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Trichonectria calopadiicola sp. nov. (Hypocreales, Ascomycota): the second species of the family *Bionectriaceae* parasitic on foliicolous lichens discovered in Tanzania

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Abstract

Trichonectria calopadiicola, a new lichenicolous fungus from tropical Africa is described. The species is the second member of nectrioid fungi known as parasitic on foliicolous lichens. It is characterized by often aggregated, dark reddish brown, subglobose, richly setose perithecia, covered by obtuse, septate, thick-walled setae, and by hyaline, narrowly fusiform, 1-septate ascospores. The mycoparasite causes evident damages in the ascomata of its host.

Keywords: Africa, biodiversity, lichenicolous fungi, new species, mycoparasites, phyllosphere, Usambara Mountains

Introduction

The forests of the East Usambara Mountains in north-eastern Tanzania are considered to be one of the most important forest blocks in Africa. The area is comparable to the Galapagos Islands in terms of their endemism and biodiversity (Rodgers & Homewood 1982; Howell 1989; Iversen 1991). The Mtai Forest Reserve is situated in Muheza District, Tanga Region, extending between 180 and 1016 m, encompassing lowland and submontane forest. Despite of its conservation status the area covered by woodland is decreasing. It currently covers only 3107 ha, although 6107 ha was originally gazetted in 1913 (Doggart *et al.* 1999). The first author collected lichens in the area in the framework of the Integrated Usambara Rain Forest Project in 1986 and 1989 (Farkas 1987, 1991; Hamilton & Bensted-Smith 1989; Doggart *et al.* 1999). A high importance of the area is also confirmed by the frequency of foliicolous lichens of high bioindicator value in the area (Lücking 1997), and a new lichen species described by the first author from the same site: *Coenogonium subdilucidum* Farkas & Vězda (Farkas 2014). As well as *Lyromma multisetulatum* Flakus & Farkas collected there was found as new to the continent Africa (Farkas & Flakus 2015). During recent studies on foliicolous lichens from the region an interesting lichenicolous fungus has been discovered on the partly damaged apothecia of *Calopadia* cf. *puiggarii* (Müll. Arg.) Vězda (1986: 215), identified as a member of the family Bionectriaceae Samuels & Rossmann in Rossmann *et al*. (1999: 15). A unique set of characters justify its description as a species new to science. Within the family Bionectriaceae previously only one species parasitic on foliicolous lichens, *Nectriopsis porinicola* Samuels was known (Samuels 1988: 43). Bionectriaceae is one of the three families of hypocrealean fungi representing three major morphological lines and correlating phylogenetic clades revealed by Rehner & Samuels (1994, 1995), Ogawa *et al.* (1997) and Spatafora & Blackwell (1993). The family is characterized by taxa with uniloculate perithecial (rarely cleistothecial), usually pale, white to brown or yellow to red ascomata (without stroma or immersed in the substratum), not changing colour in KOH or lactic acid. The genus *Trichonectria* Kirschst. (1907: 60) is currently containing about 20 species described from various hosts and geographic areas. However, only 12 are known as lichen parasites as summarized in Table 1.

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Material and Methods

The studied material of the new species is deposited in VBI. We examined the morphology and the anatomy using an Olympus SZX9, a NIKON SMZ 800, an ECLIPSE 80i (DIC) and an Eclipse/NiU (DIC) microscope. Sections were prepared by a freezing microtome Thermo Scientific Microm HM430 (combined with BFS-MP freezing stage and BFS-3MP controller). The anatomy was studied in water, 10% KOH solution (K), lactophenol cotton blue (LPCB), and the ascus structures in Lugol's iodine solution without (I) or with pre-treatment with K (K/I) and in Congo red. The measurements of microscopic structures were made in water.

The New Species

Trichonectria calopadiicola Farkas & Flakus, *sp. nov.* (Figs. 1A–J) Mycobank MB 817763

Diagnosis: Differs from the otherwise similar *Trichonectria pertusariae* Etayo & van den Boom in having darker and larger ascomata, larger, thicker setae, and in the host selection.

Type:—TANZANIA, **East-Usambara Mts**, Mtai Forest Reserve, on the E slopes of Mtai ridge, W of Maramba village, alt. 250–500 m, in semideciduous forest and secondary dry vegetation, on apothecia of epiphyllous *Calopadia* cf. *puiggarii*, 13 Nov. 1986, *E. Farkas* 86245 (VBI 06082—holotype!).

Description. *Mycelium* hyaline, inconspicuous, at the base of the perithecia, of thin-walled hyphae, 1–3 µm wide. *Ascomata* perithecioid, lichenicolous on foliicolous *Calopadia*, immersed (when young) to superficial, situated on the surface of the host apothecia, 90–190 µm in diam, non-stromatic, not immersed in mycelium, often aggregated, dark reddish brown (when young) to almost blackish brown (when older), subspherical, occasionally flattened but generally not cupulate when drying, with a central ostiole, richly setose throughout; external surface of the perithecial wall composed of almost isodiametric cells, resembling more or less textura epidermoida, though not clearly seen due to setae hiding the surface and giving the appearance of a textura intricata. *Setae* obtuse, 2–5-septate, 12–40 × (3–)4–7 µm, usually constricted at the septa, thick-walled, with a hyaline wall but covered by a granular reddish brown pigment (K–), rarely branched, composed of cells with the lumen 1–5 µm wide and the wall 1–1.5 µm thick. *Ascomatal wall* 15–30 µm thick, paraplectenchymatous, consisting of several (5–10) layers of angular cells; outer layer reddish brown to orange (K–) due to a granular pigment scattered on the surface of the broadly ellipsoid (rarely almost isodiametric) cells, 3–9 × 1.5–4 µm; internal layer of hyaline and strongly elongated cells, 10–15 × 0.5–2 µm. *Periphyses* 6–15× 0.5–1.0 µm. *Hamathecium* gelatinized I–, K/I–. *Asci* 8-spored, 34–48 × 8–12 µm, I–, KI–, narrowly clavate, functionally unitunicate, not thickened at the apex, apical ring absent; ascogenous cells isodiametric, 4–7 µm in diam. *Ascospores* narrowly fusiform, sometimes slightly curved, ends tapering, usually constricted at the septum, smooth-walled, 1-septate, hyaline, with thin gelatine coat, usually with (1–)2–3 large guttules in each cell, biseriate to slightly spirally arranged in ascus, (10–)14–19(–21) × 2.5–3(–4) µm (n=50).

Host. The species inhabits the apothecia of foliicolous *Calopadia* cf. *puiggari*, causing destruction of the hymenial tissue.

Etymology. This new species is named after its host.

Distribution and habitat. The species is known only from the type location, where it grows epiphyllous in a semideciduous forest.

Notes. The main diagnostic feature of *Trichonectria calopadiicola* are the dark reddish brown, subglobose perithecia, which are richly covered by obtuse, septate, thick-walled setae, and narrowly fusiform ascospores. In addition the ascomata of the species are characteristically aggregated on the apothecia of its host and cause evident damages in its hymenial tissue.

Lichenicolous species of *Trichonectria* are mainly associated with macrolichens and only two species growing on crustose, corticolous lichens are known currently (Rossmann *et al.* 1999; Etayo & van den Boom 2005). *T. calopadiicola* most closely resembles *T. pertusariae* Etayo & van den Boom, growing on *Pertusaria ophthalmiza* and *P. amara* var. *slesvicensis*, because of its similar, narrowly fusiform ascospores. However, *T. pertusariae* has paler, pink-orange to brown ascomata, thinner ascomatal wall (10–12 µm wide) and setae (3–4 µm wide), and also smaller ascospores,

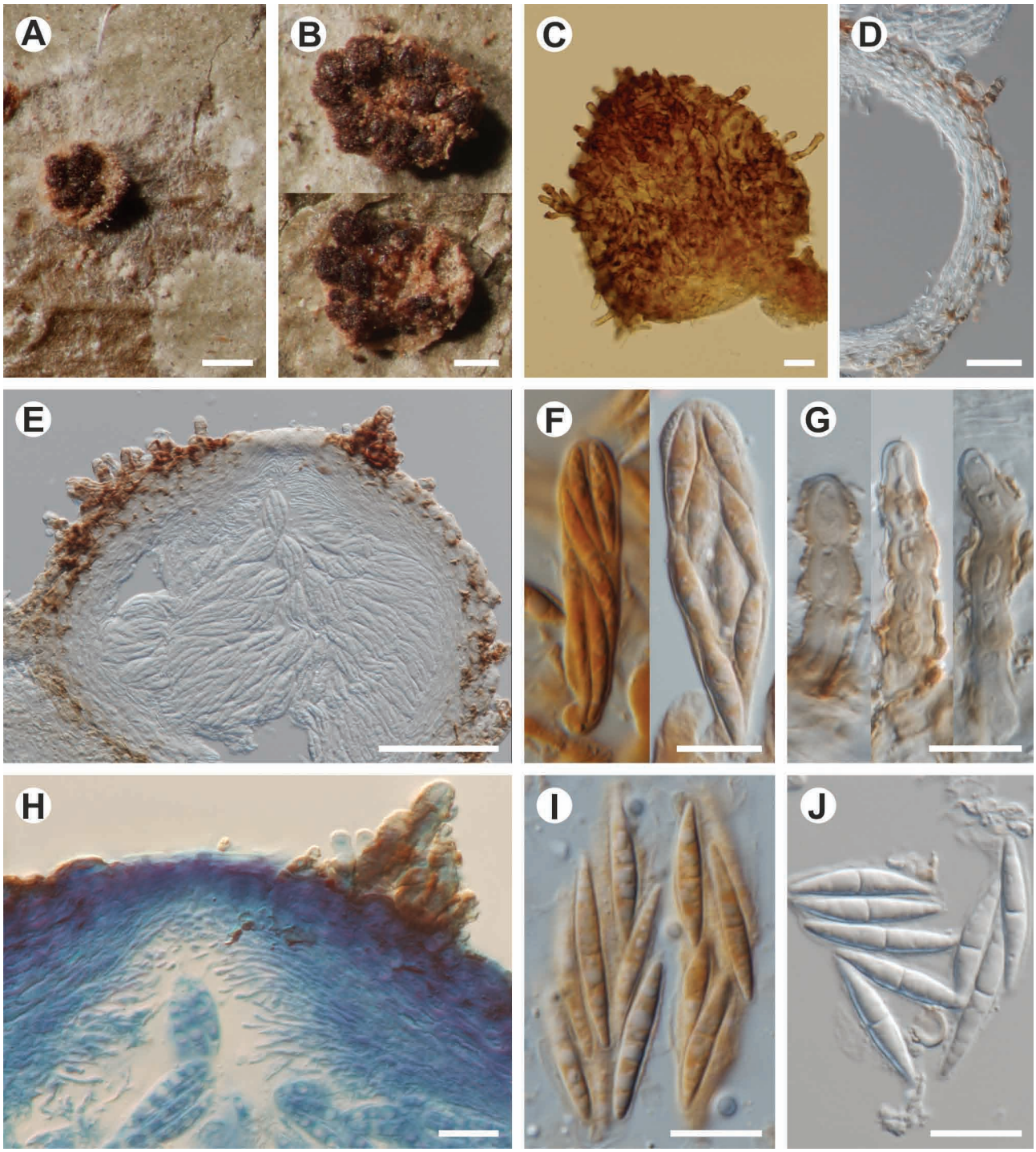

FIGURE 1. *Trichonectria calopadiicola* (*holotype*). A–B, a habit of reddish brown perithecia aggregated on apothecia of the host. C, external surface of the perithecia. D, cross section of the ascomatal wall showing a hyaline paraplectenchymatous tissue with pigmented external layer. E, cross section of an ascoma. F, asci (mounted in Congo Red). G, septate and thick-walled setae, with hyaline wall covered by a granular reddish brown pigment. H, periphyses (mounted in LPCB). I, ascospores with 2–3 oil droplets per cell (mounted in Congo Red). J, ascospores with thin gelatine coat (in tap water). Scales: A– B—200 µm, C–D—20 µm, E—50 µm, F–J—10 µm.

(8–)10–11(–15) × 2–3 µm (Etayo & van den Boom 2005). The second species, associated with crustose lichens, is *Trichonectria hirta* (A. Bloxam) Petch, reported from *Lecanora conizaeoides*, *Lepraria incana, Micarea prasina, Placynthiella dasea* and *P. uliginosa*, among others. It can be distingushed easily by its pale orange ascomata and larger (45–120 × 4.5–8 µm), very characteristic (vermiform and multiseptate) ascospores (Hawksworth 1978; Rossman *et al.* 1999; Kukwa & Czarnota 2006).

TABLE 1. Lichenicolous species of *Trichonectria*.

Species name	Publications	Ascomata diam.	Colour of ascomata	Setae	Ascospores size	Hosts	Distribution
T. anisospora (Lowen) van den Boom & Diederich	Lowen (1989: 248), (1990: 461); Sérusiaux *et al.* (2003: 33)	140–200 µm	bright orange	non-septate to few septate, hyaline, 12–32 × 3.5–5.5 µm, in groups of 4 or 5	14.5–17 × 4–6.5 µm	on Parmeliaceae (*Hypogymnia physodes*, *H. tubulosa*)	Europe, North America (USA)
T. australis Etayo 2008	Etayo & Sancho (2008: 253)	100–170 µm	orange	non-septate, hyaline, 29–33 × 3.5–5 µm	8–11 × 2–3 µm	on Parmeliaceae (*Usnea sp., Menegazzia globulifera*)	South America (Argentina)
T. cladoniicola (Alstrup & Svane) Alstrup	Alstrup & Svane (1998: 23); Alstrup *et al.* (2004: 45)	100–200 µm	bright orange	non-septate, hyaline, 50–90 × 6–8 µm	26–30 × 3–5 µm	on Cladoniaceae (*Cladonia stygia*, *C. merochlorophaea*)	Europe
T. furcatosetosa Brackel	Brackel (2014: 386)	150–250 µm	pale orange	septate at the base, hyaline, furcate, 35–55 × 3–4.5 µm	(10–)10.9–13.2(–14.5) × (3–)3.4–4.1(–4.5) µm	on epiphytic lichens (*Melanelixia sp., M. subaurifera, M. glabratula*, *Parmelia sulcata*, *Physcia tenella*, *Candelariella reflexa*)	Europe
T. hirta (A. Bloxam) Petch	Bloxam in Currey (1863: 158); Saccardo (1878: 307), (1883: 542); Massee & Crossland (1904: 4); Rossman (1983: 77); Rossman *et al.* (1999: 77); Brackel (2014: 389)	230–300(–500) µm	white to pale orange (pinkish salmoncolour)	white, prominent, (43–)80–100 × 5–8 µm	45–85(–120) × 4.5–8(–10) µm	on crustose lichens (*Lecanora conizaeoides*, *Lepraria incana*, *Micarea prasina*, *Placynthiella dasea*, *P. uliginosa, Scoliciosporum chlorococcum*)	Europe
T. hypotrachynae Etayo	Etayo (2002: 132)	100–120 µm	brownish orange	2-4-septate, 25–42 × 4.5–6 µm	11–13 × 5–6 µm	on Parmeliaceae (*Hypotrachyna*)	South America (Colombia)
T. leptogiicola Etayo	Etayo (2001: 221)	150–200 µm	rose to orange	non-septate, 15–30 × 3.5–5 µm	8–10.5 × 3–4 µm (ornamented)	on Collemataceae (*Leptogium phyllocarpum*)	South America (Ecuador)
T. pertusariae Etayo & van den Boom	Etayo & van den Boom (2005: 160)	100–130 µm	pink-orange to brown	septate, concolorous with ascomatal wall, 15–20(–30) × 3–4 µm	(8–)10–11(–15) × 2–3 µm hyline, 1-septate,	on epiphytic crustose lichens (*Pertusaria amara* var. *slesvicensis, P. ophthalmiza*)	Europe
T. pyrenaica Gardiennet & Lechat	Gardiennet & Lechat (2011: 296)	120–150 µm	pale orange to pale orange-brown	25–30 × 15–20 µm (papillae)	(9–)11–14(–15) × 3–4.5 µm	on Parmeliaceae (*Usnea*)	Europe
T. rubefaciens (Ellis & Everh.) Diederich & Schroers	Ellis & Everhart (1887: 116); Etayo & Sancho (2008: 256)	80–160 µm	subhyaline, pallid, then orange-red	0–1 septate, hyaline	(12–)14–18 × 2.5–3(–4) µm	on Parmeliaceae (*Flavoparmelia*, *Parmelia*, *Parmotrema*, *Punctelia* and others)	Europe
T. setadpressa Etayo	Etayo (2002: 134)	100–130 µm	orange to brownish orange	septate, concolorous with ascomatal wall, 5–13 × 2–4 µm	6–8 × 2–3 µm	on Lobariaceae (*Lobariella pallida*)	South America (Colombia)
T. usneicola Etayo	Etayo (2002: 137)	150–170 µm	vivid orange	1–2-septate, concolorous with ascomatal wall, 12–48 × 4–5.5 µm	14–16.5 × 4.5–5 µm	on Parmeliaceae (*Usnea*)	South America (Colombia)

One of the most common species of the genus, *Trichonectria rubefaciens* (Ellis & Everh.) Diederich & Schroers, is similar to *T. calopadiicola* in the size of the ascospores, but differs in having subhyaline to red-orange, smaller (80–160 µm) ascomata, with thinner ascomatal wall (10–12 µm wide), shorter, 0–1-septate, hyaline setae, scattered around the ostiole, and is growing on members of *Parmeliaceae* (Sérusiaux *et al.* 1999; Lowen 1995).

It is worth pointing out that only one species of nectrioid fungi, *Nectriopsis porinicola*, is known as a parasite of foliicolous lichens. *N. porinicola* was described from ascomata of foliicolous *Porina epiphylla* Fée s.l. collected in Indonesia, and has yellow to almost white, small (to 80 µm in diam.) ascomata, furnished with setae composed of 2–3 agglutinated hyphae, and small ascospores (9–)9.5–10.7(–11.5) × 2.2–3(–3.3) µm, significantly distinguishing it from *T. calopadiicola* (Samuels 1988). Another species, *Trichonectria erythroxylifoliae* (Viégas) Samuels was described from the same habitat, the leaves of *Erythroxylum suberosum*, from Brazil. Although the species has fusiform ascospores of similar size with those of the new species, it has paler ascomata, becoming cupulate when dry, covered by non-septate, acute setae, and is probably not fungicolous (Samuels 1988).

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