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Hyperparasitism in caves: bats, bat flies and ectoparasitic fungus

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1. Introduction

Bat flies (order Diptera) are highly specialized bloodsucking ectoparasites living on bats (Fig. 1).

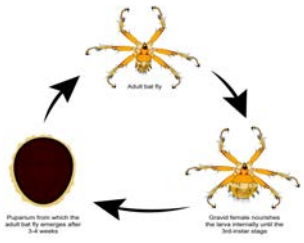

Fig. 1. Illustration of Bat flies (Family: Nycteribiidae) life cycle.

Some ectoparasitic bat flies are infected with an ectoparasitic fungus of the genus *Arthrorhynchus* (order Laboulbeniales) (Fig. 2).

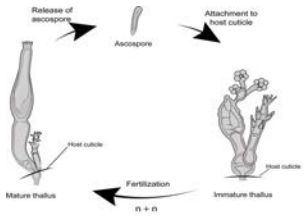

Fig. 2. Illustration of the fungus *Arthrorhynchus* spp. life cycle.

Ascospores of the fungi attach to the cuticle of the bat fly and presumably develop an haustorium, which penetrates into the bat fly tissue getting its nutrition. This interaction converts the fungus into an hyperparasite.

Both the bat fly and the fungus are obligate ectoparasites and cannot live separate from their hosts. This unique case of fungus-insect-vertebrate hyperparasitism interaction remains highly unknown.

2. Material and Methods

Bat flies were collected in different seasons from 2016 to 2018 at 11 different roost sites in caves of Portugal in a standardized way during monitoring programs of cave dwelling bats (Fig. 3 & 4).

Fig. 3. Bat collecting

Fig. 4. Location of sampling sites in Portugal

Bat flies were identified to species level and screened for Laboulbeniales under a stereomicroscope.

The fungus was studied under Differential Interphase Contrast microscopy (DIC) and the contact zone between the fungus and the bat fly was studied under Transmission Electron Microscopy (TEM).

3. Results and discussion

Bat flies were found on 239 bats belonging to six species. On these bats, 428 bat flies were collected, belong to six species of the family Nycteribiidae (Table 1).

Among the 428 bat flies studied, only 10 were infected with the fungus *Arthrorhynchus nycteribia*, an infection rate of 2.3%. *Penicillidia conspicua* was the most abundant bat fly and nine specimens were infected with the hyperparasitic fungus, while only one specimen of *P. dufourii* was infected.

Table. 1. Number of bat species, number of bat fly species per bat species, number of infected bat flies.

Bat species	Nº of individuals (bats)	Bat fly species	Nº of individuals (bat flies)	Nº of infected bat flies
Miniopterus schreibersii	127	*Nycteribia schmidlii*	34	-
		Penicillidia conspicua	135	9
		Penicillidia dufourii	7	-
Myotis bechsteinii	1	*Nycteribia vexata*	1	-
Myotis blythii	59	*Nycteribia latreillei*	3	-
		Nycteribia vexata	12	-
		Penicillidia conspicua	2	-
		Penicillidia dufourii	95	-
Myotis daubentonii	1	*Nycteribia kolenatii*	1	-
Myotis escaleraí	2	*Penicillidia dufourii*	2	-
Myotis myotis	49	*Nycteribia latreillei*	1	-
		Nycteribia vexata	11	-
		Penicillidia conspicua	1	-
		Penicillidia dufourii	123	1

Sex ratio: There is an excess of female bat flies with a sex ratio of males to females at 74.7. The same skewed sex ratio is found in the fungus infected bat flies. Of the 10 infected bat flies, 7 are females and only 3 are males. This gives a sex ratio of infected males to females at 42.9.

Host specificity: There is a clear host specificity in the collected bat flies (Fig. 5). All Laboulbeniales infected bat flies of the species *P. conspicua* were found on the bat species *Miniopterus schreibersii*. The infected bat fly of the species *P. dufourii* was found on the bat species *Myotis myotis*.

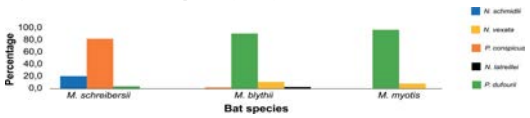

Fig. 5. Host specificity of bat flies

Position of the fungus in the batfly: No evidence of strong position specificity has been observed. The fungus is mostly found in the junctions between sclerites on the abdomen or in the posterior genital region (Fig. 6).

Structure of the haustorium: TEM images reveal that the haustorium of *A. nycteribiae* penetrates the tissue of the bat flies (Fig. 7).

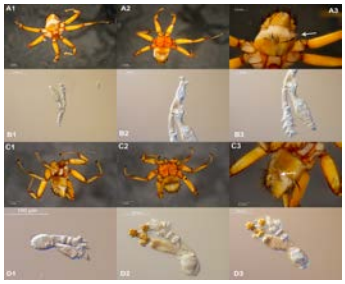

Fig. 6. Position of the fungus in infected bat flies (A, C), and thalli overview (B, D).

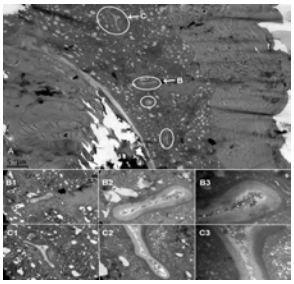

Fig. 7. TEM overview of the haustorium inside the bat fly (B, C).

- Laboulbeniales infected bat flies exhibits a generally low infection rate (Blackwell 1980; Haelewaters *et al*. 2017), matching the infection rate of 2.3% found in this study. The low infection rate in the winter season may be explained by the hibernation of bats, which will affect the life cycle of the bat fly and consequently the of the fungal hyperparasite.

- Out of 11 sampling sites, infected bat flies were only found on 3 sites, indicating that there is a low fungal dispersal, between bat shelters.

- Research on the seasonal differences will illuminate the effect of bat hibernation on the life-cycle of the hyperparasite.

References

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