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## Insect pathogenic Entomophthorales from Nepal and India

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Six species of Entomophthorales new to Nepal and a probably undescribed species from India are listed together with their main characteristics. The latter attacks the sugarcane pest *Pyrilla perpusilla*. The most interesting findings in Nepal concern *Entomophthora byfordii*, known only from Switzerland and England, and a species with dimensions intermediate between *Erynia aquatica*, known from the western hemisphere, and *E. chironomi*, known from China. Since the latter species might be identical with *E. aquatica*, it was attributed to this species.

Keywords. Insect pathogenic fungi, Entomophthorales, India, Nepal, new records.

### INTRODUCTION

Entomopathogenic fungi play a significant role in the natural regulation of insect and mite populations and have an enormous potential to be used as microbial control agents. The most significant ones belong to the Zygomycetes (order Entomophthorales), Ascomycetes (genera *Cordyceps* and *Torrubiella*) and Hyphomycetes, now considered as anamorphic Ascomycetes (genera *Beauveria*, *Hirsutella*, *Lecanicillium*, *Metarhizium*, *Paecilomyces* and others). Some species of Hyphomycetes have already been developed to officially registered plant protection products available on the market (Butt *et al.* 2001). Species of *Beauveria* and *Metarhizium* are the most exploited ones in respect to the use as myco-insecticides and a lot of knowledge and experience is available. Therefore, they offer an interesting opportunity especially for developing countries and for organisations aiming to get rid of harmful insecticides and to develop their own products, to create small industries and to reach a certain independency from the international plant protection market. A prerequisite is the knowledge of the existing species of entomopathogenic fungi and their distribution.

Projects in this direction were recently initiated in India (Deshpande *et al.* 2003) and in Nepal (Yubak Dhoi GC & Keller 2003; Yubak Dhoi GC 2006). In both countries *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokin were isolated from soil samples the latter also from insects. In addition to that *Lecanicillium lecanii* (Zimm.) Zare & Gams was collected in April 2004 from numerous unidentified aphids on maize, probably *Rhopalosiphum* sp., at the National Maize Research Programme, Rampur, Chitwan, Nepal (Keller, unpubl.). In the course of this project work attention was given to Entomophthorales which resulted in the detection of six species belonging to five genera new to Nepal and a species of *Batkoa* probably new to India. They are described in this paper.

## MATERIAL AND METHODS

Dead insects with symptoms of fungus infections were removed from plants or other supports and placed in small tubes with 70 % ethanol and taken to the laboratory. They were carefully teased and mounted either in 1 % aniline-blue or in lactophenol-aceto-orcein (0.5% orcein) (Keller 1987). Measurements of fungal structures and counts of nuclei were based on 25 objects per host. If available, several specimens (series) of the same species were examined to assess variability. The number of series is given after the range of the mean values, the range of the extreme values (in brackets) and the ratio length/diameter (L/D). Genera and species are listed in alphabetical order.

## RESULTS

*Batkoa* sp.

Hyphal bodies subspherical, ovoid to slightly elongate. Conidiophores unbranched, terminal enlargement missing or indistinct, long «neck», contain (6–) 9.7 (–14) nuclei with a diameter of (4–) 4.7 (5.5)  $\mu\text{m}$  (1 series). Primary conidia on insect body (22–) 24.1 (–27) x (18–) 20.9 (–24)  $\mu\text{m}$ , L/D = 1.16 (1 series), contain (7–) 10.0 (–14) nuclei (1 series) with a diameter of (4–) 4.6 (–5.5)  $\mu\text{m}$ , primary conidia on wings (25–) 30.9 (–36) x (23–) 27.2 (–33)  $\mu\text{m}$ , L/D = 1.14 (1 series). Secondary conidia on wing (24–) 25.4 (–26) x (19–) 21.4 (–23)  $\mu\text{m}$ , L/D = 1.19.

*Host:* *Pyrilla perpusilla* Walker (Homoptera, Fulgoridae).

*Distribution:* Ashoknagar (200 km SE of Pune), India. The species was collected in September 2000.

*Remarks:* The species recorded from this important pest of sugar cane in India, Sri Lanka and Pakistan resembles *Batkoa amrascae* (Villacarlos & Keller 1997) but the available data do not allow to unequivocally attribute it to this species. *Pyrilla perpusilla* was already mentioned as host of an entomophthoralean fungus (Varma *et al.* 1990). No morphological data were given but the illustrations show different symptoms so that the two fungi very probably are different.

*Entomophaga grylli* (Fres.) Batko (1964)

The primary conidia measured (30–) 36.2 (–42) x (24–) 28.6 (–34)  $\mu\text{m}$ , L/D = 1.27 (1 series).

*Host:* Orthoptera, Acrididae: Unidentified species.

*Distribution:* The species was found in a single grasshopper in a rice field near Rampur, Chitwan, Nepal.

*Erynia aquatica* (Anderson & Anagnostakis) Humber (1989)

Primary conidia (36–) 40.2–40.6 (–46) x (12–) 14.3–14.6 (–16)  $\mu\text{m}$ ; L/D = 2.78–2.82 (2 series), bent; papilla conical, basal diameter less or equal length, asymmetric.

*Host:* Diptera, Nematocera: Unidentified midges.

*Distribution:* On stones in lake Phewa close to the water level, Pokhara, Kaski, Nepal, February 25, 2003.

*Remarks:* The species was attributed to *E. aquatica* although it does not match exactly the data given in the original description. This gives the dimensions for the primary conidia as 35–40  $\mu\text{m}$  x 17  $\mu\text{m}$ . However, the dimensions vary in a wide range. According to Bałazy (1993) the primary conidia measure 30–40  $\mu\text{m}$  x 15–18  $\mu\text{m}$  and according to Keller (1991) they measure on average 31–43  $\mu\text{m}$  x 12–16  $\mu\text{m}$  with an L/D-ratio of 2.16–2.90. These data overlap with *E. chironomi* (Fan & Li) Li & Fan (Fan & Li, 1994), whose primary conidia measure on average 41–48  $\mu\text{m}$  x 13–17  $\mu\text{m}$  with an L/D-ratio of 2.5–3.3. Although *E. chironomi* has longer conidia, an identity with *E. aquatica* can not be excluded. Further studies, especially genetic analysis, are needed to clarify the situation.

The species caused an epizootic among small midges. In a few conidiophores the number of nuclei could be counted, it was between 9 and 16.

*Entomophthora byfordii* Keller (2002)

The fungus on the collected sciarids was not in a sporulating stage when placed in ethanol. However, the developing conidia had the campanulate shape typical for the genus *Entomophthora*. The conidiophores contained (4–) 5.9 (–8) nuclei (1 series) and allowed an unequivocal identification of the species.

*Hosts.* Diptera: Unidentified Sciaridae.

*Distribution:* Campus of the Institute of Agriculture and Animal Sciences (IAAS) Campus, Rampur, Chitwan, Nepal.

*Entomophthora culicis* (Braun) Fresenius (1858)

The typical binucleate conidia measured (13–) 14.7 (–17) x (9–) 10.1 (–11)  $\mu\text{m}$ , L/D = 1.46 (1 series).

*Host:* Diptera, Nematocera: Unidentified midges.

*Distribution:* The species was found in high densities associated with *E. aquatica* on stones in lake Phewa close to the water level, Pokhara, Kaski, Nepal.

*Pandora neoaphidis* (Remaudière & Hennebert) Humber (1989)

The primary conidia measured (21–) 24.2–26.5 (–31) x (10–) 11.5–12.4 (–13)  $\mu\text{m}$ , L/D = 1.99–2.17 (5 series). A few rounded secondary conidia (type 1b secondary conidia) were present and measured 16–18 x 11–13  $\mu\text{m}$ .

*Hosts.* Homoptera, Aphididae: Unidentified aphid species on mustard and maize (probably *Rhopalosiphum maidis*).

*Distribution:* IAAS and Campus of National Maize Research Programme (NMRP), Rampur, Chitwan, Nepal.

*Zoophthora cf. radicans*.

The primary conidia measured (22–) 23.7–24.2 (–27) x (7–) 8.6–8.9 (–10)  $\mu\text{m}$ , L/D = 2.66–2.82 (3 series). The capilliconidia and the type Ia secondary conidia measured (23–) 26.3 (–29) x (6–) 7.6 (–9)  $\mu\text{m}$ , L/D = 3.48 (1 series) and (17–) 19 (–20) x (8–) 9.4 (–11)  $\mu\text{m}$  (1 series), respectively.

*Hosts.* Homoptera, Aphididae: Unidentified aphid species on maize, probably *Rhopalosiphum maidis*.

*Distribution:* IAAS Campus and NMRP Campus, Rampur, Chitwan, Nepal.

*Remarks:* The species is a typical member of the *Z. radicans* group. However, the species from aphids should be considered as different from *Z. radicans*, whose type host is *Pieris rapae* (Lepidoptera) (Keller 2006).

## DISCUSSION

So far, no species of arthropod-pathogenic Entomophthorales have been reported from Nepal (GC, pers. comm.). Four of the six species are world-wide distributed namely *Entomophaga grylli*, *Entomophthora culicis*, *Pandora neoaphidis* and *Zoophthora* cf. *radicans*. Their presence in Nepal could be expected. Noteworthy are the two rare species, *Erynia aquatica* and *Entomophthora byfordii*. They represent the first findings in Asia. However, the dimensions of *E. aquatica* do not exactly match those of the original description and further investigations are needed to attribute it with certainty to this species and to separate it clearly from *E. chironomi* described from China. The finding of *Entomophthora byfordii* is the first record of this species outside Europe.

From India there are some scattered reports on Entomophthorales which are included in the monograph compiled by Bałazy (1993). The records of Entomophthorales from these two countries are certainly far from being representative since systematic samplings are missing. The Indian subcontinent especially the northern part with a wide variety of ecozones is well known for its richness of insect species. Therefore, we can assume that the richness of potential hosts is a good prerequisite for a wide variety of entomopathogenic fungus species including the Entomophthorales. Research in this area is neglected in this region, however, it would be worth to know more about entomopathogenic fungi in order to better exploit their regulatory potential and to use it for pest control.

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