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Fungal and rickettsial infections of some East Asian trapdoor spiders

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Abstract
While published data on fungal and rickettsial infections of spiders give the impression of a wide range of hosts infected by particular agents, field collections of trapdoor spiders from East Asia reveal a clear distinction: representatives of Mesothelae were infected by rickettsiae, while specimens of the genus *Latouchia* (Mygalomorphae, Ctenizidae) were found to be infected by the hyphomycete fungus *Nomuraea atypicola*.

Key words: Mesothelae, Ctenizidae, infection, Rickettsiales, *Nomuraea*

INTRODUCTION

Mesothelae have not been spared by predators, parasites and diseases. Besides ravaging animals like skinks and centipedes which regularly come across those soil dwelling spiders, there are reports on parasitic laelapid mites like *Liunghia bristowei* Finnegan, 1933 living on *Liphistius malayanus* Abraham, 1923 and there are parasitic flies and wasps (Sawaguti and Ozi 1937; Bristowe 1976; Schwendinger 1990) which attack Mesothelae along with other spiders. Mesothelae are also subject to parasitation by mermithid nematodes (pers. obs.).

Infections found in some species of Mesothelae are compared to those in other trapdoor spiders of the genus *Latouchia* (Mygalomor phae: Ctenizidae).

MATERIAL AND METHODS

Specimens of Mesothelae and Ctenizidae (genus *Latouchia*) collected in Kyushu, in Okinawa and in Malaysia were brought to the laboratory and kept there for ethological and morphological studies. The determination of mesothele spiders is according to Haupt (1983). Specimens of *Latouchia* were only determined to the rank of genus which is sufficient for the present work. The fungus *Nomuraea atypicola* (Yasuda, 1915) Samson, 1974 (syn. *Isaria atypicola, Cordyceps atypicola*) (Deuteromycotina: Hyphomycetes) was identified according to Kobayasi (1982). Rickettsiales could only be identified as an order, according to the electron microscopic appearance of the cells.

As in several species the collected material was quite numerous, it represented a good occasion to study the prevalence of different pathogens. The present study was carried out on living spiders, except for *Nomuraea* infections in which the perithecial stroma grows out from the dead spider and opens the trapdoor from inside the burrow, thus indicating the infection.

Trapdoor spiders live inside their burrows in moist soil, therefore the observer easily misses the date when a specimen dies. In the

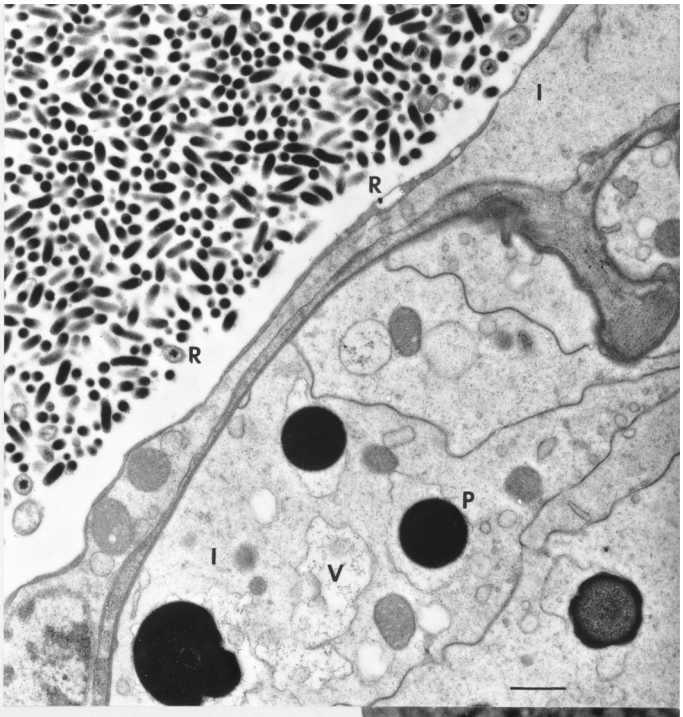

Fig. 1. Electron microscopic section through the opisthosoma of *Liphistius malayanus cameroni* with intestinal cells (I) and rickettsiae in intermediate cell (R). P: vacuoles containing protein, V: vacuoles. Scale 10 µm.

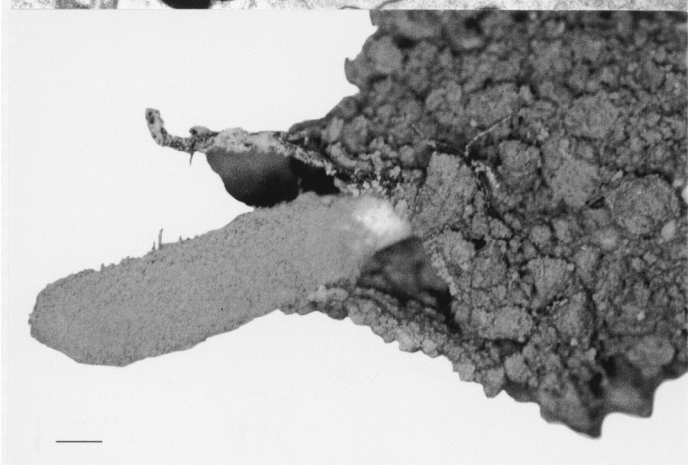

Fig. 2. Perithecial stroma of *Nomuraea atypicola* emerging from the burrow of *Latouchia* sp. (Ctenizidae) by opening the trapdoor from inside. Scale 2 mm.

moist environment dead spiders may become infected secondarily by fungi from the soil: such cases were not studied in the present paper.

For light microscopic study, the opisthosoma of infected specimens was submersed in Bouin's fluid and embedded in paraplast. Sections were stained with Haematein, Azan or Haematoxylin/Chromotrop after Dobell. Procedures for electron microscopy were as described previously (Haupt 1996), semi-thin sections (1 µm) were stained with Kristallviolett (Merck). Specimens of *Nomuraea atypicola* were deposited in the Botanical Museum, Berlin.

RESULTS

Histological and electron microscopic results

In Mesothelae, rickettsial infection is very obvious in the intermediate cells of the hepatopancreas (Fig. 1), while neighbouring cells of the midgut remain uninfected. The intermediate tissue is known to function as storage organ

Table 1. Number of specimens of Mesothelae and *Latouchia* (Ctenizidae) collected (N) compared to those infected by different pathogens.

	N	Infected by Rickettsiales	Infected by *Nomuraea*	%
Heptathela kimurai kimurai (Kishida, 1920)	52	1	-	1.9
Heptathela kimurai yanbaruensis Haupt, 1983	164	3	-	1.8
Ryuthela nishihirai nishihirai (Haupt, 1979)	199	3	-	1.5
Liphistius malayanus cameroni Haupt, 1983	134	6	-	4.5
Latouchia sp.	22	-	4	18.2

analogous to the fat body of insects. Mainly lipids and glycogen are found in these cells (Ludwig & Alberti 1988), but the histological study also reveals numerous vesicles containing protein.

The first rikettsial stages to be detected are narrow and electron dense. Apparently, they grow inside the host cells to form larger, less electron dense cells. This stage may or does undergo binary fission thus multiplying the parasitic population in the host cell. Finally, all intermediate cells in the hepatopancreas are filled with nothing but rickettsial cells: all organelles and even the nuclei have disappeared. Apparently, the rikettsiae use up the storage products for their own purpose, and moulting becomes impossible for the spider.

These later stages of infection can be recognized easily by the whitish opaque opisthosoma of host spiders, which is typical for rikettsial infections. Finally, the intermediate cells rupture and as the spider dies and the whole opisthosoma dissolves, the pathogens spread into the soil. In this final phase the opisthosoma is so inflated that dissection becomes impossible. Even a careful touch results in the disruption of the cuticle and a milky fluid appears from inside.

So far, no experimental infection of Mesothelae along with food has been successful.

The other group of trapdoor spiders, living in the same habitats, belongs to the genus *Latouchia* (Ctenizidae). Among them, one may regularly observe infections by the fungus *Nomuraea atypicola*. Its hyphae grow through the whole body of the spider and finally, within a few hours, form a long stalk with conidium production. Generally, this stalk, the perithecial stroma, grows along the burrow, opens the trapdoor from inside, and reaches a height of 20 to 30 millimeters. In the upper part it bears numerous purple ascospores (Fig. 2), which now, outside the spider's burrow, are subject to aerial dispersal.

Instead, during 20 years of breeding Mesothelae, out of 549 specimens taken from the field not a single one was found to be infected by *Nomuraea atypicola*.

Infection rates

There are regular infections by Rikettsiales in Mesothelae (Table 1) found in Kyushu, Ryukyu, or Malaysia. The infection rate must be considered low, as far as most localities are concerned. Nevertheless, when comparing infection rates of the same species from different localities, there were striking differences: Rickettsial infection rates of *Ryuthela nishihirai nishihirai* were almost 6% (n = 52) in Suyeyoshi, Naha, Okinawa, but almost negligible in Ryutan, Naha, Okinawa. Both places, only a few kilometers from each other, are or were residues of rather natural habitats with similar soil conditions, but the locality in Suyeyoshi is situated in a valley of a brooklet, while Ryutan is close to a hill top, more exposed to wind and this place appears much drier.

Trapdoor spiders of the genus *Latouchia* (Ctenizidae) were infected by the hyphomycete *Nomuraea atypicola*, and to a much larger degree. The 22 specimens of *Latouchia* proved to be infected at a rate of about 18%.

DISCUSSION

Many fungal infections have been described in connection with spiders (Evans & Samson 1987), but unfortunately, in many cases either the spider or the fungus remained undetermined. The hyphomycete fungus *Nomuraea* certainly occurs in a wide range of soil dwelling mygalomorphs, and it has also been found in araneomorph spiders (for literature, see Coyle et al. 1990). In North America a broad range of spider species could be infected experimentally by an isolate of *Nomuraea* from ascospores originating from an infected Brazilian trapdoor spider. By applying a conidial suspension containing a detergent, 20 out of 27 spider species were successfully infected (Greenstone et al. 1987). In Okinawa, on the other hand, infectious diseases in trapdoor spiders seem to be clearly linked to different families: while *Latouchia* (Ctenizidae) specimens were found to be infected by *Nomuraea atypicola*, rickettsial infections were limited to Mesothelae. So far, the experimental infection of Ryukyuan mesothele spiders by *Nomuraea* (without using detergents) turned out to be impossible.

Among Rikettsiales, the genus pathogenic in arthropods has been named *Rikettsiella*. Very similar pathogens as in Mesothelae have been reported from intermediate (interstitial) cells of a Japanese funnel web spider (*Paracoelotes luctuosus*) (Osaki 1973) and from a linyphiid spider (Suhm 1995). These pathogens are comparable to infections described from the hepatopancreatic caeca in a buthid scorpion (Morel 1976).

In acarids (Reinhardt et al. 1972), insects, diplopods (Schlueter & Seifert 1985) and woodlice (Vago et al. 1970) the occurence of similar pathogens is quite frequent, and they have been reported as intracellular parasites from different tissues, such as labial glands (*Lepisma saccharina*, pers. obs.), Malpighian tubules (Schlueter & Seifert 1985) and adipous tissue (Götz 1972; Vago et al. 1970). In the case of woodlice, there is strong evidence that specimens from moist and periodically wet habitats are most likely to be infected (Federici 1984), which is in line with our observations on *Ryuthela nishihirai*.

The wide distribution does not automatically allow any conlusion on specific identity. At present it seems doubtful whether pathogenic Rickettsiales represent only one species. Transmission of Rickettsiales from a scorpion to other arachnids, even to members of other scorpion families, turned out to be impossible (Morel 1978). Our studies on infections of trapdoor spiders point to the same direction. The redefinition of the group based on serological data has been strongly suggested previously (Louis et al. 1977).

In general, the infection rate with Rickettsiales is low. A 5% infection rate in scorpions (Morel 1976) is well in line with our results. Such low prevalence of infection may also be the reason that early stages of infections are hardly found in the intermediate cells of free-living Mesothelae; only the late stage of infection is obvious.

There are interesting reports on Rickettsiales and unidentified virus particles occurring in acarid ovaries (Lewis 1979), in insect sperm, spider coenospermia, and mite spermatozoa (Afzelius et al. 1989). Apparently, still other paths of infection are used beside food, and *Wolbachia pipiens* is known to commonly infect ovary cells, thus being transmitted in a vertical manner (Bourtzis & Braig 1999).

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