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First Report of a Species of *Folliculovarium* Gu & Shen, 1983 (Bucephalidae: Prosorhynchinae) from European Marine Waters, with the Description of *F. mediterraneum* n. sp.

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Abstract

Folliculovarium mediterraneum n. sp. is described and figured in detail from the intestine of the Mediterranean moray eel *Muraena helena* (Muraenidae) in the Scandola Nature Reserve, off Corsica in the western Mediterranean. This new species closely resembles *F. gymnothoracis* Gu & Shen, 1983 and *F. xishaense* Gu & Shen, 1983 from marine fishes off China, but it may differ from both in not having specialized circum-rynchal spines. It differs from the former by having slightly larger eggs and a longer esophagus, and from *F. xishaense* by having tegumental spines, a longer esophagus, and a nonrelated host (an eel rather than a serranid). The status of *Folliculovarium*, which was recently treated as a genus *inquirendum* by Overstreet & Curran (2002), is clarified by its multilobed rather than follicular ovary and other features, and remains in the Prosorhynchinae (Bucephalidae).

Introduction

During the course of our research in the Scandola Nature Reserve, off Corsica in the western Mediterranean, we found in the digestive tract of the Mediterranean moray eel *Muraena helena* L. some specimens of an unknown bucephalid digenean species belonging to

the subfamily Prosorhynchinae Nicoll, 1914. In contrast to virtually all other prosorhynchine species, which have an entire or a weakly lobed ovary, this species is characterized by a distinctly multilobed ovary. A similar and unusual form of the ovary, depicted as follicular, has already been described by Gu & Shen (1983) for two species from marine fishes off China. For those, the authors erected the genus *Folliculovarium* Gu & Shen, 1983. Because of the atypical and uncertain form of the ovary, *Folliculovarium* was treated by Overstreet & Curran (2002) as a *genus inquirendum* in their revision of the Bucephaloidea Poche, 1907. This study of Mediterranean specimens demonstrates that the ovary, at least in the present species, is not really follicular but multilobate. Despite such an apparently distinct difference, the new specimens agree well with the diagnosis of *Folliculovarium*. In this paper, we describe the Mediterranean species and discuss the status of the genus.

Materials and methods

Mediterranean moray eels were collected alive in the Scandola Nature Reserve, off Corsica, and dissected immediately after being killed. The helminths collected were studied both live and as permanent preparations. Specimens were fixed in Bouin's fluid under slight coverslip pressure and stored in 70% alcohol. They were stained in acetic carmine, dehydrated in an ethanol series, cleared in eugenol, and mounted in Canada balsam. Studies were conducted using differential interference microscopy, and illustrations were made with the aid of a drawing tube. Measurements are given as the range in micrometers, with the mean in parentheses. Type and paratype specimens have been deposited in the Natural History Museum, London.

Family Bucephalidae Poche, 1927

Subfamily Prosorhynchinae Nicoll, 1914

***Folliculovarium mediterraneum* n. sp.**

Type-host: *Muraena helena* Linnaeus (Anguilliformes, Muraenidae).

Type-locality: Scandola Nature Reserve, off Corsica, France (42°23'N; 8°33'E).

Site: Intestine.

Level of infection: Prevalence: 2 of 10 fish (20%) in August, 2002; abundance: 0.8; mean intensity: 4.0; range: 2–6 specimens per fish. Prevalence 2 of 6 fish (33.3%) in June 2003; abundance 1.3; mean intensity 4.0; 3–5 specimens per fish.

Material studied: 2 large specimens with many eggs, 2 medium-sized, 2 relatively small with few eggs and 1 immature.

Type-material: Holotype BMNH Reg. No. 2003.2.6.1; paratypes: BMNH Reg. Nos 2003.2.6.2–3.

Etymology: The adjectival name *mediterraneum* refers to the Mediterranean Sea as the presumed range of the species.

Description (Figures 1–8)

General morphology (Figures 1–2, 5–8; see Table 1 for measurements). Body fusiform, more pointed at posterior than anterior extremity.

Tegument. Entire body surface densely covered with spines. Spines in anterior body of mature worms 12–16 long, acuminate, slightly curved, flattened, wider (3–6) at base in lateral view; in posterior body, spines slightly shorter, less curved and with narrower base in lateral view.

Rhynchus. In form of shallow muscular glandular cone, capped with muscular disc which is usually flat, without circum-rhynchal rows of special spines and devoid of lobes (Figure 5). Longitudinal muscles well developed. “Gland-cells” and associated ducts extending posteriorly past rhynchus into parenchyma up to about 1 rhynchal length.

Digestive system. Mouth on ventral surface, in posterior half of body. Prepharynx relatively short, thin-walled. Pharynx spherical, 58–65% of body-length from anterior extremity. Esophagus distinct, about as long as pharynx, surrounded by esophageal gland-cells (Figures 3–4). Intestinal caecum simple, sac-like, directed anteriorly from pharynx, with relatively tall columnar gastrodermal cells.

Oral gland. Oral gland large, with numerous relatively large swollen ducts containing products, distinct from esophageal gland, widespread in ventral parenchyma immediately anterior to mouth, distinct in immature or small adult specimens (Figure 6), obscured by eggs in large specimens; ducts lead into short prepharyngeal region between pharynx and mouth.

Male reproductive system. Testes 2, entire, spherical, post-equatorial, not contiguous, on either side or slightly posterior to level of pharynx, symmetrical (3 specimens) or slightly oblique (left testis slightly anterior to right in 2 specimens; vice versa in 2 specimens); right testis in ventral body field; left testis more dorsal. Vasa efferentia fuse at base of cirrus-sac. Cirrus-sac conspicuous, thick-walled, sinistral. Seminal vesicle elongate, 2 or more times as long as wide, obliquely oriented relative to axis of cirrus-sac, with posterior (distal) extremity opening into male duct (Figure 7) which immediately reflexes. Male duct with proximal portion a seminal duct which passes anteriorly, connected to middle portion by short narrow duct which reflexes; middle portion of male duct a well-developed broad pars prostatica, rectilinear or slightly sinuous, lined by thick layer of anuclear structures; distal ejaculatory duct short, thin-walled, contractile, opening directly into genital atrium at base of genital lobe. Genital atrium thin-walled, wide, enclosing a mitten-shaped genital lobe (Figure 4), surrounded by abundant gland-cells. Genital pore clearly ventro-subterminal.

Figure 1. *Folliculovarium mediterraneum* n. sp. from the intestine of *Muraena helena*, Scandola Nature Reserve, off Corsica, France, western Mediterranean (same data for all figures). Holotype, slightly flattened, ventral view (only a small proportion of eggs figured).

Table 1. *Folliculovarium mediterraneum* n. sp. dimensions (in micrometers) of different sized individuals

No.	2	2	2	1
Size of specimen	Large	Medium with moderate number of eggs	Small with few eggs	Small and immature
Figures	Figures 1–2	Figures 3–4		
Body length	2,860; 2,670	1,722; 1,327	1,596; 1,343	1,248
Body-width	1,185; 1,011	506; 561	395; 295	411
Rhynchus	240 × 266; 170 × 243	131 × 176; 160 × 166	145 × 144; 141 × 134	134 × 138
Pharynx	125 × 136; 118 × 112	96 × 109; 88 × 96	80 × 99; 82 × 96	80 × 96
Caecum	218 × 218; 368 × 158	192 × 154; 240 × 150	195 × 170; 205 × 112	180 × 160
Esophagus	224; 208	125; 112	131; 106	138
Right testis	229 × 229; 308 × 235	173 × 128; 191 × 124	166 × 118; 131 × 102	150 × 96
Left testis	254 × 146; 337 × 292	150 × 138; 159 × 159	157 × 122; 134 × 102	144 × 112
Ovary	286 × 368; 400 × 267	234 × 192; 235 × 191	230 × 182; 160 × 166	192 × 156
Diameter of ovarian lobes	125; 178	87; 90	90; 68	72
Cirrus-sac	486 × 173; 448 × 166	288 × 99; 259 × 117	205 × 92; 229 × 81	—
Thickness of cirrus-sac wall	13; 10	7; 5	5; 3	—
Seminal vesicle	279 × 89; 235 × 108	99 × 42; —	90 × 38; —	—
Atrium genital length	128; 256	154; 138	118; 92	—
Uncollapsed distal eggs	24–25 × 16–17 (24.5 × 16.4)	24–25 × 15–17 (24.4 × 16.0)	25–26 × 15–16 (—)	—
From anterior body extremity to:				
– anterior limit of uterus	585; 537	635; 365	743; 699	—
– anterior limit of vitellarium	806; 790	603; 489	552; 464	486
– anterior limit of caecum	1,296; 1,185	768; 559	698; 572	533
– mouth	1,738; 1,659	1,066; 914	1,041; 867	857
– anterior limit of testes	1,810; 1,548	1,080; 679	1,009; 946	800
From posterior body extremity to:				
– posterior limit of uterus	174; 158	127; 159	508; 330	—
– posterior limit of vitellarium	1,230; 1,106	591; 476	622; 565	470
– mouth	1,122; 869	648; 451	578; 499	413
– posterior limit of testes	838; 901	527; 425	387; 267	304
– genital pore	216; 241	77; 93	77; 79	67

Figure 2. *Folliculovarium mediterraneum* n. sp. Large adult, dorsal view.

Figure 3. *Folliculovarium mediterraneum* n. sp. Small adult, ventral view.

Figure 4. *Folliculovarium mediterraneum* n. sp. Small adult, dorsal view.

Figure 5. *Folliculovarium mediterraneum* n. sp. Rhynchus and associated gland-cells of small specimen with few eggs, ventral view.

Figure 6. *Folliculovarium mediterraneum* n. sp. Oral gland of immature specimen, ventral view.

Figure 7. *Folliculovarium mediterraneum* n. sp. Terminal genitalia of holotype, ventral view. Note that seminal duct is connected to the pars prostatica by a short, narrow duct which is not illustrated in this figure.

Figure 8. *Folliculovarium mediterraneum* n. sp. Ovary and female complex of small adult specimen, dorsal view.

Female reproductive system. Ovary dextral, at level of right testis, more or less anterolateral and dorsal to right testis, multilobate (but may appear follicular in whole-mounts); lobes distinct, fused centrally, numbering 7 (1 specimen), 8 (3 specimens) or 9 (3 specimens), not isolated as follicles. Mehlis' gland located posterior to ovary (Figures 1–4, 8). Laurer's canal oriented posteriorly (Figure 8), passes ventrally to Mehlis' gland before opening dorsally on dextral margin of body, completely surrounded by gland-cells. Uterine seminal receptacle observed in holotype (Figure 1). Uterus winding throughout full width of body in large specimens between level just anterior to vitelline field and level of genital pore. Distal portion of uterus not differentiated as metraterm, opening into genital atrium near opening of ejaculatory duct. Eggs numerous, relatively thick-shelled, operculate. Vitellarium distinctly anterior to gonads in large specimens, but contiguous with or overlapping gonads in small adults. Vitelline follicles 24–29 in number, in middle of body or just anterior, mostly distributed in ventral field; lateral fields unite anteromedially and give appearance of horseshoe-shaped arch around caecum. Lateral vitelline ducts posteriorly directed, uniting as common duct just anterior to Mehlis' gland. Vitelline reservoir inconspicuous.

Excretory system. Excretory vesicle I-shaped, arcuate, situated to right side of cirrus-sac, clearly distinct in small specimens but can be obscured by eggs in large ones, with anterior

extremity reaching level of mouth in small specimens but only halfway along cirrus-sac in large ones. Excretory pore terminal.

Discussion

Among the Bucephalidae Poche, 1907, all species have either an entire or an indistinctly lobed ovary, except for two, which are said to have an “ovary lobed as scattered follicles” (Gu & Shen, 1983). These two marine species, *F. xishaense* Gu & Shen, 1983 from *Epinephelus fasciatus* (Forsskål) (Perciformes: Serranidae), the type-species,¹ and *F. gymnothoracis* Gu & Shen, 1983 from *Gymnothorax undulatus* (Lacepède) (Anguilliformes: Muraenidae), were reported from off the coast of Guangdong Province, China, and were allocated their own genus, *Folliculovarium*, primarily on the basis of the follicular ovary. The unusual and peculiar nature of the ovary (considered as “suspect” by Overstreet & Curran, 2002, p. 87) has been questioned by the latter authors, who argued that a possible misinterpretation was possible between ovarian follicles and either a testis, Mehlis’ gland or both. Figures 1–4 and 8 show clearly that the ovarian lobes in *F. mediterraneum* n. sp. cannot be confused with either but that they are a single multilobed organ rather than separated follicles. We assume the same structure occurs in the Chinese species.

Overstreet & Curran (2002) considered *Folliculovarium* a *genus inquirendum* because of the uncertainty of the shape and relative position of the ovary. Nevertheless, they considered it an acceptable genus and considered the ovary, regardless of its features and even if follicular, to represent no more than a generic feature. Consequently, they considered the Folliculovarinae Wang, 1995 [*sic*: it should be Folliculovariinae] a junior synonym of the Prosoerhynchinae. We agree with that decision here and leave the genus in the Prosoerhynchinae.

Based on our specimens of *F. mediterraneum*, we recognize a difference in the relative size and location of some organs when comparing small specimens having relatively few eggs with larger specimens packed densely with many eggs. Consequently, the overall range in size and arrangement of the vitellarium, testes, and excretory vesicle all exhibit a wide range. Nevertheless, all three members of *Folliculovarium* now recognized appear quite similar and clearly belong in a single genus.

Of the two other species, *F. gymnothoracis* was differentiated from *F. xishaense* by Gu & Shen (1983) by having a larger body, two rather than one circular rows of special rhynchal spines, the vitelline follicles in a single arch rather than in two separate groups, and non-related hosts. We are not certain about the rhynchal spines in these two species from off Guangdong Province, China, because the distal portion of glandular ducts running through the rhynchus of many bucephalids appear like spines. Even if special circum-rhynchal spines are present, we consider *F. mediterraneum* without them to be included in the same genus. The shape and extent of the vitellarium, as indicated above, could result from the size of the specimens. Based on the text descriptions, *F. gymnothoracis* has a sinistral ovary (even though the English version of the generic diagnosis suggests it is dextral) and a dextral cirrus sac. However, if figure 8 of Gu & Shen (1983) actually represents a dorsal aspect, as suggested by the uterus being illustrated on top of the mouth, then the position of the ovary and cirrus-sac is similar in all three species.

F. mediterraneum, a small species like *F. xishaense*, differs from it having a body covered with tegumental spines. It differs from *F. gymnothoracis*, also from a moray eel (*G. undulatus*) like *F. mediterraneum* rather than a serranid (*E. fasciatus*) like *F. xishaense*, by having slightly larger eggs as in *F. xishaense* (24–26 rather than 21–24 μm long). It apparently has a longer esophagus, 0.11–0.22 mm for specimens of 1.3–2.9 mm in length, than either *F. xishaense* (0.05–0.09 for specimens 2.1–2.6 mm long) or *F. gymnothoracis* (0.09–0.10 for specimens 3.7–4.8 mm long). A reexamination of the described specimens of the two Chinese species as well as additional material may show either similar or no distinctive esophageal and oral glands, as described above for *F. mediterraneum*, as well as the status of the rhynchal spines, ovary, vitellarium, cirrus-sac, and presence of an ejaculatory duct (neither described nor illustrated for either species). In any event, the description of *F. mediterraneum* clarifies much about the genus.

The presence of a species of digenean, whose congeners occur in Chinese marine waters, in a Mediterranean fish is unusual. It is also surprising that, despite the fact that *Muraena helena* is an eel and occurs throughout the Mediterranean and neighboring Atlantic waters, as far as we are aware, no bucephalid appears to have previously been reported from this host.

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Note

1. Overstreet & Curran (2002) correctly stated that *F. xishaense* was the type-species on page 90, but they also presented the type-species as *F. gymnothoracis* on page 108 and illustrated that species mislabeled as *F. xishaense* (Figure 15.33) on page 109.

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