

REDESCRIPTION OF THE ADULT STAGE OF *HYPODERAEUM CONOIDEUM* (BLOCH, 1782) (TREMATODA: ECHINOSTOMATIDAE) AND NEW RECORD IN SPAIN

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ABSTRACT: Malacological prospections carried out in the Albufera Natural Park of Valencia (Spain) allowed the collection of *Lymnaea peregra* (Müller, 1774) (Gastropoda: Lymnaeidae) naturally infected with larval echinostomes and shedding echinostome cercariae. Metacercariae were obtained experimentally from the renopericardial sac of laboratory raised snails: *L. peregra*, *L. truncatula* (Müller, 1774), *L. palustris* (Müller, 1774) (Gastropoda: Lymnaeidae), *Physa acuta* (Draparnaud, 1805) (Gastropoda: Physidae) and *Gyraulus chinensis* (Dunker, 1848) (Gastropoda: Planorbidae). Adult worms were obtained experimentally from chicks and ducks, but not from rats, mice and golden hamsters, and classified as *Hypoderaeum conoideum* (Bloch, 1782). The adult morphology of this species is redescribed and compared, as well as differentiated from other *Hypoderaeum* species reported to date. This is the second record of this adult echinostomatid in Spain, and the first record of the cercaria in this country.

KEY WORDS: *Hypoderaeum conoideum*, Echinostomatidae, Trematoda, Spain.

INTRODUCTION

Malacological prospections were carried out as part of a project to investigate the life cycle of the digenean trematodes found in the small mammals of the Albufera Natural Park of Valencia (Spain). *Lymnaea peregra* (Müller, 1774) (Gastropoda: Lymnaeidae) was found to be naturally infected with larval echinostomes of the genus *Hypoderaeum* Dietz, 1909. This material belongs to the group of *Hypoderaeum* with 47-54 collar spines, and was identified as *H. conoideum* (Bloch, 1782). This is a common and widespread parasite of fowls and, eventually, other vertebrates including humans (YOKOGAWA, HIRANASUTA & CHAROENLARP, 1965), and has been reported in many helminthological surveys across the world. Despite these many records, in Spain this species has only been cited by GALLEGO, FELIU & TORRES (1984) parasitizing *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae) in the Ebro Delta (Tarragona, Spain).

The adult morphology of *H. conoideum* has been studied by several authors (MATHIAS, 1925; REES, 1932; BEVERLEY-BURTON, 1961; DIAZ-DIAZ, 1976; GALLEGO, FELIU & TORRES, 1984; KHAN & AHMAD, 1991) though certain morphological details remain confused and the differentiation from others species of *Hypoderaeum* is difficult. The aim of the present paper is to complete the description of the adult of *H. conoideum*, with new data with particular reference to female genital system, and to establish comparisons with other representatives of the genus *Hypoderaeum*.

MATERIAL AND METHODS

Lymnaea peregra (Müller, 1774), *L. truncatula* (Müller, 1774) and *L. palustris* (Müller, 1774) (Gastropoda: Lymnaeidae), *Physa*

acuta (Draparnaud, 1805) (Gastropoda: Physidae) and *Gyraulus chinensis* (Dunker, 1848) (Gastropoda: Planorbidae) were collected in different rice fields within the Albufera Natural Park of Valencia. Only *L. peregra* was found to be naturally infected with larval stages and shedding cercariae of *H. conoideum*. The other species of snails, though listed by several authors as first intermediate host of *H. conoideum*, were not seen to shed cercariae of this echinostomatid.

Experimental studies were conducted for the specific determination of this material. Metacercariae were obtained from the renopericardial sac of laboratory raised snails (*L. peregra*, *L. truncatula*, *L. palustris*, *Physa acuta* and *Gyraulus chinensis*) that had been previously exposed to cercariae. In order to obtain the adult stage, the following laboratory reared animals were fed with 100 metacercariae each: a) 30 one-week old chicks (*Gallus gallus* Linnaeus, 1758); b) 10 one-week old ducks (*Anas platyrhynchos* Linnaeus, 1758); c) 15 two-weeks old albino mice (*Mus musculus* Linnaeus, 1758); d) 15 one-week old albino rats (*Rattus norvegicus*); e) 5 one-week old golden hamsters (*Mesocricetus auratus* Waterhouse, 1839).

Sixteen chicks and 7 ducks presented the presence of eggs in faeces after 11-14 days post-infection and the worms lived between 3 and 4 weeks. All three rodents were refractory to infection.

The experimental definitive hosts were killed by dislocation after 20 days post-infection, and the adult specimens collected were directly flattened in Bouin's solution between slide and coverslip under slight pressure, stored in alcohol 70%, stained with Grenacher's boric carmine and mounted in Canada balsam. Adults were individually examined using light microscope and Nomarski interference optics. Figures were made with the aid of a camera lucida.

RESULTS

Hypoderaeum conoideum (Bloch, 1782)

Definitive host: natural: unknown; experimental: *G. gallus* and *A. platyrhynchos*.

First intermediate host: *L. peregra*.

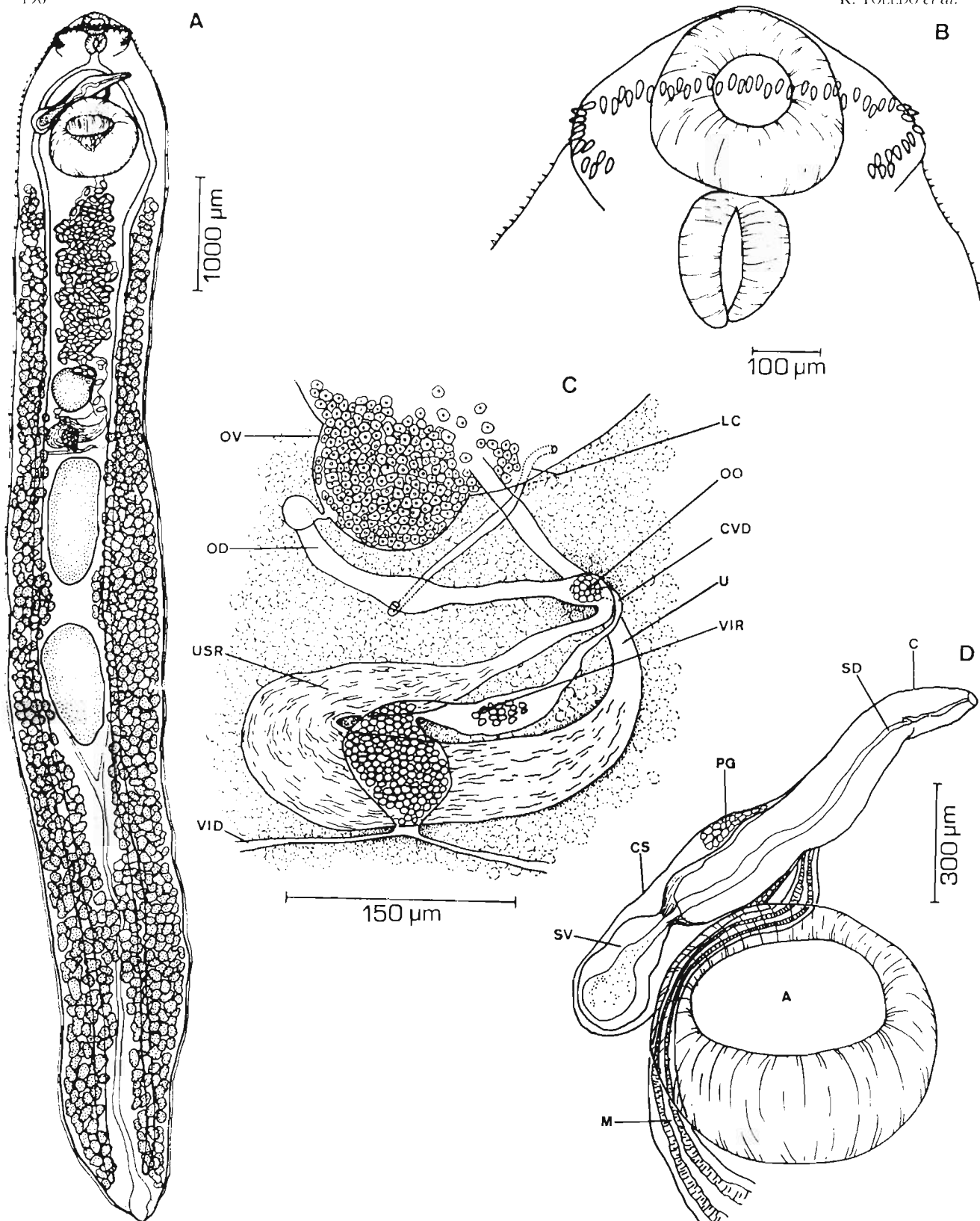


Fig. 1. *Hypoderacum conoideum* obtained from an experimentally infected chick: A) 21-day-old adult in ventral view; B) spined collar arrangement; C) terminal male organs and final part of uterus in ventral view; D) ovarian complex in ventral view. A = acetabulum; C = cirrus; CS = cirrus sac; CVD = common vitelline duct; LC = Laurer's canal; M = metraterm; OD = oviduct; OO = ootype; OV = ovary; PG = prostatic glands; SD = sperm duct; SV = seminal vesicle; U = uterus; USR = uterine seminal receptacle; VID = vitelline ducts; VIR = vitelline reservoir. Scale bars: A: 1000 µm; B: 100 µm; C: 150 µm; D: 300 µm.

Second intermediate host: natural: unknown; experimental: *L. peregra*, *L. truncatula*, *L. palustris*, *P. acuta* and *G. chinensis*.

Site: posterior half of the small intestine.

Locality: Albufera Natural Park, Valencia, Spain.

Material studied: 100 specimens (60 from *G. gallus* and 40 from *A. platyrhynchos*). Voucher specimens are deposited in the Department of Parasitology, Faculty of Pharmacy, University of Valencia, Spain.

Morphology

Body elongated and attaining a maximum width at the approximate level of the junction between the first and second thirds of the body. The anterior part of the body covered with minute spines extending to half-point of acetabulum on ventral side, and to half-point to the pharynx on the dorsal side (Fig. 1A). The collar is poorly developed with 47-54 spines (mean: 52). Arrangement of spines: four corner spines in each ventral lappet (2 oral and 2 aboral) and 39-45 spines in two rows alternating oral and aboral spines (Fig. 1B).

Oral sucker subterminal ventral. Acetabulum in the first sixth of the body. Sucker diameter ratio 1:4. Prepharynx very short, with a muscular pharynx and oesophagus bifurcating in front of the ventral sucker; the two intestinal caeca extend to near the posterior end of the body. The

testes are arranged in tandem, and are smooth or slightly lobulated, intercaecal in the posterior half of the body and often contiguous. Cirrus sac situated dextrally to acetabulum, without extending beyond its posterior margin, and containing a bipartite or saccular seminal vesicle, *pars prostatica* and a conspicuous coiled cirrus (Fig. 1D). Genital pore immediately anterior to the acetabulum and opening into a genital atrium. Each testis is connected to the cirrus sac by single sperm duct. Ovary median and spherical or slightly ovoid. Seminal receptacle absent. Laurer's canal arises from the oviduct and opens to the dorsal surface. Oviduct (with oviscapts originating after emerging from the ovary) widens into ootype, which is surrounded by the Mehli's complex, and uterus. Proximal part of uterus forms a uterine seminal receptacle that is often filled with sperm (Fig. 1C). The uterus runs intercaecally between anterior testis and acetabulum, containing many eggs, and finally forming a muscular metraterm connecting with genital atrium (Fig. 1C). Vitellarium follicular in lateral fields overlapping caeca and extending from about just behind the posterior end of acetabulum to near the posterior extremity. Vitelline ducts connecting above the anterior testis and forming an elongated vitelline reservoir, which connects with oviduct just before the ootype, through a short common vitelline duct (Fig. 1C). The excretory bladder opens at the posterior end into an excretory pore terminal and medial.

	Specimens collected from <i>Gallus gallus</i>	Specimens collected from <i>Anas platyrhynchos</i>
Body length	7618-11427 (9498±891)	7332-9462 (8383±704)
Body width	1057-1482 (1315±118)	1161-1604 (1308±114)
Collar across	379-689 (527±74)	437-540 (486±33)
Corner spines	26-34 (31±3)	26-34 (29±3)
Oral spines	17-34 (26±5)	18-33 (26±5)
Aboral spines	20-31 (23±4)	20-31 (24±4)
Oral sucker length	242-296 (266±17)	211-257 (239±14)
Oral sucker width	168-242 (208±23)	171-239 (199±19)
Ventral sucker length	804-954 (870±42)	758-974 (869±60)
Ventral sucker width	701-942 (819±60)	678-885 (790±62)
Sucker surface ratio	0.069-0.071	0.072-0.079
Sucker diameter ratio	1:4	1:4
Sucker distance	437-827 (616±116)	414-678 (521±74)
Pharynx length	162-231 (191±20)	148-194 (179±12)
Pharynx width	151-202 (179±11)	122-201 (163±16)
Anterior testis length	620-1183 (1012±138)	610-1057 (863±179)
Anterior testis width	322-643 (468±97)	207-586 (462±97)
Posterior testis length	701-1183 (1022±119)	379-1996 (952±354)
Posterior testis width	287-655 (421±90)	117-540 (365±118)
Cirrus sac	735-1206 (970±133)	686-1110 (894±109)
Ovary length	310-494 (410±55)	172-414 (342±42)
Ovary width	241-414 (356±53)	115-345 (393±58)
Uterine eggs length	69-115 (93±10)	37-94 (78±15)
Uterine eggs width	46-80 (60±11)	25-63 (51±11)
Anterior end - Ventral sucker distance	655-1046 (864±109)	589-926 (725±98)
Ventral sucker - Anterior testis distance	1781-4067 (2497±568)	1882-3927 (2309±423)

Table 1.— Measures (in μm followed by averages \pm SD in parentheses) of 40 experimentally obtained 20-day-old specimens (20 from chicks and 20 from ducks) of *Hypoderaeum conoideum*.

Measures

Table 1 shows the measures obtained (μm , followed by averages \pm SD in parentheses) based on forty 20-day old specimens (20 from chicks and 20 from ducks).

DISCUSSION

The morphological characteristics of this species justifies its inclusion in the genus *Hypoderaeum*, within the family Echinostomatidae Rudolphi, 1809. This genus includes species that are differentiated by adult dimensions, the relative position and dimensions of organs and, in particular, by the number and arrangement of collar spines and the sucker diameter ratio. The systematic position of these species has been revised and discussed by several authors, such as BASHKIROVA (1941, 1947), MENDHEIM (1943), SKRJABIN & BASHKIROVA (1956), YAMAGUTI (1971), BYCHOVSKAYA-PAVLOVSKAYA (1978) and ISKOVA (1985). According to these revisions, only 6 species exhibit a similar morphology and have collar spines numbering in the range of the fluke described in this paper: *H. conoideum* (Bloch, 1782); *H. gnedini* Bashkirova, 1941; *H. skrjabini* Oshmarin, 1946; *H. microspina* (Singh, 1954); *H. essexensis* (Khan, 1960); and *H. dingeri* Lie, 1964.

H. conoideum was first described by BLOCH (1782) as *Cucullamus conoides*. DIETZ (1909) established the genus *Hypoderaeum* and included this trematode as *H. conoideum*. Since then, this particular fluke has been reported in different countries of Europe (VEYERS, 1923; MATHIAS, 1924; DUBOIS, 1929; REES, 1932; WESENBERG-LUND, 1934; BAYLIS, 1939; OWEN, 1951; VOJTECHOVSKA-MAYEROVA, 1952; BYCHOVSKAYA-PAVLOVSKAYA, 1953; KASIMOV, 1953; SOLIMAN, 1955; BEZUBIK, 1956; DAWES, 1956; RYZHIKOV, 1956; WIKGREN, 1956; RYSAVY, 1957; ALISAUSKAITE, 1958; GINECINSKAYA, 1959; KOPRIVA, 1959; BEVERLEY-BURTON, 1961; ZAJICEK & PAV, 1961; GINECINSKAYA & DOBROVOI'SKIJ, 1964; MEYER, 1964; WILLIAMS, 1966; SMIRNOVA & IBRASHEVA, 1967; SITKO, 1968; RICHARD, 1971; NAZAROVA-SARODYNOVA, 1974; DIAZ-DIAZ, 1976; BUSTA, 1980; GALLEGO, FELIU & TORRES, 1984; SKOVRONSKY, 1984; GRABDA-KAZUBSKA & KISELIENE, 1990; ADAM & LEWIS, 1993; HAAS *et al.*, 1995). Asia (MORISHITA, 1929; YAMAGUTI, 1934; HSÜ & CHOW, 1938; YAMAGUTI & MITUNAGA, 1943; YOKOGAWA, HARINASUTA & CHAROENLARP, 1964; KUMARAN & PETER, 1973; KHAN & AHMAD, 1991) and America (STUNCKARD & DINIHUE, 1935; GROWER, 1937; CANNON, 1938; CERECERO, 1944; CRICHTON & WELCH, 1972; TURNER & THRELFALL, 1975; CANARIS, MENA & BRISTOL, 1981; FARIAS & CANARIS, 1986). However, many of these reports are possibly inadequate, as in numerous cases no exhaustive studies have been made of the corresponding morphology, biology and ecology.

Observations of the life cycle of *H. conoideum* were

first presented by RAILLIET (1893), who found larval stages of this trematode in several genera of snails. VEYERS (1923) fed ducklings with the liver of *Lymnaea stagnalis* (Linnaeus, 1774) infected with echinosotome metacercariae, though the adults recovered were not described by this author. The life cycle of *H. conoideum* was completed by MATHIAS (1924, 1925). This author described adult flukes obtained from the posterior half of the small intestine of naturally infected ducks. He characterized this species as having 47-53 spines in a double row, and a sucker diameter ratio of 1:4. No description of the female genital system was provided in the text.

REES (1932) redescribed *H. conoideum* and its life cycle. This species was collected from *L. peregra* naturally infected with metacercariae in South Wales, United Kingdom. The author mentioned the presence of a «receptaculus seminis» often filled with sperm, arising from the oviduct and opening into the ootype, though the uterine seminal receptacle was not noted. Moreover she described a head collar with 43-45 spines.

BASHKIROVA (1941) described *H. gnedini* collected from *Anas* spp. in the old USSR. According to this author, this species possesses a larger ratio between the diameters of two sucker than *H. conoideum*, and its body dimensions are smaller.

OSHMARIN (1946 in SKRJABIN & BASHKIROVA, 1956) described *H. skrjabini* from *Aythya ferina* (Linnaeus, 1758) in Russia and differentiated it from *H. conoideum* on the basis of the sucker diameter ratio (1:5), and because the vitellaria of *H. skrjabini* meets behind the testes.

SINGH (1954) described *Echinostoma microspina* from *Anas acuta* Linnaeus, 1758, in India. SKRJABIN & BASHKIROVA (1956) transferred this species to the genus *Hypoderaeum*. According to SINGH (1954), this species characteristically presents 47 collar spines in a single row.

BEVERLEY-BURTON (1961) collected *H. conoideum* from several wildfowl species in the United Kingdom. This author gave a brief description of this species and suggested that *H. gnedini* and *H. skrjabini* may be a synonym of *H. conoideum*.

KHAN (1960, 1962) completed the life cycle of *H. essexensis*. The adult described was recovered from the first half of the intestine of several species of experimentally infected fowls. He reported 49 collar spines in a double row, with a group of 5 corner spines and a ratio between the diameter of its oral and ventral sucker of 1:3.

LIE (1964) in Malaysia collected *L. rubiginosa* (Michelin, 1831) naturally infected with echinosotome larval stages and completed the life cycle of what he described as *H. dingeri*. He obtained adult stages of this fluke from ducklings and goslings experimentally infected and characterised this parasite by possessing 49-54 spines arranged in a double row, with the exception of the dorsal spines, which were arranged in a single row. The sucker diameter ratio given was 1:3.3, and an uterine seminal receptacle is reported for the first time in this genus.

In his Doctoral Thesis, DIAZ-DIAZ (1976) redescribed

the life cycle of *H. conoideum*. The adult specimens were not described, though the author mentioned that his materials agreed with those described by REES (1932), with the exception of the previously commented «*receptaculus seminis*». In this sense, DIAZ-DIAZ (1976) reported the presence of a true uterine seminal receptacle.

GALLEGO, FELIU & TORRES (1984) provided the first record of *H. conoideum* in Spain. These authors found one specimen of *R. norvegicus* (rate of infection: 0.6%) naturally infected with one slightly gravid specimen of *H. conoideum* in the Ebro Delta. The description given is in agreement with the materials of MATHIAS (1924, 1925).

KHAN & AHMAD (1991) have briefly described the morphology of the adult of *H. conoideum* collected from *A. platyrhynchos* in India. The description is very poor, however, and shows several inconsistencies with respect to other published reports.

The *Hypoderaeum* species reported in the present paper appears to be closely related to both *H. microspina* and *H. conoideum*, and differs from *H. gnedini*, *H. skrjabini*, *H. essexensis* and *H. dingeri*.

H. gnedini differs from our specimens in the sucker diameter ratio (1:5 vs 1:4 in our specimens), body length (5400-7520 vs 7332-11427) and in collar diameter (320-370 vs 379-689). Comparison of our specimens with the description given by OSHMARIN (1946) for *H. skrjabini* shows that the morphology of this parasite is very similar to that of materials described in the present paper, and differs mostly in the sucker diameter ratio (1:5 vs 1:4 in our specimens) and collar diameter (220 vs 379-689). Our materials in turn differ from *H. essexensis* in the sucker diameter ratio (1:3 vs 1:4 in our series), body length (4050-6520 vs 7332-11427), size of the cirrus sac (352-430 vs 753-1206) and location within the definitive host (first vs last half of the small intestine). Finally, *H. dingeri* differs from our material in the sucker diameter ratio (1:3.3 vs 1:4 in our specimens) and in the arrangement of the collar spines (dorsal collar spines in a single row vs all collar spines in a double row).

Comparison of the present species with *H. microspina* shows that both are closely related. According to SINGH (1954), *H. microspina* possesses 47 collar spines arranged in a single row. However, examination of the figures provided by this author does not clearly demonstrate this. Moreover, on studying the description by SINGH (1954), including the sucker diameter ratio reflected in the figures, no significant differences are seen with respect to *H. conoideum*. It is thus suggested that the validity of *H. microspina* is questionable and that it closely resembles *H. conoideum*. Indeed an examination of the type specimens of this form would possibly lead to synonymy with *H. conoideum*.

The materials presented in the present work are in good agreement with the most relevant descriptions of *H. conoideum*. Nevertheless, the study of those papers that describe this species reveals several inconsistencies in relation to the number of collar spines, body dimen-

sions, sucker ratio and the structure of the female genital system.

DIETZ (1909) proposed a range of 47-53 collar spines for *H. conoideum*, a range that has been confirmed by most authors, with the exception of REES (1932) and BEVERLEY-BURTON (1961). These authors respectively reported 43-45 and 47-49 spines, and considered the range of collar spines for *H. conoideum* to be 43-53. In the present paper the appropriate range was found to be 47-54 spines, though the difficulties entailed in visualizing and counting the spines, together with intraspecific variation and the existence of several species that exhibit the same number range prevent us from using this feature as a diagnostic characteristic in the genus *Hypoderaeum*.

It is well established that the sucker diameter ratio in *H. conoideum* is 1:4. Moreover, this feature is regarded by different authors as the most important characteristic in the specific diagnoses of members belonging to the genus *Hypoderaeum*. We suggest that those descriptions in which this ratio is either missing or different should be revised. In this context, we advocate that the ascriptions of BEVERLEY-BURTON (1961) and KHAN & AHMAD (1991) should be reconsidered. BEVERLEY-BURTON (1961) provided neither the sucker diameter ratio nor any figure of *H. conoideum* in her work. In order to compare our specimens with the material described by this latter author, we calculated the sucker surface ratio (a ratio not given in the description but calculated posteriorly from data provided by this author); the results show that both material sources differ in terms of this ratio (0,094-0,096 vs 0,071-0,080 in our study). In turn, KHAN & AHMAD (1991) reported a different sucker diameter ratio to *H. conoideum* (1:5,45 vs 1:4 in our material). Moreover, these authors do not indicate the number and arrangement of the collar spines, and report the absence of both prepharynx and pharynx. All these aspects point to the need of a revision of these descriptions in order to establish the definitive specific diagnoses.

The female genital system has been only poorly described. REES (1932) reported the presence of a seminal receptacle, yet no organ such as a uterine seminal receptacle is mentioned in the text. The study of our materials shows that none of our specimens present a seminal receptacle. In our specimens the ootype enlarges to form a wide, sperm-filled uterine seminal receptacle that is actually part of the uterus; a true seminal receptacle is absent, however. This observation has been confirmed by DIAZ-DIAZ (1976), who described a similar anatomy of the female genital complex. After considering the figures provided by REES (1932), we suggest that the apparent error of this author may be attributed to the misinterpretation as a true seminal vesicle of a dilation of the uterus where spermatozoa are bound into a ball. In this sense, the uterine seminal receptacle, when partly filled with sperm, may resemble and be mistaken for a true seminal receptacle.

In conclusion, we identify the echinostomatid found in Valencia as *H. conoideum*. This is the second record of

this adult fluke in Spain, and the first record of the cercaria in this country. We consider that the adult of this species may be differentiated from other *Hypoderaeum* species exhibiting a similar number of collar spines on the basis of several characteristics such as the body dimensions and sucker ratio.

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