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Amended description and new combination for *Entomophthora nebriae* Raunkiaer, (1893), a little known entomopathogenic fungus attacking the ground beetle *Nebria brevicollis* (Fabricius, 1792)

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Abstract

A fungus attacking the ground beetle *Nebria brevicollis* (Fabricius, 1792) (Coleoptera, Carabidae) was collected in north-western Germany. The fungus was identical to *Entomophthora nebriae* Raunkiaer, 1893 (Entomophthoromycotina, Entomophthoraceae), described in 1893 from Denmark and so far only known from the type collection. We provide an amended description of *E. nebriae* based on the new collection and transfer the species to the genus *Erynia* as *Erynia nebriae* **comb. n.**

Zusammenfassung

Eine grössere Anzahl verpilzter Laufkäfer von *Nebria brevicollis* (Fabricius, 1792) wurden im Spätherbst 2016 in Nordrhein-Westfalen, Deutschland, gesammelt. Der Pilz wurde unter Beizug von Typusmaterial als *Entomophthora nebriae* Raukiaer, 1893 (Entomophthoromycotina, Entomophthoraceae) identifiziert. Dieser Pilz wurde 1893 in Dänemark beschrieben und seither nie mehr nachgewiesen. In der vorliegenden Arbeit geben wir eine erweiterte Beschreibung der Art, die wir auf Grund der morphologischen Merkmale in die Gattung *Erynia* transferieren als *Erynia nebriae* **comb. n.** Angesichts der Häufigkeit und der weiten Verbreitung von *N. brevicollis* in der Schweiz ist zu erwarten, dass *E. nebriae* auch hier vorkommt.

Introduction

Raunkiaer (1893) described *Entomophthora nebriae* as a pathogen of the ground beetle *Nebria brevicollis* F. (Coleoptera, Carabidae). Since its description in 1893, the fungus has not been reported in the literature. Recently, it was observed from near Bochum in Germany by Keller (2013). In the last three years (2014–2016) infected individuals of *N. brevicollis* were encountered regularly in a forest in the vicinity of Witten between November and January. But only at the end of 2016 was sufficient fresh material collected to enable examination of the fungus more thoroughly. In the present paper we report data of these new collections of *Entomophthora nebriae* upon which we base an amended description and propose a new combination, *Erynia nebriae*.

At the time, when Raunkiaer (1893) described *E. nebriae*, all entomophthoralean species were placed either in the genus *Entomophthora* Fresenius (1856) or in the genus *Empusa* Cohn (1855) the latter subsequently became a synonym of the former. Today the family Entomophthoraceae consists of three subfamilies, the Entomophthoroideae (four genera including *Entomophthora*) and the Erynioideae (six genera including *Erynia*) being the most important (Keller and Petrini 2005). They differ mainly by the conidiophores and the number of nuclei in the conidia. The former has unbranched conidiophores and multinucleate conidia, the latter has branched conidiophores

and uninucleate conidia. In addition to that the genus *Entomophthora* Fres. (1856) has campanulate primary conidia, a single type of secondary conidia, and cystidia are absent when conidia are produced. On the other hand the genus *Erynia* (Nowakowski ex Batko) Remaudière & Hennebert (1980) has ovoid to elongate primary conidia and two types of secondary conidia produced on short conidiophores, and long and thick cystidia.

Material and methods

Eighteen beetles with recently sporulating fungus were collected between November 19 and December 16, 2016 along a forest path in a deciduous forest dominated by beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.). The forest belongs to the recreation area Hohenstein near Witten, Nordrhein-Westfalen, Germany, situated at an altitude of about 130 m. The collection site is defined by the coordinates 51.432241N and 7.353456E.

During the collection period the day temperatures varied between 2 and 14°C and the night temperatures between minus 6 and 12°C. Freshly collected beetles with sporulating fungus were individually placed in humid chambers with a slide placed 1–2 mm above the cadaver to collect the projected primary conidia. Some of these slides with primary conidia were subsequently placed in humid chambers with a second slide 1–2 mm above the one with primary conidia to collect the projected secondary conidia. The cadavers with sporulating fungus were placed in 70% ethanol. Additionally further examination of the fungal material was achieved by removing small portions of the fungus and carefully dissecting them into thread-like pieces.

The fungal material was mounted in lactophenol-cotton blue (LPCB) or in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). All measurements were based, if not otherwise stated, on 25 structures per individual host, designated as one series. From each structure, usually more than one series was studied to assess variation. 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).

Type material, labelled "Museum Botanicum Hauniense C-F-70834" was obtained from the University of Copenhagen. The paper envelop was labelled by hand: "Entomophthora Nebriae sp. n., på Nebria brevicollis Fab., Dyrehaven 11-1888, leg. C. Raunkiaer". It contained fragments of the exoskeleton of a beetle and dust-like material adhering to the paper. Small pieces of the exoskeleton and dust-like material scratched from the paper were mounted in LPCB and microscopically examined.

Results

The infected beetles were found on bare soil (about 85%), on wood sticks, on fallen leaves or on stones (Fig. 1). The abdomina of the sporulating cadavers were extremely swollen with the forewings spread and the hindwings lifted upwards (Fig. 2). Fresh mycelium was white (Fig. 2), older mycelium yellowish (Fig. 3). The mycelium covered the entire abdomen and the posterior part of the thorax. The cadavers could easily be removed from the substrate which infers that the rhizoids did not fix them strongly to the surface.

Only a few rhizoids were encountered. They were monohyphal with a diameter ranging from 14 to 30 µm (n = 7) ending with short root-like branches (Fig. 4). The conidiophores were densely packed, hyaline and branched. The spherical nuclei had a diameter of 7.0 (6–8.5) µm. The primary conidia measured 29.6–32.4 (27–37) µm × 9.7–11.5 (8–13) µm. They were uninucleate, bitunicate, elongate subcylindrical to slightly ellipsoidal to fusiform, sometimes slightly asymmetrical, with a large central vacuole. The papilla was rounded and joined smoothly the conidial body (Figs. 6 and 7). The L/D ratio ranged from 2.82–3.11 (5 series). Secondary conidia measured 23.2 (21–26) µm × 11.7 (11–13) µm, L/D = 1.98 (1 series). Young resting spores with simple wall were spherical and measured 33.9–35.0 (31–41) µm (2 series). The number of nuclei in young resting spores varied between 7 and 16 with an average of 11.1 (Fig. 8). However, since the nuclei only stained faintly and were not clearly demarcated from the surrounding material, this number may be slightly inaccurate. Mature resting spores were spherical and measured 32.4–35.6 (27–40) µm (5 series). The spore wall was 3.5–5.0 µm thick, yellow to slightly brown and smooth (Fig. 9). Resting spores were present in all examined cadavers. The cystidia were long and thick, the diameter above the conidial layer was 23.8 (14–39) µm (1 series). They distinctly towered above the conidial layer (Figs 2, 4) and contained many nuclei (Fig. 4). More details are given in Table 1. The fungal material was deposited at the herbarium ZT, accession number ZT Myc 58425.

Table 1. Dimensions of the fungal structures (pc = primary conidia, sc = secondary conidia, yRS = young resting spores, mRS = mature resting spores, s.d. = standard deviation).

Structure	Length (L) (s.d.) min-max	Diameter (D), (s.d.), min-max	Ratio L/D	Stain
pc 1	30.8 (2.21) 27-37	10.4 (1.18) 9-13	3.11	LPAO
pc 21	29.6 (1.75) 27-33	10.2 ((0.88) 8-12	2.90	LPAO
pc 22	30.2 (1.27) 28-33	9.7 (0.85) 8-12	3.11	LPAO
pc 23	31.7 (1.61) 28-35	10.5 (0.62) 9-12	3.01	LPCB
pc 24	32.4 (2.46) 28-37	11.5 (0.64) 11-13	2.82	LPCB
sc 25	23.2 (1.52) 21-26	11.7 (0.62) 11-13	1.98	LPCB
yRS 7		35.0 (2.09) 31-41		LPAO
yRS 8		33.9 (1.81) 31-38		LPAO
mRS 4		32.4 (2.41) 28-37		LPAO
mRS 6		33.1 (2.54) 27-39		LPAO
mRS 9		33.9 (1.62) 29-37		LPAO
mRS 14		35.6 (2.36) 31-40		LPAO
mRS 15		34.2 (2.04) 31-39		LPAO
Cystidia		23.8 (6.32) 14-39		LPAO

Figures 1–3. 1. *Nebria brevicollis* at an early stage of fungus sporulation. At this stage the beetle is still able to move it's legs and antennae (nat. length of the beetle about 13 mm). **2.** Beetle with fully sporulating fungus showing the extremely swollen abdomen. The strong cystidia are clearly visible at the edge of the fungus mass. **3.** At the end of the sporulating period the mycelium turns yellowish (Photos: T. Hülsewig).

The slide with type material contained some resting spores but no other fungal material. The resting spores were spherical, smooth-walled and measured 33.6 (29–41) µm (n=35).

Discussion and conclusion

Raunkiaer (1893) found *E. nebriae* on the ground beetle *Nebria brevicollis*. He described the conidia as ellipsoidal to fusiform, asymmetrical, often slightly curved, 28–37 µm long, 10–13 µm broad, hyaline and smooth, and the resting spores as spherical, 35–50 µm diameter, hyaline to pale brown developing outside the host. Our measurements and observations match Raunkiaer's data though minor differences to the original description were noted concerning the dimensions of the resting spores. Own measurements of resting spores of the type material, however, completely matched the dimensions of resting spores present in the material collected in Germany. The place of the formation of the resting spores (outside the host) was not examined in the present study. Considering the correspondence of all comparable data (symptoms, dimensions of conidia and resting spores, host species, season of the collections) we are convinced that the examined fungus is identical with the one described by Raunkiaer.

Present data also clearly show that *E. nebriae* is not a species of *Entomophthora* but is a typical member of the genus *Erynia*. This was already recognized by Humber and Ben-Ze'ev (1981) who proposed the new combination *Erynia nebriae* but did not publish it validly (ICN 2011, Melbourne Code, Art. 45.1; see also MycoBank number 111468).

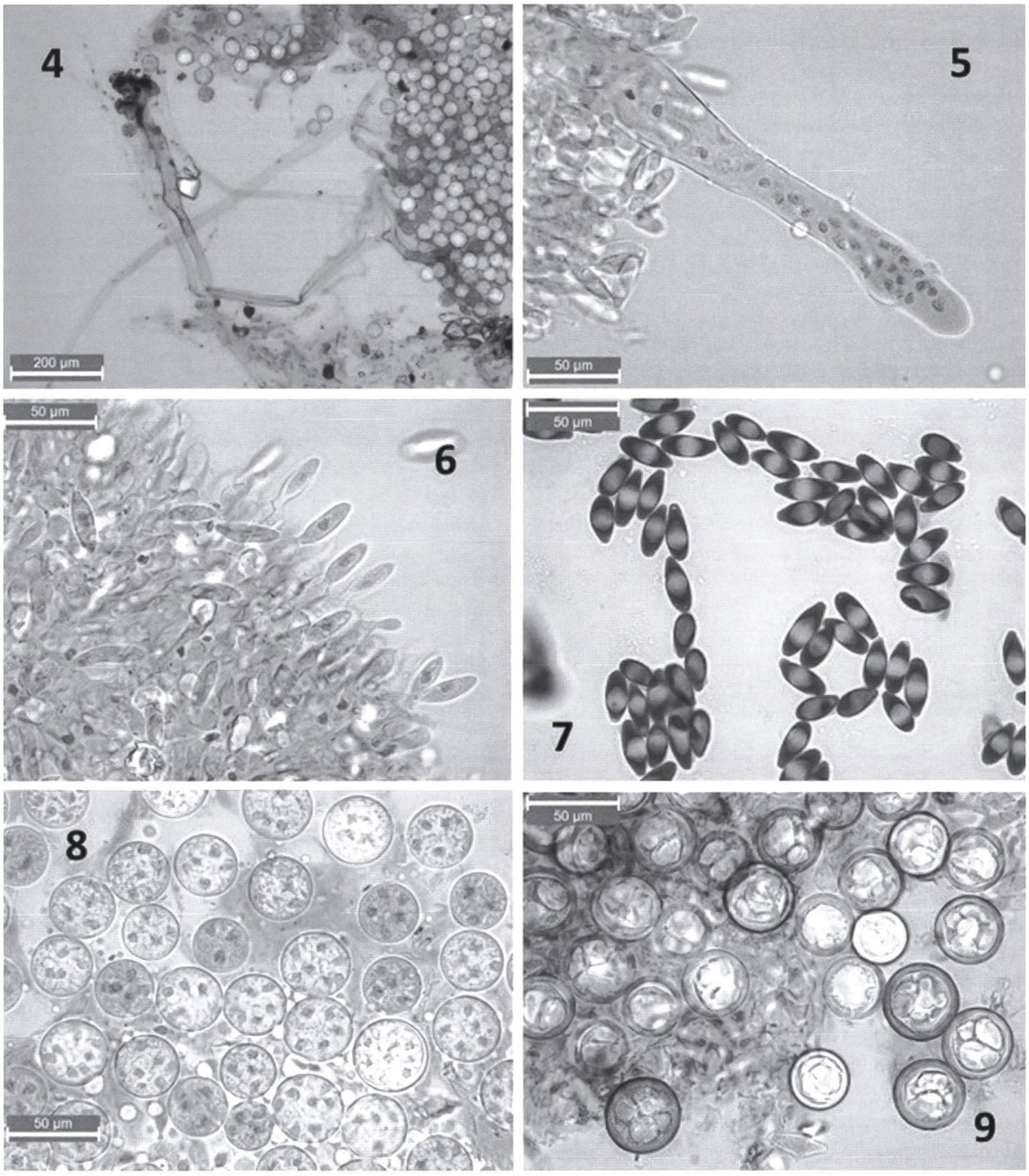
Therefore, we validate the name as

***Erynia nebriae* (Raunkiaer) S. Keller, comb. n.**
MB822120

Basionym: *Entomophthora nebriae* Raunkiaer, 1893, Bot. Tidsskr. 18: 109-110.

Syn.: *Zoophthora nebriae* (Raunkiaer) Batko (1966) MycoBank number 341184.

An aspect of the presented findings is noteworthy. The fungus was found at an epizootic level (in addition to the examined 18 individuals, there were more fungus-killed beetles, but in late state of sporulation or post sporulation) at the end of the seasonal activity of the host beetle. Even low temperatures at night, including below zero, did neither impede the epizootic nor kill the fungus. It is likely that the beetles at this time of year (late in the season) are more susceptible to the attack of the fungus. Another



Figures 4–9. 4. Rhizoid with dark and sparsely branched ending together with resting spores (LPAO). **5.** Cystidium with numerous nuclei (LPAO). **6.** Dense layer of conidiophores with developing primary conidia (LPAO). **7.** Primary conidia with prominent central vacuole (LPCB). **8.** Young resting spores with nuclei (LPAO). **9.** Mature resting spores with thick walls and one to several vacuoles depending on the stage of maturation (LPAO).

explanation is that the beetles became infected earlier in the season, before moving to their overwintering habitats. It is plausible that the fungus developed slowly within the beetles located in overwintering habitats at the low temperatures and that just prior to sporulation the fungus altered the host behaviour causing the infected beetles to leave the overwintering sites in order to die in the open environment. It is well established that many species of Entomophthorales manipulate the behaviour of their hosts to benefit their own survival and transmission (Roy et al. 2006).

Ground beetles are hosts of only two species of Entomophthorales with known taxonomic position. *Erynia nebriae* is the only one known to attack adult beetles while *Furia zabri* attacks larvae of *Zabrus tenebrioides*. The reason for this rareness may be the strong exoskeleton of the adults on one hand and the subterranean life of the larvae of most carabid species. Another species of Entomophthorales with unknown taxonomic position, *Tarichium jaczewskii* Zaprometov, was described from *Zabrus gibbosus*. According to Ben-Ze'ev and Kenneth (1982) *T. jaczewskii* is possibly the resting spore state of *F. zabri*.

Nebria brevicollis is among the best known, most widely distributed, and most frequently encountered carabid beetles in Europe. It prefers hedgerows, field borders and deciduous forests (Luka et al. 2009). In Switzerland the species is very common and widely distributed up to altitudes of about 1200 m (Luka et al. 2009). Under these circumstances we can expect the presence of *E. nebriae* in Switzerland. Unfortunately, most coleopterists are not interested in "mouldy" beetles but it would be advantageous to encourage recording of diseased beetles. This would improve our ecological knowledge of these fascinating fungi, particularly for antagonists such as Entomophthorales, and additionally contribute to improved understanding of the population dynamics of their hosts.

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