APOCREADIUM GALAICUS SP. N. (DIGENEA : APOCREADIIDAE), A PARASITE OF THE THICKBACK SOLE *MICROCHIRUS VARIEGATUS* (SOLEIDAE, OSTEICHTHYES) FROM N.W.SPAIN

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Summary :

A new species of digenetic trematode, *Apocreadium galaicus* (Apocreadiidae), parasitic in the intestine and occasionally the stomach of the thickback sole *Microchirus variegatus* (Soleidae, Osteichthyes), is described. To date, *Apocreadium* has been reported only from American and Asian coastal waters; this is the first report of a species from European coastal waters. The most similar taxa to the new species are *A. foliatum*, *A. mexicanum*, *A. synagris* and *A. balistis*. *A. galaicus* can be distinguished a) from *A. foliatum*, because the oral sucker of this species is larger than the acetabulum, while in *A. galaicus* it is smaller; b) from *A. mexicanum* by the simple (not sphincter-like) structure of the anterior quarter of its pharynx and by its larger eggs, c) from *A. synagris* by the position of the oral sucker and the restriction of cuticular spines to the pretesticular region in *A. galaicus* and d) from *A. balistis* by its longer post-testicular region (55.3 – 69.6% of body length), by the densely spiny cuticle of the acetabular region and its larger eggs. In the study area, prevalence of infection by *A. galaicus* showed a clear peak between November and January, and dropped to zero in May / June.

KEY WORDS : *Apocreadium galaicus* sp. n. *Microchirus variegatus*. Galicia. Spain.

Résumé : *APOCREADIUM GALAICUS* SP. N. (DIGENEA : APOCREADIIDAE), UN PARASITE DE *MICROCHIRUS VARIEGATUS* (SOLEIDAE, OSTEICHTHYES) DANS LE NORD-OUEST DE L'ESPAGNE

Une nouvelle espèce de trématode (Digenea) est décrite, *Apocreadium galaicus* (Apocreadiidae). Elle parasite l'intestin et occasionnellement l'estomac de *Microchirus variegatus* (Soleidae : Osteichthyes). Jusqu'à présent le genre *Apocreadium* n'a été trouvé que dans les eaux des côtes américaines et asiatiques. Les taxons les plus proches de la nouvelle espèce sont *A. foliatum*, *A. mexicanum*, *A. synagris* et *A. balistis*. On peut les distinguer de *A. galaicus* : *A. foliatum*, parce que dans cette espèce la ventouse orale est plus grande que l'acetabulum, au contraire de ce qui arrive chez *A. galaicus* ; *A. mexicanum* par la structure simple du quart antérieur de son pharynx et par la taille des oeufs qui sont plus grands ; *A. synagris* par la position de la ventouse orale et par les épines cuticulaires qui sont restreintes à la région pretesticulaire ; *A. balistis* par la longue région posttesticulaire (55,3 – 69,1% de la longueur du corps), par la cuticule de la région de la ventouse ventrale, qui a des épines très serrées, et par les oeufs qui sont plus grands. Dans la zone étudiée la prévalence de l'infestation par *A. galaicus* montre un pic évident entre les mois de novembre et janvier et cette prévalence tombe à zéro les mois de mai et juin.

MOTS CLÉS : *Apocreadium galaicus* sp. n. *Microchirus variegatus*. Galice. Espagne.

INTRODUCTION

The genus *Apocreadium* was erected by Manter (1937) to accommodate *A. mexicanum* and *A. longisinosum*. This author originally included the genus in the subfamily Megasoleninae in view of the presence of a well-developed lymphatic system, although it differs from the other genera of this subfamily in not having a hermaphroditic sac. Over the subsequent 20 years, seven species were assigned to *Apocreadium* : *A. balistis* Manter, 1947, *A. synagris* Yamaguti, 1953, *A. caballeroi* Bravo, 1953, and *A. coili*, *A. angustum*, *A. uroproctoferum* and *A. bravoii*, described by Sogandares-Bernal (1959). The latter author proposed

the following subclassification of the genus :

a) Oral sucker with two lateral lobes; vitellaria confluent anterior to acetabulum - *A. coili*, *A. angustum*, *A. bravoii*.

b) Oral sucker without lobes; vitellaria not confluent anterior to acetabulum.

i) without uroproct - *A. mexicanum*, *A. longisinosum*, *A. synagris*, *A. caballeroi*, *A. balistis*.

ii) with uroproct - *A. uroproctoferum*.

Siddiqi and Cable (1960) erected the genus *Neoapocreadium* to accommodate the species of the first group (which thus became *N. coili*, *N. angustum* and *N. bravoii*). Four species have subsequently been assigned to *Apocreadium* : *A. cryptum* Overstreet, 1969, *A. manteri* Overstreet, 1970, *A. viguerasi* Zhukov, 1983 and *A. vinodae* Ahmad, 1985. In addition, Overstreet (1969) transferred *Homalometron foliatum* Siddiqi et Cable, 1960 to *Apocreadium*, since this species has a lymphatic system. *A. viguerasi* has recently been transferred by Ahmad (1987) to *Neoapocreadium*.

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	Holotype	Sexually mature specimens			Sexually immature* specimens		
		Paratypes (Range)	X**	SD _{n-1} **	Range	X	SD _{n-1}
Body L. × Max. W.	6450×760	3500-7260×420-995	5235×709	1255×153	1662-3938×295-520	2793×388	724×79
Oral sucker L.× W.	315×295	325-371×185-363	303×275	41×45	148-260×128-250	209×188	33×37
Acetab. L.× W.	445×400	308-545×300-521	407×390	63×54	185-352×175-337	274×257	55.6×53
O.sucker/Acetab. Width ratio	1:1.4	1:1.1 - 1:1.5	1:1.4	1:0.1	1:1.1 - 1:1.7	1:1.3	1:0.7
O. sucker/Acetab. area ratio	1:1.9	1:1.4-1:2.2	1:1.9	1:0.2	1:1.4-1:2.3	1:1.8	1:0.3
D. O. Sucker - Acetab.	640	190-632	430	114	168-653	335	130
PT. Region L.	3900	2075-4800	3192	927	820-2125	1462	466
PT. Region (% of Body L)	60.5	55.3-69.6	60.4	3.8	47.5-58	51.8	5.4
D. Anterior E. - TestisA	1650	860-1760	1322	247	710-1350	975	193
D.Acetab. - TestisA	300	56-356	234	83	52-180	114	49
D. Anterior E. - O. Sucker	35	14-55.3	37	14	12-52	34	12
Prepharynx	54	22-48	37.5	9.8	14-38	28.5	8.6
Pharynx	185×140	120-221×110-182	158×134	29×19	70-136×70-112	103×89	20×16
Oesophagus	75	15.8-64	36	20	10-38	23	9
Sem. Ves. L. × W.	-	363-545×150-245	467×199	66×39	-	-	-
Sem. Rec. L. × W.	276×132	88-379×48-184	215×112	86×39	40-100×34-58	77×47	28×12
Ovary L. × W.	275×225	120-276×100-248	213×171	63×45	52-136×42-116	86×71	26×26
TestisA L. × W.	475×465	210-552×170-476	360×316	108×99	82-288×71-196	156×145	50×63
Testis P L. × W.	500×475	260-640×148-456	423×302	113×91	96-300×73-212	181×128	62×44
Mehlis. G. L. × W.	100×52	76-180×24-152	113×67	42×35	-	-	-
Eggs L. × W.	-	84-95×48-62	89×54	4.5×4.5	-	-	-

Table I. – Measurements of *Apocreadium galaicus* sp. n. (in µm)

*Specimens with no eggs forming were considered sexually immature.

**Means (X) and Standard deviations (SD) are for both the holotype and paratypes.

Dimensions : D= distance; L= length; W= width. Parts of body : Acetab = acetabulum; Anterior E = anterior end of body; Mehliis G = Mehliis' gland; O Sucker = oral sucker; PT Region = post-testicular region; Sem Rec = seminal receptacle; Sem Ves = seminal vesicle; Testis A = anterior testis; Testis P = posterior testis.

The genus *Apocreadium* as currently recognized thus contains 10 species. On Yamaguti's (1971) classification, *Apocreadium* forms part of the family Apocreadiidae, alongside *Choanodera*, *Neapocreadium* and *Neomegasolenia*.

Here we report a new species of this genus, infecting the thickback sole (*Microchirus variegatus*) from coastal waters of the northwest Iberian Peninsula.

with iron acetocarmine (Georgiev *et al.*, 1986) cleared in a 1:1 mixture of beech creosote and ethanol (96°) and mounted in Canada balsam. Additional specimens were mounted in Hoyer's fluid to allow reliable measurement of eggs, or stained (in transverse, longitudinal and sagittal sections) with 1% Mayer's haematoxylin-eosin or Wheatley's Trichrome stain to allow examination of the lymphatic system and other organs.

MATERIALS AND METHODS

We examined 150 individuals of *Microchirus variegatus* caught between October 1987 and September 1988, and a further 183 individuals caught between October 1993 and March 1994, in the Ría de Arousa (an estuarine bay in Galicia, northwest Spain). In all cases examination consisted of careful observation of the viscera under a stereomicroscope. All trematodes found were extracted, washed in physiological saline, relaxed and killed in Berland's fluid, fixed in Bouin's fluid (24 h minimum) and conserved in ethanol (70%) until preparation for microscopy. Specimens were stained

RESULTS

DESCRIPTION OF *APOCREADIUM GALAICUS* SP. N. (APOCREADIIDAE)

Measurements in table I.

Body elongate and slender, dorsoventrally flattened except in the acetabular region, where it is more cylindrical. Cuticle densely spiny between the anterior end of the body and the acetabulum, sparsely spiny between the acetabulum and the ovary or anterior testis; otherwise smooth.

Fig. 1. – *Apocreadium galaicus*. a) Paratype. b) Eggs.

Oral sucker ventral, slightly separated from the anterior end (Fig. 1a). Acetabulum located in the anterior quarter of the body, postbifurcal; sucker area ratio 1:1.4 – 1:2.3 (obtained as indicated by Mas-Coma *et al.*, 1984) (table I). Both suckers bear several papillae. Prepharynx short, pharynx longer than it is wide. Oesophagus short, bifurcating to form two caeca which extend almost to the posterior end of the body (Fig. 1a).

Lymphatic system comprising four vessels running anteroposteriorly, branching in the hindbody, difficult to see in adults, except in specimens in which the posterior ramifications are particularly well developed and in very young, sexually inactive specimens.

Testes tandem, postacetabular, oval to rectangular, located in the anterior half of the body (Fig. 1a), posterior testis generally slightly greater than anterior testis. Post-testicular region 55.3 – 69.6 % of body length in mature (i.e. egg-bearing) specimens and in immature specimens longer than 2.9 mm, and 47.5 – 49 % of body length in shorter specimens.

Cirrus and cirrus pouch absent. Seminal vesicle pyriform, dorsal to acetabulum, with prostatic wall surrounded by glandular cells, fusing with the distal part of the uterus anterior to the acetabulum, giving rise to a genital sinus. Genital pore located close to the anterior edge of the acetabulum.

Ovary globular, smaller than and anterior to testes, generally slightly to the left of the midline (Fig. 1a), though median in some immature specimens. Seminal receptacle globular, dorsal to ovary. Mehlis' gland located between ovary and anterior testis. Laurer's canal opening to surface dorsally, just posterior to the acetabulum. Uterus with a single ascending branch, folded several times (Fig. 1a), extending from about the level of the ovary to that of the acetabulum. Eggs large, operculate, with a protuberance or spine at the anopercular pole (Fig. 1b).

Vitellarium follicular, located throughout the hindbody, laterally and between caeca, extending anteriorly as far as the anterior testis or, in some cases, the posterior edge of the ovary, feeding into two ducts which run intracaecally through the post-ovarian region and fuse to form a vitelline reservoir, which is confluent with Mehlis' gland, between the levels of the ovary and the anterior testis (Fig. 1a).

Excretory vesicle I-shaped, terminating just behind the posterior testis; excretory pore at posterior end of body (Fig. 1a).

OTHER INFORMATION ON TYPE SPECIMENS

Type host : *Microchirus variegatus* (Donovan, 1808) (Soleidae, Osteichthyes)

Site : Intestine, occasionally stomach.

Fig. 2. – Prevalence and intensity of parasitization by *A. galaicus*.

Specimens examined :

- Holotype : Deposited in the Museo Nacional de Historia Natural, Madrid, n° 402/7.
- Paratypes : 24 specimens deposited in the Museo Nacional de Historia Natural, Madrid, n° 402/8-15.
- Other specimens deposited in the Laboratory of Parasitology, Faculty of Pharmacy, Santiago de Compostela, Spain.

Type location : Ria de Arosa, Galicia, northwest Spain. The specific name refers to the region, Galicia, where the host specimens were caught.

SEASONAL VARIATION IN PREVALENCE AND INTENSITY OF INFECTION

A. galaicus was detected in 33% of the 150 specimens of *M. variegatus* caught between October 1987 and September 1988, and in 34% of the 183 specimens caught between October 1993 and March 1994. Overall mean prevalence was 34%. Breaking down the data by months (Fig. 2), mean prevalence was highest in November, December and January and lowest (zero) in May and June; from July onwards prevalence begins to rise again, presumably reflecting reinfection by larvae. Mean intensity of infection peaked (23 parasites per host) in January.

DISCUSSION

Part of the examined material corresponds to that mentioned by Quinteiro *et al.* (1988) in the same host. We believe that the specimens mentioned by these authors in *S. lascaris* also belong to the same species, but this will be the object of further studies.

On the basis of published descriptions of the members of *Apocreadium*, the most similar species to *A. galaicus* are *A. foliatum*, *A. mexicanum*, *A. synagris* and *A. balistis*. *A. galaicus* can be distinguished from *A. foliatum* (Siddiqi et Cable, 1960) because in this species the oral sucker is larger than the acetabulum, while in *A. galaicus* it is smaller. It differs from *A. mexicanum* (Manter, 1937) by its larger eggs and by the simple structure of its pharynx; the anterior quarter of the pharynx of *A. mexicanum* is formed by a sphincter-like, circular muscle.

A. galaicus differs from *A. synagris* (Yamaguti, 1953) in the position of the oral sucker, which is completely ventral (slightly separated from the anterior end) in the first species, and subterminal in the second, and in the cuticle being spiny only anterior to the testes, not over the whole body. Finally, *A. galaicus* can be distinguish-

shed from *A. balistis* (Manter, 1947) by its longer post-testicular region, by its larger eggs and by its cuticle; that of *A. balistis* is densely spiny only from the anterior end of the body to the posterior edge of the pharynx, and sparsely spiny in the acetabular region.

The members of the genus *Apocreadium*, including *A. galaicus*, can be distinguished with the aid of the following key :

1. a) Oral sucker terminal, more or less conical **2**
 b) Oral sucker usually subterminal or ventral, spherical or subspherical **3**
2. a) Oral sucker much larger than acetabulum (sucker ratio 1:0.4 - 1:0.5); post-testicular region about 30 - 40 % of body length ***A. cryptum***
 b) Acetabulum and oral sucker of similar size (sucker ratio 1:1 - 1:1.2); post-testicular region very short (about 18 - 19 % of body length) ***A. vinodae***
3. a) Cuticle with neither scales nor spines ***A. longisinosum***
 b) Cuticle more or less covered with scales, each bearing 1 - 6 spines. Oral sucker smaller than acetabulum (sucker ratio 1:1.3 - 1:1.5); vitellarium extending to posterior edge of acetabulum; post-testicular region 26 - 35% of body length. ***A. manteri***
- c) Cuticle entirely or partially covered with simple spines **4**
4. a) With uroproct. ***A. uroproctoferum***
 b) Without uroproct **5**
5. a) Testes deeply lobulate ***A. caballeroi***
 b) Testes more or less irregular or oval in outline, not deeply lobulate **6**
6. a) Oral sucker subterminal; cuticle more densely spiny in forebody than in hindbody. ***A. synagris***
 b) Oral sucker ventral, slightly separated from the anterior end. Only forebody cuticle spiny **7**
7. a) Anterior quarter of pharynx formed by a sphincter-like, circular muscle ***A. mexicanum***
 b) Anterior quarter of pharynx not sphincter-like **8**
8. a) Post-testicular region near or over 50% of body length **9**
 b) Post-testicular region less than 50% of body length; cuticle densely spiny anterior to the posterior edge of the pharynx, sparsely spiny in the acetabular region ***A. balistis***
9. a) Oral sucker smaller than acetabulum (sucker area ratio 1:1.4-1:2.2; width ratio 1:1.1-1:1.5 in mature specimens) ***A. galaicus***
 b) Oral sucker larger than acetabulum (sucker width ratio 1:0.76). ***A. foliatum***

As regards distribution, all species of *Apocreadium* described to date are from American coastal waters (Pacific, Caribbean, Gulf of Mexico and Atlantic), except *A. synagris* (reported only from Indonesia; Yamaguti, 1953; Sogandares Bernal, 1959), *A. vinodae* (reported only from the Arabian Sea near Bombay; Ahmad, 1985) and *A. balistis* (reported from the Gulf of Mexico, the Caribbean and the Indian Ocean; Toman, 1992). *A. galaicus* is the first species to be reported from European waters.

Prevalence of infection of *M. variegatus* by *A. galaicus* peaks over the period November-January and reaches a minimum in May and June. Although the life cycles of the species of this genus have not been

determined yet, it is possible that this variation is related with the absence of the appropriate intermediate hosts in the diet of the thickback sole in the spring months, so when the adults die out, prevalence becomes zero. If we have in mind that all the other species of *Apocreadium* live in warm, tropical waters, it would seem that *A. galaicus* shows a surprisingly marked preference for cold water, but we must remember that the Atlantic coast of Spain and Portugal are on the course of the Gulf Stream, which makes the temperature of its waters much milder in winter than would be suspected according to its latitude. No information is available on prevalence of infection of other members of the genus.

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