 2 zones of the Argentinean 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). Of these species, 16 fish are common prey of the South American sea lion (Koen-Alonso et al., 2000; N. A. García, pers. comm.). Fish were sampled onboard Argentine hake trawlers during 2006–2007 from the same area where sea lions were collected. Samples of fish were frozen at −20 C for later examination or kept on ice to be examined fresh upon arrival at the laboratory. The skin, epaxial and hypaxial muscles, abdominal cavity, liver, intestine and intestinal ceca, stomach, swimbladder, gonads, heart, head, brain, and gills were examined for helminths by stereomicroscopy. Typical microhabitats for metacercariae in species of the subgenus *Ascocotyle* in fish, i.e., walls of the stomach, intestine, and other visceral organs (Martin and Steele, 1970; Font et al., 1984; Ostrowski de Núñez, 2001), bulbus arteriosus (Schroeder and Leigh, 1965; Ostrowski de Núñez, 2001; Santos et al., 2007), and muscle (Font et al., 1984) were pressed between 2 Petri dishes and subsequently examined using a stereomicroscope.

DESCRIPTION

Ascocotyle (*Ascocotyle*) *patagoniensis* n. sp.

(Figs. 1–8)

Diagnosis (based on 18 specimens for general morphology and 12 additional specimens for morphology of circumoral spines): With characters of *Ascocotyle* Looss, 1899, subgenus *Ascocotyle*, sensu Sogandares-Bernal and Lumsden (1963). Body tear-shaped, 622 ± 112 (489–860, n = 18) long and 211 ± 35 (160–302, n = 18) wide, maximum width at level of ovary (Figs. 1, 7, 8). Tegument spinose. Preoral lobe well-developed, 29 ± 10 (17–43, n = 14) long. Oral sucker subterminal, 45 ± 11 (26–68, n = 16) long and 49 ± 12 (22–68, n = 16) wide. Oral sucker surrounded by 2 rows of circumoral spines (Figs. 2–6). Anterior row with 17–23 spines (n = 17); 10 specimens with complete anterior rows, with a distribution of spines per specimen as follows: 18 (n = 6; Fig. 2); 19 (n = 1; Fig. 3); 20 (n = 2; Fig. 5); and 23 (n = 1; Fig. 6). Posterior row with 17–20 spines (n = 8); 3 specimens with complete posterior row, with a distribution of spines per specimen as follows; 18 (n = 2; Figs. 2, 4) and 19 (n = 1; Fig. 3). Spines in anterior row longer than in posterior row, 15 ± 2 (13–17, n = 28 from 5 specimens) long; spines in posterior row 11 ± 2 (10–13, n = 18 from 3 specimens) long (Figs. 2–6). Oral sucker with conical posterior prolongation, prolongation 88 ± 30 (48–149, n = 14) long, more elongated in long-necked specimens (Figs. 1, 7, 8). Prepharynx 117 ± 43 (56–200, n = 16) long. Pharynx strongly muscular, oval, 57 ± 7 (47–72, n = 20) long and 37 ± 8 (20–49, n = 20) wide. Esophagus not observed. Intestinal ceca short, wide, ending anterior to ventral sucker (Figs. 7, 8). Ventral sucker oval, medial, slightly postequatorial, 57 ± 9 (47–77, n = 9) long and 59 ± 8 (43–68, n = 9) wide; opening of ventral sucker small.

Testes irregularly ellipsoidal, symmetrical, situated near posterior extremity, similar in size, 51 ± 9 (43–72, n = 15) long and 69 ± 15 (39–89, n = 15) wide. Seminal vesicle voluminous, 75 ± 14 (43–96, n = 12) long and 111 ± 28 (79–170, n = 12) wide, sinuous with dextral dorsoventral loop connecting 2 large sacs, ventral more elongated than dorsal (Figs. 7, 8). Ejaculatory duct dorsal, curved proximally. Ventro-genital sac with slit-like opening; gonotyl simple muscular, situated antero-laterally (dextrally) to ventral sucker, without papillae, 12 ± 1 (11–13, n = 3) long and 34 ± 7 (28–43, n = 3) wide (Fig. 7). Ovary oval, tenuous, situated medially between seminal vesicle and seminal receptacle (Figs. 7, 8), 37 ± 7

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*Centro Nacional Patagónico CONICET, Boulevard Brown 3600 (9120), Puerto Madryn, Argentina.

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TABLE I. Sampling details of fish species that were examined for the presence of species of *Ascocotyle* in Patagonia, Argentina (measurements in centimeters).

Host family	Scientific name	n	Total length (mean ± SD)
Bramidae	*Brama brama* (Bonnaterre, 1788)	2	60.5 ± 2.1
Bovichtidae	*Cottoperca gobio* (Günther, 1861)	8	30.2 ± 9.3
Centrolophidae	*Seriolella porosa* Guichenot, 1848	34	33.0 ± 5.6
Cheilodactylidae	*Nemadactylus bergi* (Norman, 1937)	32	25.6 ± 5.5
Congiopodidae	*Congiopodus peruvianus* (Cuvier, 1829)	15	23.9 ± 2.0
Merlucciidae	*Macruronus magellanicus* Lönnberg, 1907	3	56.7 ± 23.0
	Merluccius hubbsi Marini, 1933	79	28.1 ± 4.2
Mullidae	*Mullus argentinae* Hubbs and Marini, 1933	2	20.7 ± 0.4
Nototheniidae	*Patagonotothen ramsayi* (Regan, 1913)	84	24.9 ± 3.5
Ophidiidae	*Genypterus blacodes* (Forster, 1801)	44	42.1 ± 9.9
	Raneya brasiliensis (Kaup, 1856)	16	21.2 ± 1.4
Paralichthyidae	*Paralichthys isosceles* Jordan, 1891	15	27.2 ± 5.5
	Xystreurys rasile (Jordan, 1891)	29	32.8 ± 5.9
Percophidae	*Percophis brasiliensis* Quoy and Gaimard, 1825	8	45.3 ± 4.9
Pinguipedidae	*Pseudopercis semifasciata* (Cuvier, 1829)	31	26.5 ± 2.7
Scombridae	*Scomber japonicus* Houttuyn, 1782	13	42.7 ± 5.0
Sebastidae	*Helicolenus lahillei* Norman, 1937	6	28.8 ± 2.6
Serranidae	*Acanthistius brasilianus* (Cuvier, 1828)	16	30.0 ± 2.6
Stromateidae	*Stromateus brasiliensis* Fowler, 1906	73	27.5 ± 3.6
Triglidae	*Prionotus nudigula* Ginsburg, 1950	32	23.1 ± 2.8

(30–43, n = 3) long and 49 ± 5 (43–52, n = 3) wide. Seminal receptacle voluminous, round to ellipsoidal, pretesticular or slightly sub-median (dextral), 77 ± 28 (51–128, n = 6) long and 107 ± 14 (86–123, n = 6) wide (Fig. 7). Vitelline follicles small, scattered, extending into lateral fields from anterior margin of ventral sucker to posterior body end (Figs. 1, 7, 8). Transverse vitelline ducts joining at ovary level. Uterus tubular, forming several loops from region between pharynx and ventral sucker to posterior margin of body (Figs. 1, 7, 8). Eggs ellipsoidal, operculated, 19 ± 1 (17–22, n = 61 from 20 specimens) long and 11 (10–15, n = 61 from 20 specimens) wide. Excretory vesicle Y-shaped with pretesticular lateral branches. Excretory pore sub-terminal, slightly dorsal.

Taxonomic summary

Type host: South American sea lion *Otaria flavescens* (Shaw, 1890) (Carnivora: Otariidae).

Type locality: North Patagonia (40°43′–43°20′S, 63°04′–65°07′W), Chubut, Argentina.

Site in host: Intestine.

Specimens studied: Thirty mounted specimens.

Type specimens: Holotype (NHMUK 2012.2.13.1), 5 paratypes (NHMUK 2012.2.13.2–6), 4 paratypes (USNPC 105290), 6 paratypes (MNCN 4.02/52–4.02/57), 14 vouchers (MZU, EF2 11592–11605).

Infection parameters: Infection parameters could not be reliably determined (see Materials and Methods); about 4,500 specimens were collected from 2 host specimens.

Etymology: The epithet *patagoniensis* indicates the geographical region where the parasite was collected.

Remarks

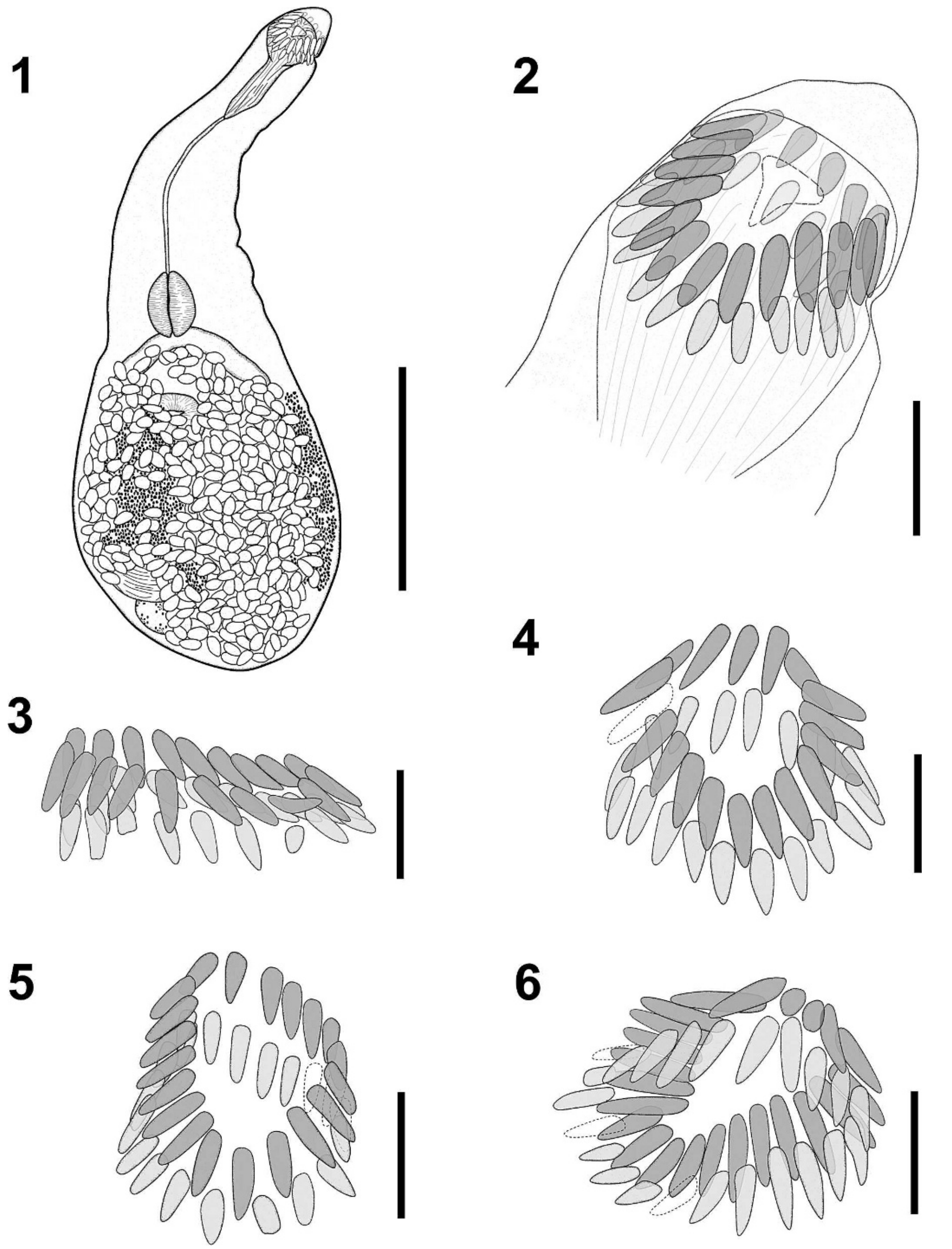
Currently, 10 valid species are recognized in *Ascocotyle* (*Ascocotyle*): *Ascocotyle* (*A.*) *branchialis* Timon-David, 1961; *Ascocotyle* (*A.*) *coleostoma* Looss, 1896; *Ascocotyle* (*A.*) *felippei* Travassos, 1928; *Ascocotyle* (*A.*) *gemina* Font, Heard and Overstreet, 1984; *Ascocotyle* (*A.*) *leighi* Burton, 1956; *Ascocotyle* (*A.*) *pachycystis* Schroeder and Leigh, 1965; *Ascocotyle* (*A.*) *paratenuicollis* Nasir, Lemus de Guevara and Díaz, 1970; *Ascocotyle* (*A.*) *secunda* Ostrowski de Núñez, 2001; *Ascocotyle* (*A.*) *sexidigita* Martin and Steele, 1970; and *Ascocotyle* (*A.*) *tertia* Ostrowski de Núñez, 2001 (see Santos et al., 2007; Table II). *Ascocotyle* (*A.*) *patagoniensis* can be distinguished morphologically from these species by the number of circumoral spines (18 to 23 per row; 17 spines were found only in apparently incomplete rows). *Ascocotyle* (*A.*) *paratenuicollis* has only 11 spines per row, whereas 5 species have 16 spines per row and 4 species ≥22 spines per row (maximum number 32 in *A.* (*A.*) *gemina*) (Table II). The number of spines per row of *A.* (*A.*) *patagoniensis* only overlaps with that of *A.* (*A.*) *pachycystis*, but they differ from one another in the maximum number of spines (23 and 29, respectively) and the seminal receptacle size. *Ascocotyle* (*A.*) *patagoniensis* has the widest seminal receptacle of all described species, both in absolute and relative terms (Table II). The new species also differs from *A.* (*A.*) *gemina*, *A.* (*A.*) *secunda*, *A.* (*A.*) *sexidigita*, and *A.* (*A.*) *tertia* in lacking papillae in the gonotyl (Table II).

No metacercariae of *Ascocotyle* spp. were found in any of the fish examined.

DISCUSSION

Species belonging to the subgenus *Ascocotyle* possess 2 rows of circumoral spines, vitellarium extending to ventral sucker, uterus mainly confined to the area posterior to ventral sucker, and parapleurolophocercous cercariae (Sogandares-Bernal and Lumen, 1963). These diagnostic traits are shared with *A.* (*A.*) *patagoniensis*, although the type of cercaria is unknown for the new species. Additionally, apart from the morphological differences, the new species is the first in this subgenus found from a marine mammal. The other species occur mainly in fish-eating birds in freshwater habitats or salt marshes; 2 species have also been reported in terrestrial carnivores (raccoons) feeding on aquatic prey (Table II).

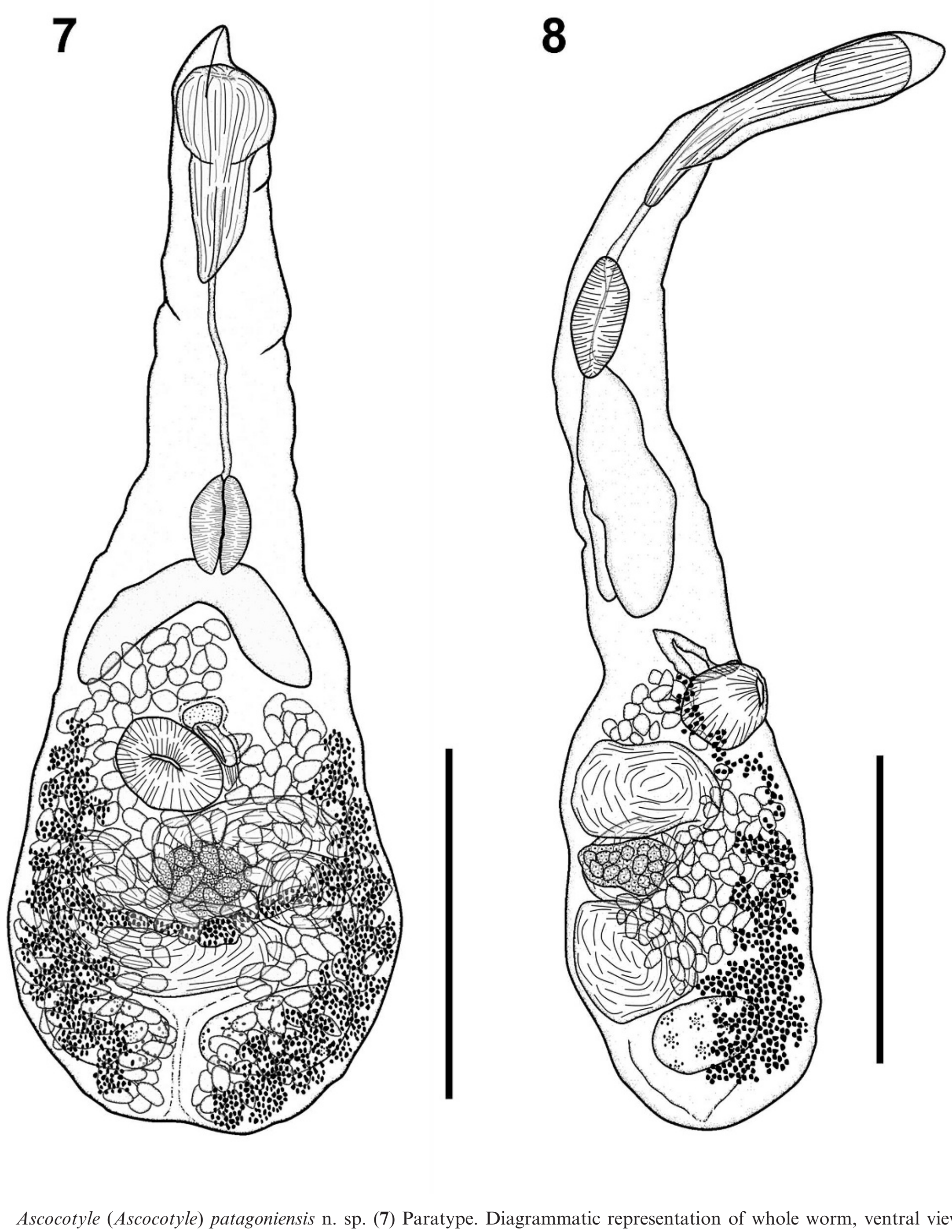
The number of circumoral spines is the most useful morphological character to differentiate species within the subgenus *Ascocotyle* (Sogandares-Bernal and Lumsden, 1963; Santos et al., 2007). *Ascocotyle* (*A.*) *patagoniensis* is unique in having 2 complete circles of 18 to 23 spines each (Figs. 2, 3). Travassos (1928) described *A.* (*A.*) *felippei* as also having 18 spines per row. Santos et al. (2007) re-examined the original specimens described by Travassos (1928) (who did not designate a holotype), synonymized with other species as *A.* (*A.*) *puertoricensis* and *A.* (*A.*) *tenuicollis*, and concluded that no specimen possessed more than 16 spines per row. Circumoral spines are often difficult to



FIGURES 1–6. *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp. (**1**) Holotype. Whole worm, dorsal view. (**2**) Holotype. Detail of oral sucker showing the double row of 18 spines each. (**3–6**) Paratypes. Details of the circumoral spines with different combinations of numbers per rows (possible missing spines have been reconstructed with dotted lines): 19 spines each (3); 17 spines in anterior row and 18 in posterior row (4); 20 spines in anterior row and 17 in posterior row (5); and 23 spines in anterior row and 20 in posterior row (6). Scale bars: Figure 1 = 200 μm; Figures 2–6 = 50 μm.

count due to their small size and visual overlapping in mounted specimens. Moreover, spines may be missing because of the post-mortem decomposition of the tegument (Santos et al., 2007). Many specimens of *A.* (*A.*) *patagoniensis* were in relatively poor condition, and the upper and lower rows of circumoral spines were complete in just 10 and 3, respectively, of 629 mounted specimens (Figs. 2–6). The problem of post-mortem maceration is widespread in digeneans of marine mammals because the host is usually difficult to obtain and process because, frequently, it may have been dead for a long time, or frozen, before worms can be collected (Gibson, 2005). In fact, spines are often lost in other typical structures of digeneans, e.g., the tegument, the cirrus pouch, or the metraterm (Adams and Rausch, 1989).

Other structural traits, e.g., the morphology of the gonotyl, meristic, the number and morphometric character of gonotyl papillae (or both), the size of the body, relative position of testes,



FIGURES 7–8. *Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp. (**7**) Paratype. Diagrammatic representation of whole worm, ventral view. (**8**) Paratype. Diagrammatic representation of worm, lateral view. Scale bars = 200 μm.

and extension of vitellaria have been used to separate species within the subgenus *Ascocotyle* (Schroeder and Leigh, 1965; Martin and Steele, 1970; Font et al., 1984; Ostrowski de Núñez, 2001, Santos et al., 2007). In addition, we suggest that the voluminous seminal receptacle of *A* (*A.*) *patagoniensis* could be a useful feature that separates it from other species in the subgenus because this structure was markedly consistent in fully developed specimens. Unfortunately, the seminal receptacle has rarely been measured in older descriptions; therefore, we generally obtained its dimensions from original drawings (Table II). However, even a cursory examination of drawings clearly reveals that the seminal receptacle is comparatively much more developed in *A.* (*A.*) *patagoniensis* than in any other species. The seminal vesicle was also voluminous in this species, with a particular looped shape. A folded or looped seminal vesicle has only been described in 3 other species of this subgenus, i.e., *A.* (*A.*) *pachycystis*, *A.* (*A.*) *secunda*, and *A.* (*A.*) *sexidigita* (Schroeder and Leigh, 1965; Martin and Steele, 1970; Ostrowski de Núñez, 2001). Nevertheless, the

TABLE II. Taxonomic data on the species of *Ascocotyle*, subgenus *Ascocotyle* (measurements in micrometers).

Species of *Ascocotyle* and References:	*Ascocotyle* (*Ascocotyle*) *branchialis*	*Ascocotyle* (*Ascocotyle*) *coleostoma*	*Ascocotyle* (*Ascocotyle*) *gemina*	*Ascocotyle* (*Ascocotyle*) *felippei*	*Ascocotyle* (*Ascocotyle*) *leighi*	*Ascocotyle* (*Ascocotyle*) *pachycystis*
	Timon-David, 1961; Santos et al., 2007	Travassos, 1930; Santos et al., 2007	Font et al., 1984; Scholz et al., 2001	Santos et al., 2007	Burton, 1956; Kennedy, 1988; Forrester and Spalding, 2003; Kinsella et al., 2004	Schroeder and Leigh, 1965; Kennedy, 1988; Underwood, 1990
Definitive host	Pigeon*	Fish-eating birds	Fish–eating birds	Fish-eating birds, raptors	Fish-eating birds, raccoon	Raccoon, clapper rail
Second intermediate host	Edible frog	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish	Freshwater, brackish fish
Locality	France	Palearctic	North America	America	United States	United States
Body length × width	332–475 × 147–199	700–800 × 250	402–902 × 103–284	361–655 × 123–218	283–402 × 84–122	470–679 × 127–158
No. circumoral spines (no. per row)	32 (16)	32 (16)	55–61 (27–32)	32 (16)	48–52 (24–26)	44–58 (22–29)
Gonotyl	Unknown	Unknown	With 7–10 papillae	Simple	Simple	Simple†
Seminal vesicle (length × width)	Unknown	23 × 55†	49 × 37†	32–39 × 39–55†	19 × 53†	74 × 63† ‡
Seminal receptacle (length × width)	30 × 33†	30 × 40†	69 × 82†	25–48 × 34–60	22 × 22†	37 × 33†

Species of *Ascocotyle* and References:	*Ascocotyle* (*Ascocotyle*) *paratenuicollis*	*Ascocotyle* (*Ascocotyle*) *secunda*	*Ascocotyle* (*Ascocotyle*) *sexidigita*	*Ascocotyle* (*Ascocotyle*) *tertia*	*Ascocotyle* (*Ascocotyle*) *patagoniensis* n. sp.
	Nasir et al., 1970	Ostrowski de Núñez, 2001	Martin and Steele, 1970	Ostrowski de Núñez, 2001	Present study
Definitive host	Chicken*	Chicken*	Chicken*	Chicken*	South American sea lion
Second intermediate host	Freshwater, brackish fish	Freshwater, brackish fish	Brackish, marine fish	Freshwater, brackish fish	Unknown
Locality	Venezuela	Argentina	United States	Argentina	Argentina
Body length × width	429 × 143	377–678 × 119–264	350–810 × 140–240	377–590 × 138–220	489–860 × 160–302
No. circumoral spines (no. per row)	22 (11)	32 (16)	? (29–30)	32 (16)	36–46 (18–23)
Gonotyl	Simple	With 9 papillae	With 6 papillae	With 10 papillae	Simple
Seminal vesicle (length × width)	65 × 38†‡	54 × 81†	60 × 138†	60 × 127†	43–96 × 79–170
Seminal receptacle (length × width)	50 × 54†	36 × 36†	62 × 74†	25 × 22†	51–128 × 86–123

* Adult specimens obtained only from experimental infections.
† Obtained from figures in species descriptions.
‡ Seminal vesicle not differentiated from ejaculatory duct.

morphology of these 2 saccular structures must be considered carefully, as they are thin-walled and can change shape depending on the state and position of the worm.

Because the adult stage of as many as 5 species from the subgenus *Ascocotyle* are still known only from experimental infections in chickens and pigeons (Table II), it is relevant to note that the parasites herein described were obtained from wild hosts. Moreover, 2 additional species, i.e., *A.* (*A.*) *gemina* and *A.* (*A.*) *leighi*, have been reported from fish-eating birds or mammals under natural conditions (Table II), but descriptions are available only from experimental hosts, namely chickens and ducks (Burton, 1956; Font et al., 1984). Host species have been shown to induce significant effects on morphometric traits and allometric relationships in digeneans (e.g., Kinsella, 1971; Pérez-Ponce de León, 1995, Mateu et al., 2011), and it would not be surprising that the effect is more pronounced in specimens obtained from unnatural hosts. Therefore, re-descriptions of most species of *Ascocotyle* (*Ascocotyle*) based on specimens collected from natural hosts would be required for a proper comparison of morphometric differences among species. Moreover, a genetic comparison of specimens from different hosts would be necessary to ensure the validity of the species of the subgenus *Ascocotyle* because there are no available sequences to date.

Life cycles of *Ascocotyle* (*Ascocotyle*) species involve 3 hosts, i.e., a hydrobiid snail, a teleost, and a fish-eating bird or mammal in freshwater or brackish habitats (Font et al., 1984; Ostrowski de Núñez, 2001). Thus, the occurrence of *A.* (*A.*) *patagoniensis* in sea lions represents the first record of a species from this subgenus in a marine definitive host. The low prevalence in sea lions of the present study could indicate that *O. flavescens* is not the principal definitive host of this species; however, we cannot argue this conclusively because the intensities of gravid parasites were high and, as previously mentioned, many worms could have been lost due to their small size.

Unfortunately, the detection of other stages of the life cycle of this species has hitherto been elusive. South American sea lions feed principally on marine teleosts and cephalopods (Koen-Alonso et al., 1999; N. A. García, pers. comm.). However, in the study area, we failed to find metacercariae of *Ascocotyle* spp. in 542 fish specimens from 20 species, including common fish prey for South American sea lions along the Argentine coast. Moreover, there are no records in other parasitological surveys (Timi and Poulin, 2003; Sardella and Timi, 2004; Vales et al., 2011, and references therein). Given the small size of adult worms, we cannot rule out that, in our fish sample, some metacercariae have been overlooked, particularly in microhabitats that are difficult to examine, e.g., in muscles. However, we thoroughly surveyed, by transparency, the typical microhabitats that have been reported for other species of *Ascocotyle* (*Ascocotyle*), so it is hard for us to believe that metacercariae were systematically missed. For some species, few specimens could be collected (see Table I) and, therefore, metacercariae might not have been recorded due to low sample size. Because our samples were obtained 80–330 km from the coastline, one possibility is that the life cycle is restricted to more coastal waters. Alternatively, the fish intermediate host of *A.* (*A.*) *patagoniensis* could be a euryhaline species that serves as prey for other potential definitive hosts, such as salt marsh or marine birds, and that it is seldom consumed by sea lions. In fact, Morgades et al. (2002) reported thousands of adult *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920, in South American sea lions from Uruguay, and *A.* (*P.*) *longa* is known to be a typical parasite from freshwater and marine birds (Scholz, 1999).

Ascocotyle (*A.*) *patagoniensis* is the fourth species of the *Ascocotyle*-complex that has been reported in pinnipeds, with the other 3 belonging to the subgenus *Phagicola* Faust, 1920. Apart from the record of *A.* (*P.*) *longa* Ransom, 1920, in the South American sea lions noted above, *Ascocotyle* (*Phagicola*) *sinoecum* Ciurea, 1933 has been reported in the Caspian seal *Phoca caspica* (Gmelin, 1788) (Raga, 1992; Demidenko and Korolev, 2004) and *Ascocotyle* (*Phagicola*) *septentrionalis* Van Den Broek, 1967, in the harbor seal *Phoca vitulina* (L.) from the North Sea (Van Den Broek, 1967; Borgsteede et al., 1991). The 2 latter species are apparently specific to their respective hosts, with few records in natural conditions, other than seals in the case of *A.* (*P.*) *sinoecum*. This evidence suggests the potential for host switching between birds and mammals in freshwater and marine environments, with or without subsequent speciation of the parasite in the new hosts (see Hoberg and Brooks, 2008). The same phenomenon seems to occur in other heterophyids as well, e.g., *Cryptocotyle lingua* (Creplin, 1825) in harbor seals (see Borgsteede et al., 1991) and species of *Galactosomum* Looss, 1899 in sea lions (see Dailey, 1969; Dubois and Angel, 1976; Dailey et al., 2002). In conclusion, although *A.* (*A.*) *patagoniensis* could be harbored by definitive hosts other than South American sea lions, its unique morphological traits clearly indicate that this is a new species within *Ascocotyle.*

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LITERATURE CITED

Adams, A. M., and R. L. Rausch. 1989. A revision of the genus *Orthosplanchnus* Odhner, 1905 with consideration of the genera *Odhneriella* Skriabin, 1915 and *Hadwenius* Price, 1932 (Digenea: Campulidae). Canadian Journal of Zoology **67:** 1268–1278.

Borgsteede, F. H. M., H. G. J. Bus, J. A. W. Verplanke, and W. P. J. van Burg. 1991. Endoparasitic helminths of the harbor seal, *Phoca vitulina*, in the Netherlands. Netherlands Journal of Sea Research **28:** 247–250.

Burton, P. R. 1956. Morphology of *Ascocotyle leighi* n. sp. (Heterophyidae), an avian trematode with metacercaria restricted to the conus arteriosus of the fish, *Mollienesia latipinna* Le Sueur. Journal of Parasitology **42:** 540–543.

DAILEY, M. D. 1969. *Stictodora ubelakeri*, a new species of heterophyid trematode from the California sea lion (*Zalophus californianus*). Bulletin of the Southern California Academy of Sciences **68:** 82–85.

———, R. S. DEMAREE, AND R. L. CRITCHFIELD. 2002. *Galactosomum stelleri* sp. n. (Trematoda: Heterophyidae) from the Northern sea lion, *Eumetopias jubatus* (Schreber, 1776) (Carnivora: Otariidae). Comparative Parasitology **69:** 58–61.

DEMIDENKO, L. A., AND V. A. KOROLEV. 2004. Localization of helminths in organs of digestive system in Caspian seal (*Phoca caspica* Gmelin, 1788). *In* Marine mammals of the Holarctic: Collection of Scientific Papers after the 3rd International Conferences, V. M. Belkovich (ed.). Moscow, Russia, p. 180–183.

DUBOIS, G., AND L. M. ANGEL. 1976. *Galactosomum angelae* Pearson 1973 in *Neophoca cinerea* (Pérson, 1816) the Australian sea lion. Bulletin de la Société Neuchâteloise des Sciences Naturelles **82:** 191–229.

FONT, W. F., R. W. HEARD, AND R. M. OVERSTREET. 1984. Life cycle of *Ascocotyle gemina* n. sp., a sibling species of *A. sexidigita* (Digenea: Heterophyidae). Transactions of the American Microscopical Society **103:** 392–407.

FORRESTER, D. J., AND M. G. SPALDING. 2003. Parasites and diseases of wild birds in Florida. University of Florida Press, Gainesville, Florida, 1132 p.

GIBSON, D. I. 2005. Family Brachycladiidae Odhner, 1905. *In* Keys to the Trematoda vol. 2, A. Jones, R. A. Bray, and D. I. Gibson (eds.). CAB International and Natural History Museum, London, U.K., p. 641–652.

HOBERG, E. P., AND D. R. BROOKS. 2008. A macroevolutionary mosaic: Episodic host-switching, geographical colonization and diversification in complex host-parasite systems. Journal of Biogeography **35:** 1533–1550.

KENNEDY, M. J. 1988. Synopsis of the Digenea of mammals of North America. Alberta Agriculture, Animal Health Division, Edmonton, Alberta, Canada, 83 p.

KINSELLA, J. M. 1971. Growth, development, and intraspecific variation of *Quinqueserialis quinqueserialis* (Trematoda: Notocotylidae) in rodent hosts. Journal of Parasitology **57:** 62–70.

———, M. G. SPALDING, AND D. J. FORRESTER. 2004. Parasitic helminths of the American white pelican *Pelecanus erythrorhynchos*, from Florida, U.S.A. Comparative Parasitology **71:** 29–36.

KOEN-ALONSO, M., E. A. CRESPO, S. N. PEDRAZA, N. A. GARCÍA, AND M. COSCARELLA. 2000. Food habits of the South American sea lion (*Otaria flavescens*) of Patagonia, Argentina. Fishery Bulletin **98:** 250–263.

MARTIN,W. E., AND D. F. STEELE. 1970. *Ascocotyle sexidigita* sp. n. (Trematoda: Heterophyidae) with notes on its life cycle. Proceedings of the Helminthological Society of Washington **37:** 101–104.

MATEU, P., J. A. RAGA, AND F. J. AZNAR. 2011. Host specificity of *Oschmarinella rochebruni* and *Brachycladium atlanticum* (Digenea: Brachycladiidae) in five cetacean species from western Mediterranean waters. Journal of Helminthology **85:** 12–19.

MORGADES, D., H. KATZ, O. CASTRO, D. CAPELLINO, J. M. VENZAL, L. CASAS, AND A. MORAÑA. 2002. Hallazgos parasitológicos e histopatológicos en lobos marinos en Uruguay. (Estudios preliminares). *In* Bases para la conservación y el manejo de la costa uruguaya, R. Menagra, L. Rodríguez-Gallego, R. Scarabino, and D. Conde (eds.). Vida Silvestre, Montevideo, Uruguay, p. 89–96.

NASIR, P., D. LEMUS DE GUEVARA, AND M. T. DÍAZ. 1970. Estudio sobre larvas de trematodos de agua dulce. XXIV. Ciclo vital parcial de *Ascocotyle paratenuicollis* sp. n. (Trematoda: Digenea). Acta Biológica Venezuelica **7:** 1–4.

OSTROWSKI DE NÚÑEZ, M. 2001. Life cycle of two sibling species of *Ascocotyle* (*Ascocotyle*) (Digenea, Heterophyidae) in the Neotropical Region. Acta Parasitologica **46:** 119–129.

PÉREZ-PONCE DE LEÓN, G. 1995. Host induced morphological variability in adult *Posthodiplostomum minimum* (Digenea: Neodiplostomidae). Journal of Parasitology **81:** 818–820.

RAGA, J. A. 1992. Parasitismus bei den Pinnipedia. *In* Handbuch der Säugetiere Europas, R. Duguy, and D. Robineau (eds.). Aula-Verlag, Weisbaden, Germany, p. 41–77.

SANTOS, C., S. BALMANT, E. SIMÕES, H. SANTOS, AND T. SCHOLZ. 2007. Redescription of *Ascocotyle* (*Ascocotyle*) *felippei* Travassos, 1928 (Digenea: Heterophyidae) with new synonymies. Journal of Parasitology **93:** 1468–1475.

SARDELLA, N. H., AND J. T. TIMI. 2004. Parasites of Argentine hake in the Argentine Sea: Population and infracommunity structure as evidence for host stock discrimination. Journal of Fish Biology **65:** 1472–1488.

SCHOLZ, T. 1999. Taxonomic study of *Ascocotyle* (*Phagicola*) *longa* Ramson, 1920 (Digenea: Heterophyidae) and related taxa. Systematic Parasitology **43:** 147–158.

———, M. L. AGUIRRE-MACEDO, AND G. SALGADO-MALDONADO. 2001. Trematodes of the family Heterophyidae (Digenea) in Mexico: A review of species and new host and geographical records. Journal of Natural History **35:** 1733–1772.

SCHROEDER, R. E., AND W. H. LEIGH. 1965. The life history of *Ascocotyle pachycystis* sp. n., a trematode (Digenea: Heterophyidae) from the raccoon in South Florida. Journal of Parasitology **51:** 594–599.

SOGANDARES-BERNAL, F., AND R. D. LUMSDEN. 1963. The generic status of the heterophyid trematodes of the *Ascocotyle* complex, including notes on the systematics and biology of *Ascocotyle angrense* Travassos, 1916. Journal of Parasitology **49:** 264–274.

TIMI, J. T., AND R. POULIN. 2003. Parasite community structure within and across host populations of a marine pelagic fish: How repeatable is it? International Journal for Parasitology **33:** 1353–1362.

TIMON-DAVID, J. 1961. Reserches sur la morphologie, le développement experimental et la systematique d'une metacercariae du genre *Ascocotyle* Looss 1899 (Trematoda, Digenea, Heterophyidae), parasite des branchies chez les tetrads de *Rana esculenta.* Annales de Parasitologie Humaine et Comparée **36:** 737–751.

TRAVASSOS, L. 1928. Deux nouvelles espèces du genre *Ascocotyle* Looss, 1899. Comptes Rendues de la Société Biologique de Paris **100:** 956–957.

———. 1930. Revisão de gênero *Ascocotyle* Loos, 1899. Memorias do Instituto Oswaldo Cruz **23:** 61–97.

UNDERWOOD, H. T. 1990. Determination of feeding habits from endohelminth fauna. American Zoologist **30:** 114A.

VALES, D. G., N. A. GARCÍA, E. A. CRESPO, AND J. T. TIMI. 2011. Parasites of a marine benthic fish in the Southwestern Atlantic: Searching for geographical recurrent patterns of community structure. Parasitology Research **108:** 261–272.

VAN DEN BROEK, E. 1967. *Phagicola septentrionalis* n. sp. (Trematoda: Heterophyidae) from the harbour seal, *Phoca vitulina* L. Zoologische Mededelingen **42:** 1–4.