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NEW RECORD OF *DOLLFUSINUS FRONTALIS* BIOCCA ET FERRETTI, 1958 (TREMATODA: BRACHYLAIMIDAE) IN CONTINENTAL EUROPE

M.T. GALAN-PUCHADES, M.V. FUENTES, A.M. CEREZUELA & S. MAS-COMA

Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia,
Av. Vicent Andrés Estellés s/n, 46100 Burjassot - Valencia, Spain

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ABSTRACT: Adult trematodes of the species *Dollfusinus frontalis* Biocca et Ferretti, 1958 (Digenea: Brachylaimidae) are recorded from the nasal sinuses of a specimen of Garden dormouse *Eliomys quercinus* (Linnaeus, 1766) (Rodentia: Gliridae) from the locality of Gátova, at the Sierra Calderona (prov. Valencia), Spain. The morphometry of the parasites is described and analysed. The geographical distribution of this peculiar brachylaimid is discussed, taking into account data known up to the present. This is the first report of *D. frontalis* both in the Iberian Peninsula and parasitising dormice in continental Europe.

KEY WORDS: Digenea, Brachylaimidae, *Dollfusinus frontalis*. Rodentia, Gliridae, *Eliomys quercinus*. Sierra Calderona, Valencia, Iberian Peninsula, continental Europe.

INTRODUCTION

Within multidisciplinary research activities carried out on helminths from free living mammals in the septentrional half of the Iberian Peninsula, ecological studies are being pursued on the helminth communities from small mammals inhabiting Mediterranean ecosystems which were affected by fire. Among these studies, a several year project on the postfire recolonization dynamics of insectivores and rodents in the Sierra Calderona (Comunidad Valenciana, Spain) is under way, in which the helminth parasites of these small mammals are analysed to ascertain their usefulness as biological tags for following the postfire regeneration/scarring processes in such ecosystems.

During the prospections on the helminthfauna of the small mammals from the Sierra Calderona, the digenean species *Dollfusinus frontalis* Biocca et Ferretti, 1958 (Trematoda: Brachylaimidae) was unexpectedly found in the nasal sinuses of a specimen of Garden dormouse, *Eliomys quercinus* (Linnaeus, 1766) (Rodentia: Gliridae). This parasite is worth mentioning owing to its morphoanatomic and biological peculiarities which characterise it as the most extreme among the whole family Brachylaimidae (MAS-COMA & GALLEGO, 1975b). These peculiarities explain the interest of several scientists for this species and thus the extensive literature covering different aspects of this brachylaimid species, e.g. studies on its very peculiar microhabitat in the frontal and nasal sinuses of its mammal hosts (BIOCCA & FERRETTI, 1958; MAS-COMA & KAHMANN, 1978; MAS-COMA *et al.*, 1983), the growth of its adult stage (VALERO & MAS-COMA, 1990; VALERO, DE RENZI & MAS-COMA, 1991), the pathological effects on its definitive host (MAS-COMA & KAHMANN, 1978), the development of its larval stages (TIMON-DAVID, 1964; MAS-COMA, MONTOLIU & GALLEGO, 1980; BARGUES *et al.*, 1992), its whole life cycle (TIMON-DAVID, 1965; MAS-COMA & MONTOLIU, 1978, 1987), its ecology (ESTEBAN *et al.*, 1987 a; MAS-COMA *et al.*, 1987 a; FUENTES, GALAN-PUCHADES & MAS-COMA, 1992), its evolution (MAS-COMA *et al.*, 1987 b; MAS-COMA & ESTEBAN, 1988), its presence on Mediterranean islands (MAS-COMA & GALLEGO, 1975 a; MAS-COMA, BARGUES & ESTEBAN, 1982; MAS-COMA & ESTEBAN, 1983; MAS-COMA & FELIU, 1984; ESTEBAN *et al.*, 1987 a, b), and its zoogeography (MAS-COMA, BARGUES & ESTEBAN, 1983; MAS-COMA & MONTOLIU, 1978, 1987).

Summing up, this digenean presents a triheteroxenous life cycle, in which a terrestrial snail acts as first intermediate host harbouring cercariogenous sporocysts at the level of the digestive gland or hepatopancreas, another (or the same) individual of terrestrial snail of the same or other species acts as second intermediate host harbouring non-encysted metacercariae at the level of the kidney, and small mammals as definitive hosts harbouring the adult stage in the nasal and/or frontal sinuses. Up to the present, the adult stage of this brachylaimid has been found in the following species: A) Insectivora: the European hedgehog *Erinaceus europaeus* Linnaeus, 1758 and the Algerian hedgehog *Atelerix algirus* (Lereboullet, 1842) (Erinaceidae); B) Rodentia: the Garden dormouse, *Eliomys quercinus* (Linnaeus, 1766) (Gliridae) and the Black rat *Rattus rattus* (Linnaeus, 1758) (Muridae).

The above mentioned finding of *D. frontalis* parasitising *E. quercinus* in a new geographical locality in continental Europe is the purpose of this paper.

MATERIAL AND METHODS

Geographical zone: The area under study comprises the mountains of the Sierra Calderona, located between the provinces of

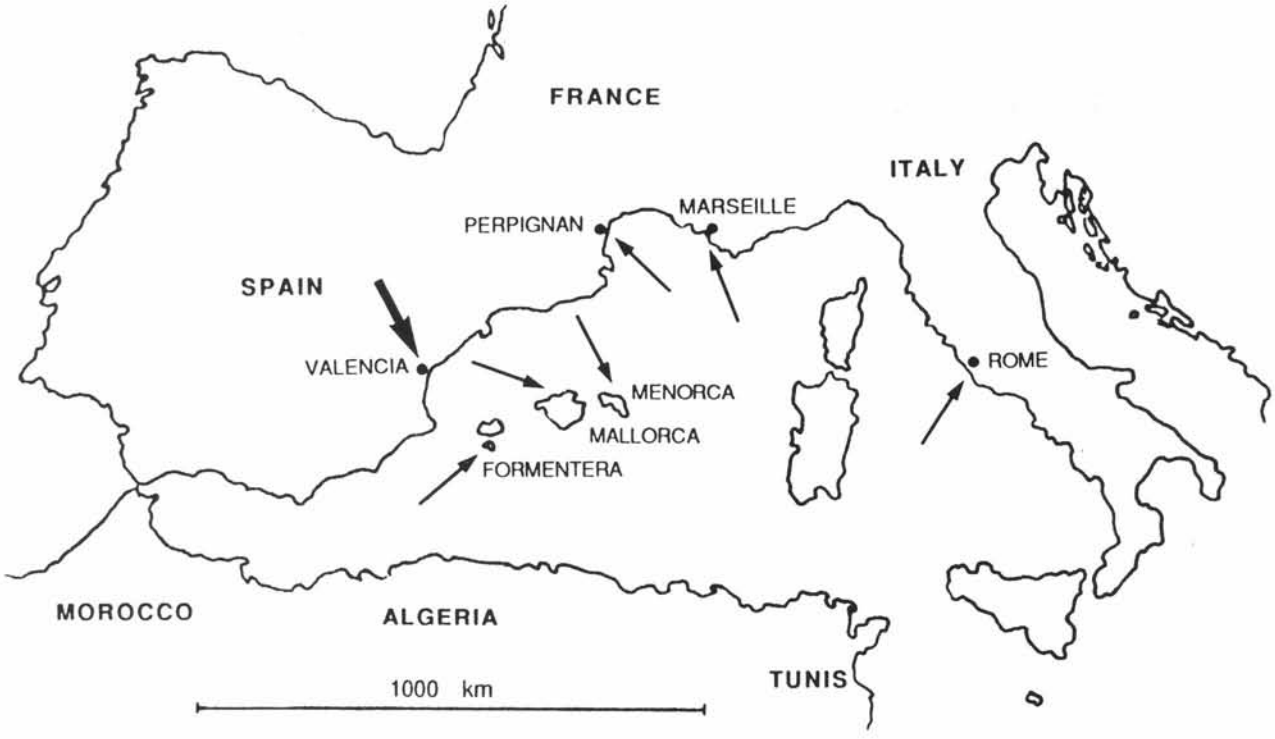

Fig. 1.– Geographical distribution of *Dollfusinus frontalis* in the Western Mediterranean region (after MAS-COMA & MONTOLIU, 1987). The new finding in the province of Valencia is marked with a thicker arrow.

Castellón and Valencia (Fig. 1). This mountain area suffered an important fire in the summer of 1992, which affected a total of 9500 ha of littoral holmoak and corkoak plantations, the most important potential vegetation, as well as abandoned cultivation zones. The parasites in question in this paper were found at Font del Rebollo, in the village of Gátova, at an altitude of 675-700 m, in the province of Valencia. The dominant vegetation in this place is continental holmoak, accompanied by white pine and fruit groves, with numerous stone walls (Fig. 2 A).

Host material: By means of 116 small mammals traps distributed in an area of 5 ha, three specimens of Garden dormice *E. quercinus* were captured alive on July 1994 in a zone not affected by the fire and located at 2 km from the burned zone. One specimen was used for the populational study and thus marked and set at liberty (Fig. 3). The other two dormice were dissected and all organs and viscera, including nasal and frontal sinuses, helminthologically studied in the laboratory under a binocular microscope.

Parasite material: Trematode and nematode parasites were found in the dormice. Trematodes were fixed with Bouin's solution, stained with Grenacher Boracic Carmine, dehydrated in different alcohols, cleared with xilol and mounted in Canada balsam. Nematodes were studied in non-permanent microscopic preparations, with lactophenol as clearing fluid. Morphometric measurements of the brachylaimid digeneans here in question were made according to the methodology proposed by MAS-COMA, MONTOLIU & VALERO (1984). The faeces of the three dormice found on the captura traps were analysed by means of the «cellophane faecal thick-smear technique» or simply «Kato-Katz method» according to KATO & MIURA (1954) modified by KATZ, CHAVES & PELLEGRINO (1972).

RESULTS

Digeneans belonging to the species *D. frontalis* were found in one of the two dormice studied helminthologically. This host specimen was captured in a partly dry canal between the holmoak zone and the cultivated fields (Fig. 2 B). It was a female dormouse of a weight of 69 g, showing a closed vulva (*anoestrus* phase of the sexual activity cycle) and the absence of uterine *maculae cianosae*.

This dormouse female was parasitised by two helminth species: *Molineus patens* (Dujardin, 1845) (Nematoda: Molineidae) at intestinal level and *D. frontalis*. Five adult specimens of this brachylaimid species were found: 3 adults were present in the right and 2 in the left nasal sinuses.

The appropriate morphoanatomic and morphometric studies allowed us to determine that these 5 individuals belonged to the species *D. frontalis*, according to the previous descriptions by BIOCCA & FERRETTI (1958), TIMON-DAVID (1965), MAS-COMA & GALLEGO (1975) and MAS-COMA & MONTOLIU (1987). Table 1 includes the results of the morphometric study.

Digenean eggs were detected in the faeces found in the traps in which the dormouse parasitised by *D. frontalis* (1200 eggs/g) and the dormouse set at liberty (1056 eggs/g) were captured. Although it was not confirmed that these eggs belonged to *D. frontalis*, by means of the

Fig. 2.– Font del Rebollo: A) a general view of the biotope; B) particular view of the zone of capture of the parasitised specimen of *Eliomys quercinus*.

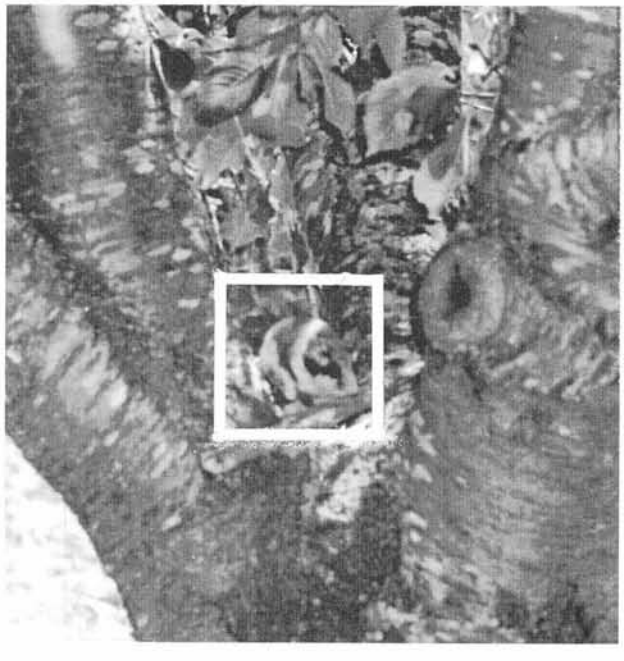

Fig. 3.– Specimen of *Eliomys quercinus* set at liberty, on a cherry tree of the biotope of Font del Rebollo.

appropriate infection of specific snails and following the whole life cycle up to the adult stage, the absence of any other digenean parasite in the first dormouse clearly suggests that they belong to this brachylaimid species.

DISCUSSION

Morphometry and life cycle

In the comparison of the morphometric data of these 5 specimens with the data furnished by other authors, two parameters appear to be worth mentioning: the sucker ratio and mainly the size of the eggs.

The sucker ratio of the specimens studied in this paper (extreme values: 0,40-0,46; mean: 0,43) is at the extreme of the whole range known in *D. frontalis*: a minimum of 0,40 in parasites of *E. quercinus* (MAS-COMA & MONTOLIU, 1987) and a maximum of 0,86 in parasites of *E. europaeus* (deduced from the data furnished by TIMON-DAVID, 1965), with mean values of 0,58 in *E. quercinus* and 0,62 in *A. algirus* (MAS-COMA & MONTOLIU, 1987).

The dimensions of the eggs in the final part of the uterus of the adult digeneans (extreme values: 32,7-39,9/14,2-21,3 μm; mean values: 38,3/18,6 μm) prove to be longer than one of the longest eggs of *D. frontalis* known so far, those of parasites from *A. algirus* from Formentera island, which have a size of 26-39,6/18-21,6 μm (mean: 30,1/18,6 μm) (MAS-COMA & MONTOLIU, 1987). The length of the eggs of *D. frontalis* ranges from a minimum of 21,6 μm found in adults parasitising *E. quercinus* up to a maximum of 39,6 μm in *A. algirus*, with mean values ranging from 28,2 in *R. rattus* to 30,1 μm in *A. algirus*. The width of *D. frontalis* eggs ranges between 15,4 and 25,2 μm (both extreme absolute values detected in *E. quercinus*), with mean values between 17,9 μm in *E. quercinus* and 18,6 μm in *A. algirus* (MAS-COMA & GALLEGO, 1975; MAS-COMA & MONTOLIU, 1987).

In the faeces found in the traps, there were digenean eggs, presumably belonging to *D. frontalis*. These eggs, detected with the Kato-Katz technique, have a size of 39,9-45,6/19,9-25,6 μm (mean: 41,8/24,7 μm) in the dormouse parasitised by *D. frontalis* and of 37,0-42,7/22,8-31,3 μm (mean: 40,8/27,0 μm) in the specimen set at liberty, that is, clearly larger than the size of the uterine eggs. This size difference can be due to not having been affected by any fixation, coloration or dehydration process, as in the case of specimens mounted in Canada balsam.

The presence of a large number of *D. frontalis* eggs in the faeces of its definitive host becomes very important for the life cycle of the parasite. Up to the present, it was thought that the eggs left the mammal host mainly directly from the nasal sinuses to the external environment (sneezing), this process being facilitated by the marked sinusitis, chronic coryza and consequent mucosity originated by the parasite in the nasal sinuses, although the

	n	E.V.	$\bar{X}$
Length	3	4124-4883	4539
Maximum width	4	1137-1260	1216
Oral sucker	4	368-425/345-368	401/359
Acetabulum	5	567-613/556-598	596/578
Sucker ratio	4	0,40-0,46	0,43
Pharynx	4	194-199/182-194	197/190
Testis I (= anterior)	5	597-781/425-563	717/515
Testis II (= posterior)	4	747-896/379-609	813/531
Ovary	5	345-425/207-322	383/283
Distance between suckers	4	379-505	434
Dist. acetabulum - testis I	5	402-1034	823
Dist. uterus - anterior end	4	540-701	655
Eggs in uterus	16	32,7-39,9/14,2-21,3	38,3/18,6
Eggs in faeces of *E.q.* with *D.f.*	6	39,9-45,6/19,9-25,6	41,8/24,7
Eggs in faeces of *E.q.* set at liberty	6	37,0-42,7/22,8-31,3	40,8/27,0

Table 1.– Measurements in µm of adults of *Dollfusinus frontalis* found in *Eliomys quercinus* on the Sierra Calderona (prov. Valencia, Spain). E.V. = extreme values; $\bar{X}$ = mean values; n = number of adults or eggs on which the measure was made.

indirect way through faeces after being swallowed was not disregarded (TIMON-DAVID, 1965; MAS-COMA & KAHMANN, 1978; MAS-COMA & MONTOLIU, 1987). The finding described in this paper, of a great number of eggs in faeces, not only confirms this second way, but indicates its possible importance in parasite transmission. If the eggs are able, after being swallowed, to follow the whole transit of the digestive tract without losing their viability and thus reach the external milieu with the faeces, it appears evident that this way can be more useful for the life cycle when taking into account the coprophagic activity of terrestrial snails, enabling their infection by eating the digenean eggs present in the faeces. Evidently, however, it remains to be demonstrated that eggs do not lose their viability after passing through the whole digestive tract, which requires infecting first intermediate host snails with eggs recovered from mammal faeces.

Definitive host specificity and geographical distribution

Up to the present, *D. frontalis* has been found in the Garden dormouse *E. quercinus* only on islands of the Balearic Archipelago, that is, on Mallorca, Menorca and Formentera (ESTEBAN *et al.*, 1987a), in spite of the several parasitological studies carried out on Garden dormice from the Iberian Peninsula, the continental part of Europe and the northern part of Africa. Similarly, this brachylaimid digenean has been found in the rat *R. rattus* only on the island of Formentera. All other findings of *D. frontalis* concern hedgehogs, both in continental Europe (*E. europaeus* in Italy and France) and on the Balearics (*A. algirus* on Formentera) (MAS-COMA & MONTOLIU, 1987).

Worth mentioning here are the statistical results obtained in ecological studies by FUENTES (1992) on the helminth communities from small mammals on the island of Formentera, where the parasite uses *E. quercinus, R. rattus* and *A algirus* as definitive hosts. FUENTES (loc cit.) demonstrates that, on this island, *D. frontalis* shows a greater specificity for the dormouse than for the rat, this brachylaimid species appearing as the dominant species for the dormouse, whereas it appears only as co-dominant species for the rat (according to the concepts of PENCE & EASON, 1980). Moreover, on Formentera the prevalence of *D. frontalis* in *A. algirus* is only 11,36% (ESTEBAN *et al.*, 1987 b) compared with 61,0% in *E. quercinus* (FUENTES, GALAN-PUCHADES & MAS-COMA, 1992). This brachylaimid species has also been found in the dormouse on all other islands of the Balearic Archipelago where it is present (MAS-COMA & MONTOLIU, 1987), as opposed to the parasitation of the hedgehog by the same brachylaimid, which is known only on Formentera (ESTEBAN *et al.*, 1987 b). Although it must not be forgotten that the number of hedgehogs examined so far on Mallorca and Menorca is unfortunately too small to reach a significant conclusion on this aspect, all data suggest that the Garden dormouse seems to be the typical and usual definitive host of *D. frontalis*, at least on the Balearics, as already mentioned by MAS-COMA & MONTOLIU (1978) and MAS-COMA, ESTEBAN & BARGUES (1983).

On the continent and up to the present, *D. frontalis* was only known in *E. europaeus*, so that its parasitation in rodents on the Balearics has been mainly explained as the consequence of the influence of the isolation phenomenon on the parasitofaunas of small mammals on islands; insularity would explain why the dormouse became the main definitive host of *D. frontalis* on the Archipelago (MAS-COMA & MONTOLIU, 1978; MAS-COMA, ESTEBAN & BARGUES, 1983; MAS-COMA *et al.*, 1987 b; MAS-COMA & ESTEBAN, 1988). Now, after the finding of this brachylaimid in dormice of Sierra Calderona, the question is posed of the greater or lesser importance of *E. quercinus* as definitive host of *D. fronta-*

lis in the south European mainland. Unfortunately, the nasal (and frontal) sinuses do not usually enter in routine helminthological post-mortem studies of small mammals, so that it is today difficult to discuss this question. At any rate, it does not seem to be prudent to consider the finding of *D. frontalis* in Sierra Calderona merely as a rare or sporadic infection of *E. quercinus* by this brachylaimid. It now appears evident that appropriate helminthological studies on *E. quercinus*, as well as on hedgehogs, in mainland zones (if possible where dormice and hedgehogs cohabit) are needed to ascertain the relative roles of both definitive hosts in the life cycle of the digenean. This would, moreover, allow us to ascertain more accurately the influence of insularity on the definitive host specificity of *D. frontalis* on the island of Formentera.

The interest of this finding lies not only in its being the first record of *D. frontalis* on the Iberian Peninsula and the first report on mainland dormice, but also concerns the possible forms of colonization followed by this parasite to reach and adapt to the different islands of the Balearic Archipelago. MAS-COMA (1978) already suggested the possibility of a colonization route following the so-called Betic-Riffian-Maghrebinian arc, and ALCOVER (1978) stressed the possibility that Garden dormice were introduced by Man to some islands several thousands of years ago. Up to the present, it has been speculated that *D. frontalis* colonized the different Balearic islands using the Algerian hedgehog as carrier. Now it is also necessary to take the Garden dormouse into account in this colonization process, which in fact gives rise to a more understandable adaptation of the parasite to the several islands by means of *E. quercinus*.

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