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FIRST RECORD OF *MARITREMA PYRENAICA* (DIGENEA: MICROPHALLIDAE) IN SPAIN (WESTERN PYRENEES) IN ITS INTERMEDIATE HOSTS

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The microphallid trematode *Maritrema pyrenaica* Deblock et Combes, 1965 has been recorded in insectivores *Galemys pyrenaicus* (Geoffroy, 1811) and *Neomys fodiens* Pennant, 1771 (Deblock S., Combes C. 1965: Bull. Soc. Zool. France 1: 101-117; Combes C., Jourdane J., Théron A. 1976: Vie et Milieu 26: 133-142) from the French Pyrenees. In the study on the life cycle of *M. pyrenaica,* Jourdane J. (1979: Ann. Parasitol. Hum. Comp. 4: 449-456) reported the hydrobiid snail *Bythinella reyniesii* (Dupuy, 1851) (Prosobranchia) and the gammarid *Gammarus pulex* (Linnaeus, 1758) (Amphipoda) as the first and second intermediate hosts, respectively. The geografical distribution of this microphallid seems to be restricted to the Pyrenean region (Jourdane 1979 - op. cit.). The absence of records of *M. pyrenaica* in Spain is probably due to scarce information on the helminth fauna of the definitive hosts of this parasite either from the Spanish Pyrenees (Mas-Coma S. 1977: Rev. Ibér. Parasitol. 37: 227-242) or the rest of the Iberian Penisula (Cordero del Campillo M., Castañón L., Reguera A. 1994: Indice Catálogo de Zooparásitos Ibéricos. Sec. Publ. Univ. León, León, 650 pp.).

Sympatric specimens of the invertebrates *B. reyniesii* and *G. pulex* were collected in the Western Spanish Pyrenees (Barranco Hungoz, Navarra) in order to determine the presence of larval stages of microphallids trematodes. The prospected biotope, situated at 660 m altitude, consisted in a cold spring of a humid forest, with abundant aquatic vegetation (bryophytes). The shrews *N. fodiens* and *Neomys anomalus* Cabrera, 1908 had been previously reported in the locality studied (Castién E., Gosálbez J. 1992: Misc. Zool. 17: 249-261). Another microphallid, *Microphallus gracilis* Baer, 1943, has also been reported from *N. fodiens* in the French Pyrenees by Jourdane J. (1977: Ann. Parasitol. Hum. Comp. 4: 403-410) who elucidated its life cycle as well. According to this autor, *M. gracilis* uses the same first and second intermediate host species as *M. pyrenaica*.

In the laboratory, 770 snails were placed individually in cell culture plates, containing 2 ml of filtered biotope's water, for cercaria emergence detection. Free cercariae, detected in 56 snails, were studied using vital stain with neutral red (Fig. 1A - see p. 252) and employed for further experimental infection assays. Out of 260 specimens of *G. pulex* dissected, 254 were infected with metacercarial cysts in different stages of development (mean intensity 6 cysts, with a maximum of 62) (Fig. 1B,C). Metacercariae were located mainly in the abdominal cavity (100% of infected gammarids) and rarely in cephalothorax and appendix. Excystement process occurred spontaneously immediately after placing mature cysts in Ringer's solution at 37°C (Fig. 1D,E). In these same conditions ovigerous worms were obtained between 1-7 days after incubation of excysted mature metacercariae. They were fixed in Bouin and stained with aluminic carmine and then were identified as *M. pyrenaica* by morphological and morphometrical features (Fig. 1F). Eggs were released gradually from 2-4 days *in vitro* cultures before death of specimens at 8 days.

With the aim to reproduce the life cycle of this digenean, infection-free gammarids, *G. pulex*, and isopods, *Proasellus coxalis* (Dolfus, 1892), reared in the laboratory, were exposed to cercariae shed by *B. reyniesii*. At room temperature immature cysts were obtained 30 days post infection (DPI) in both hosts and mature cysts at 60 DPI in *P. coxalis*. Experimental infections of 5 laboratory mice force-fed with 100 mature cysts each were unsuccessful. Hatching of miracidia (Fig. 1H) from experimentally obtained adults took place after 24 hours in Ringer's solution and 48 hours in filtered biotope's water at room temperature (18°C).

In this paper, *M. pyrenaica* is recorded for the first time in Spain as larvae infecting intermediate hosts, displaying the same life cycle pattern as in the French Pyrenees. Experimental observation of the life cycle showed that more time was necessary to complete the cycle than reported by Jourdane (1979 - op. cit.), who recovered infective meta-cercarial cysts from *G. pulex* maintained at 10°C as early as 30 DPI. It seems that the development is influenced by temperature.

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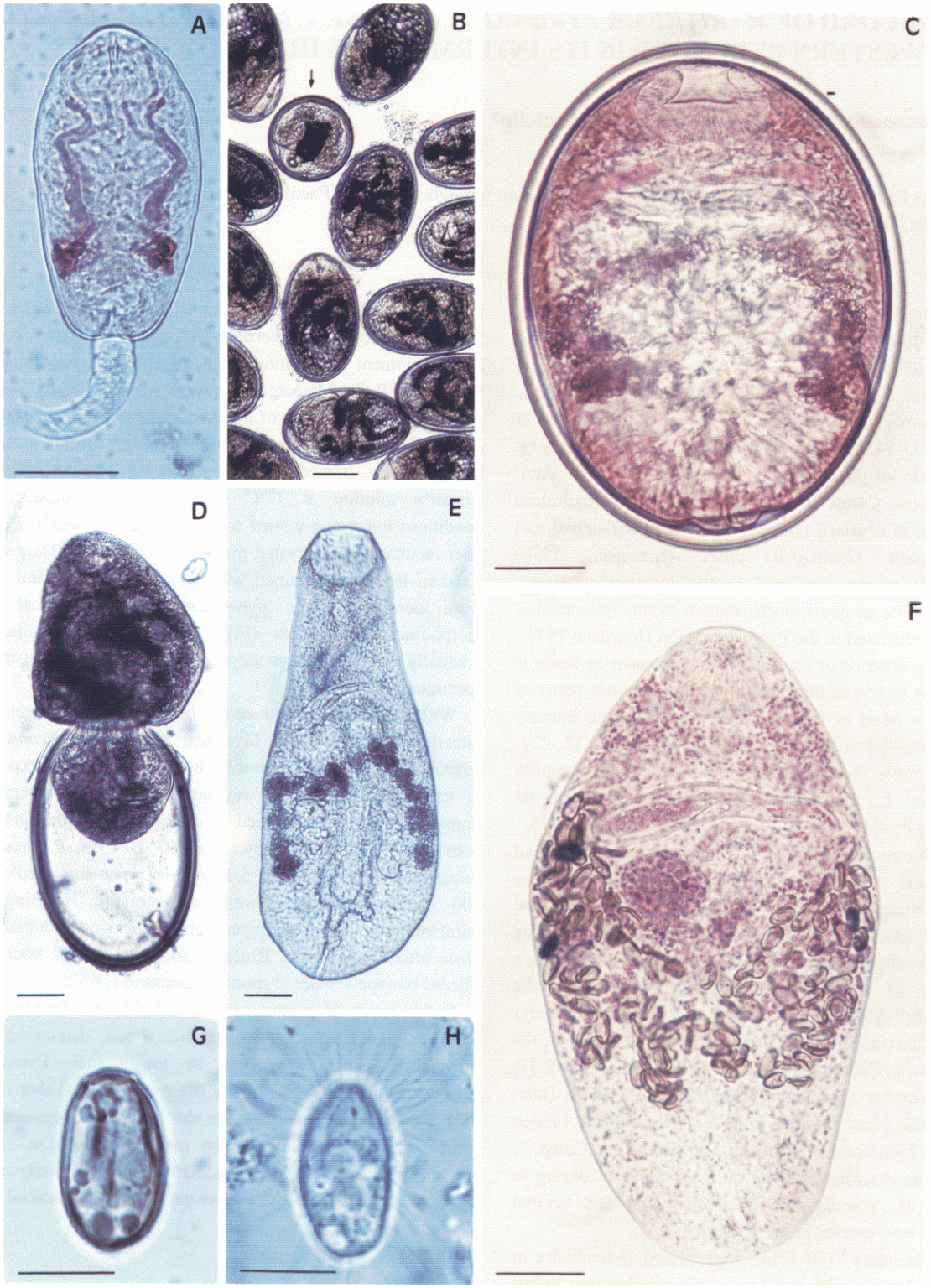

Fig. 1. Developmental stages of *Maritrema pyrenaica*. **A** - cercaria stained to show penetration glands; **B** - metacercarial cysts from naturally infected *Gammarus pulex* (arrow: cyst of another digenean); **C** - encysted metacercaria; **D** - spontaneous excystment of metacercaria in Ringer's solution; **E** - metacercaria *in vivo* excysted; **F** - experimentally recovered adult; 3 DPI; **G** - embryonated egg; **H** - hatched miracidium. Scale bars: 25 µm (A), 100 µm (B), 50 µm (C - F) and 10 µm (G,H).