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## A new species of *Laboulbenia* (Ascomycota) parasitic on an African fly (Diptera: Curtonotidae), with a brief review of Diptera-associated species of the genus

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### ABSTRACT

A new parasitic fungus, *Laboulbenia curtonoti* sp. n., associated with the endemic Madagascan fly *Curtonotum balachowskyi* Tsacas (Diptera: Curtonotidae), is described and figured. The new species is one of the very few *Laboulbeniales* which penetrate the insect's cuticle by means of more-or-less developed rhizoids. A brief review of the 23 species of the genus *Laboulbenia* associated with Diptera is presented in a tabulated form.

KEY WORDS: Ascomycota, Curtonotidae, *Curtonotum balachowskyi*, Diptera, *Laboulbeniales*, Madagascar, new species, parasitic fungi, entomopathology, review.

### INTRODUCTION

*Laboulbeniales* is a large group of ascomycete fungi which complete their entire life-cycle on living arthropods. Most are found on insects, but a few are known to parasitise mites and millipedes. These fungi are chiefly ectoparasitic, but a small number of the nearly 2,000 described species penetrate the insect's cuticle by means of more-or-less developed rhizoids.

With almost 600 described species, the genus *Laboulbenia* is by far the largest among the *Laboulbeniales* (Rossi 2011). The number of species of *Laboulbenia* parasitic on the Diptera is, however, relatively small, only 23 having been described to date. These are reported from the dipterous families Celyphidae, Curtonotidae (as Drosophilidae), Chloropidae, Diopsidae, Lauxaniidae, Psilidae, Richardiidae, Tephritidae and various unidentified flies (Balazuc 1980; Lee & Majewski 1986; Rossi & Kotrba 2004; Rossi & Ponziani 2008; Rossi 1986, 1987; Thaxter 1901, 1902, 1917, 1918). A review of all previous records of *Laboulbenia* spp. associated with the Diptera is presented in tabulated form below (Table 1). Included names are updated, although some familial names were not specified in the original publications and some may represent misidentifications. It should be borne in mind that about half of the listed associations represent records from the African continent. It is also noteworthy that only a single fungal species, *Laboulbenia diopsidis* Thaxt., has subsequently been recorded after its original description. This does not represent evidence of rarity, but rather bears witness to the scarcity of research done in this field.

During the course of an ongoing revision of Madagascan *Curtonotum* Macquart, 1844 by A. Kirk-Spriggs, numerous specimens of the Malagasy endemic species *Curtonotum balachowskyi* Tsacas, 1974, were discovered parasitised by a fungus (e.g., Figs 1, 2). Further examination revealed that this parasite represents a new species of the genus *Laboulbenia*, which is described herein.

## MATERIAL AND METHODS

Insects bearing Laboulbeniales were found in samples preserved in 95% ethanol, resulting from Mike Irwin's *Arthropod Survey of Madagascar's Protected Areas*, on loan from the California Academy of Sciences, USA (CASC). Thalli were carefully removed from the hosts with a size 3 insect pin. Permanent microscope slides of fungi were prepared following the method described by Benjamin (1971), using Amann's solution, which is a mixture of glycerol, phenol and lactic acid, as mounting medium; cover slips were ringed with transparent nail varnish.

Slides of the type series are preserved in the Botanical Museum of Florence, Italy (FI) except for the isotype, which is deposited at the National Museum, Bloemfontein, South Africa (BMSA).

## TAXONOMY

Order Laboulbeniales Lindau, 1898

Genus *Laboulbenia* Mont. & C.P. Robin, 1853

***Laboulbenia curtonoti* sp. n.**

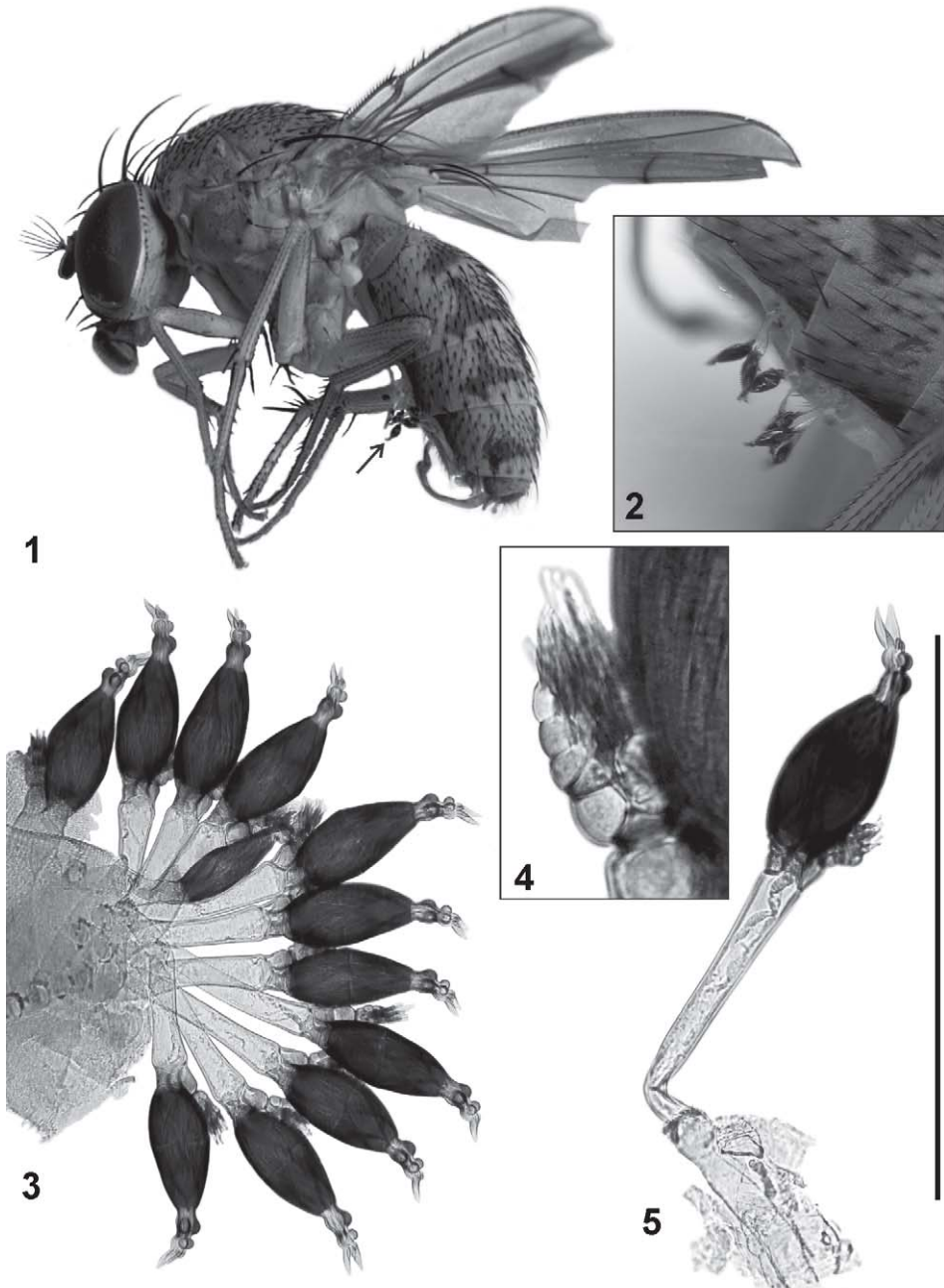
Figs 1–5

**Eymology:** The specific epithet *curtonoti* is derived from the name of the host insect (*Curtonotum*).

**Description:**

*Cellula I fere sphaerica, tota aut ex maiore parte inserta in corporem hospitis; thallus infra integumentum in magnam hypham productus. Cellula II fere hyalina, elongatum ac uniformem stipitem efficiens, sursum paulo dilatatum. Cellula appendicem sufferens grisea, sesqui longior quam lator, tertia superiore parte soluta, superficie superioris ac exterioris partis striata. Psallium clare brevius quam latitudo cellulae subiacentis. Exterior appendix grisea, constans e magna basali cellula extra prominenti et 3–4 superpositis cellulis gradatim minoribus, ex quibus confertus caespes elongatorum ac fusciorum antheridiorum gignit. Interior appendix ex una parva cellula constans ex qua oriuntur duo breves deducti ramuli singulum caespitem antheridiorum in summo ferentes; in maturis speciminibus interior appendix in tenues, hyalinos ac curvatos ramulos producit. Cellula VI grisea, striata, tam longa quam maxima latitudo. Perithecii venter solutus, fuscus, ovoideus, verticalibus septis in laxam spiram tortis. Perithecii collum abrupte angustatum, griseum, breve, incurvatum, tres protuberationes ferens. Dilute griseus apex tres protuberationes fert, in duo magna, hyalina ac paulo deducta labia, aures asini efficientia, desinens. Tota longitudo 410–520  $\mu\text{m}$ , ascosporae 55  $\mu\text{m}$ . Parasitus Curtonoti balachowskyi in Africa meridionali, in insula Madagascar nominata.*

Cell I subspherical, partially to entirely inserted within host's integument; thallus penetrating beneath integument with large haustorium (rhizomycelium). Cell II subhyaline, forming elongated stalk of uniform diameter throughout, only slightly enlarged apically. Stalk-cell of appendage (cells III+IV+V) greyish, about 1.5× longer than broad, its upper third free, although often in contact with base of perithecium; surface of upper, outer portion finely striate. Insertion cell dark, but not opaque, distinctly narrower than stalk-cell of appendage. Outer appendage greyish, with large basal cell bulging externally, followed by series of 3 or 4 gradually narrowing, superimposed cells, bearing dense tuft of elongate, brownish antheridia on inner side. Inner appendage consisting of small



Figs 1–5. (1, 2) *Curtonotum balachowskyi* Tsacas (Madagascar) bearing *Laboulbenia curtonoti* sp. n.: (1) habitus lateral (arrow indicates cluster of parasitic fungi on abdominal sternites); (2) same, detail (bulges of the insect's integument are clearly visible at the base of the thalli, caused by the spherical basal cells). (3–5) *Laboulbenia curtonoti* sp. n.: (3) cluster of thalli; (4) appendage; (5) single thallus (a portion of the large haustorium penetrating the host's abdomen is visible). Scale bar: Figs 1, 2 not to scale; Fig. 3, 650  $\mu$ m; Fig. 4, 120  $\mu$ m; Fig. 5, 500  $\mu$ m.

basal cell giving rise on either side to very short branchlets, bearing apically crown of antheridia; in older specimens antheridia replaced by slender, hyaline, curved, sterile appendages, which barely reach half the height of perithecium. Stalk-cell of perithecium (cell VI) greyish, about as long as maximum width, its surface inconspicuously striate, separated from cell below by distinctly oblique septum. Perithecial venter free, blackish brown, ovoid, wall cells slightly twisted. Neck abruptly constricted, greyish, relatively short, distinctly curved, bearing two symmetrical, rounded prominences on inner (dorsal) side and smaller and paler bulge on outer side. Tip pale grey, also bearing three prominences positioned above those of neck; two inner ones hemispherical, surmounted by two small, erect, parallel, finger-like lips; outer one larger and less protruding, giving rise to two large, hyaline, slightly diverging, pointed, horn-like lips. Total length (from insect cuticle) 410–520  $\mu\text{m}$ , ascospores 55  $\mu\text{m}$ .

Holotype: MADAGASCAR: *Majunga Prov.*: Maintirano District, Asondrodava dry forest 15 km N of Maintirano, 17°57.92'S:44°2.13'E, 200 ft [61 m], Malaise trap, 31.xii.2007–7.i.2008, M. Irwin & R. Harin'Hala, on the underside of the abdomen of *Curtonotum balachowskyi* Tsacas (Diptera: Curtonotidae), No. 3510a (FI).

Isotype: same data as the holotype, No. 3510b (BMSA).

Paratypes: MADAGASCAR: *Majunga Prov.*: Same data as the type, No. 3486; Besalamy District, Marofotofra palm forest 17 km W of Besalamy, 16°43.30'S:44°25.42'E, 35 ft [11 m], Malaise trap, 19–26.xi.2007, M. Irwin & R. Harin'Hala, Nos. 3540 & 3546 (FI).

#### DISCUSSION

The small group of species of *Laboulbenia* parasitic on Diptera is paraphyletic. From the morphological standpoint it can be divided in three groups: one in which cells III, IV and V are distinct; one with these cells undivided; and one with a distinct cell V and cells III+IV fused together.

The very peculiar distal portion of the perithecium of *Laboulbenia curtonoti* sp. n., with the two large lips resembling the ears of a donkey, makes it possible to immediately distinguish it from all other species of the genus. The only species to which it can eventually be compared is *L. dahlii* (Thaxt.) Thaxt. (= *Ceraomyces dahlii* Thaxt.), described from an unidentified “flower fly” from New Britain (Thaxter 1901). This latter parasite also penetrates the host with a long haustorium and shares the undivided stalk cell of the appendage, but differs from *L. curtonoti* in almost every other respect.

Preservation for extended periods in 95% ethanol, as these specimens had been, results in host insects becoming stiff and brittle, and as a result, it was not possible to extract the penetrating haustorium intact. It was possible, however, to observe that the basal portion of the haustorium is still quite large (diameter *ca* 30  $\mu\text{m}$ ) and undivided after 420  $\mu\text{m}$ . From this it can be inferred that it is long and ramified in the distal portion as in *L. dahlii* (Thaxter 1908, pl. XLIII, fig. 4).

From preserved museum specimens it is also impossible to ascertain the degree of damage caused by the parasite to its host. Experimental studies undertaken with *Stigmatomyces ceratophorus* Whisler indicated that the life span of its host, the fly *Fannia canicularis* (L.), was not influenced by the presence of Laboulbeniales (Whisler 1968). This example provides only limited evidence, however, as *S. ceratophorus* is a “pure” ectoparasite and flies reared under experimental condition are not subject to predator pressure or food shortages. It can be assumed in the present case that the presence of large haustoria of *L. curtonoti* sp. n. may be detrimental to the host, especially when these occur in critical numbers on a single host fly (Fig. 3).

TABLE 1  
Species of *Laboulbenia* parasitic on Diptera.

Fungus species	Host family	Host (of type)	Country (of type)	Reference
<i>admirabilis</i> Y.B. Lee & T. Majewski	Celyphidae	<i>Spaniocephylus</i> sp.	Malaysia	Lee & Majewski (1986)
<i>anguifera</i> Thaxt.	Chloropidae	<i>Hippelates</i> sp.	Grenada	Thaxter (1917)
<i>apotropinae</i> W. Rossi & Ponziani	Chloropidae	<i>Apotropina panamensis</i> Malloch	French Guyana	Rossi & Ponziani (2008)
<i>clavulifera</i> Thaxt.	?Lauxaniidae	? <i>Physogenia</i> sp.	Cameroon	Thaxter (1918)
<i>crispata</i> Thaxt.	Chloropidae	<i>Hippelates</i> sp.	Panama	Thaxter (1917)
<i>curtonoti</i> sp. n.	Curtonotidae	<i>Curtonotum balachowskyi</i>	Madagascar	this paper
<i>cyrtomatis</i> Balazuc	Curtonotidae	<i>Cyrtona sensu lato</i> sp.	Senegal	Balazuc (1980)
<i>dahlia</i> (Thaxt.) Thaxt.	?family	flower fly [ <i>sic!</i> ]	New Guinea	Thaxter (1901)
<i>diopsidis</i> Thaxt.	Diopsidae	<i>Diopsis longicornis</i> Macquart	Liberia	Thaxter (1902)
<i>gratiellae</i> W. Rossi	Diopsidae	<i>Cyrtodiopsis dalmanni</i> (Wiedemann)	Malaysia	Rossi (1987)
<i>lagarocerina</i> Thaxt.	Chloropidae	<i>Lagaroceras</i> sp.	Gambia	Thaxter (1918)
<i>lomaensis</i> W. Rossi	Tephritidae	gen. sp.	Sierra Leone	Rossi (1986)
<i>matilei</i> Balazuc	Curtonotidae	<i>Cyrtona sensu lato</i> sp.	Central African Rep.	Balazuc (1980)
<i>muiriana</i> Thaxt.	Chloropidae	gen. sp.	Papua New Guinea	Thaxter (1918)
<i>muscaria</i> Thaxt.	Lauxaniidae	<i>Xenochaetina flavipennis</i> (Fabricius)	Guatemala	Thaxter (1917)
<i>pachylophi</i> Thaxt.	Chloropidae	<i>Pachylophus frontalis</i> Loew	Tanzania	Thaxter (1918)
<i>pectinulifera</i> Thaxt.	?Lauxaniidae	? <i>Physogenia</i> sp.	Cameroon	Thaxter (1918)
<i>porrigens</i> Thaxt.	?family	gen. sp.	Cameroon	Thaxter (1901)
<i>psilina</i> Thaxt.	Psilidae	? <i>Psila</i> sp.	Cameroon	Thaxter (1901)
<i>richardiana</i> W. Rossi & Kotrba	Richardiidae	<i>Richardia teevani</i> Curran	French Guyana	Rossi & Kotrba (2004)
<i>sapromyzae</i> Thaxt.	Lauxaniidae	<i>Sapromyza triseriata</i> Coquillett	Guatemala	Thaxter (1917)
<i>sphyracephalae</i> W. Rossi	Diopsidae	<i>Sphyracephala detrahens</i> Walker	Philippines	Rossi (1987)
<i>steleoceri</i> Thaxt.	Chloropidae	<i>Mepachymerus baculus</i> Speiser	Gambia	Thaxter (1918)
<i>tsacasii</i> Balazuc	Curtonotidae	<i>Cyrtona sensu lato</i> sp.	South Africa	Balazuc (1980)

Three additional species of *Laboulbenia* have been reported on flies of the family Curtonotidae. These fungi, which were all found on unidentified species of *Cyrtona* Séguy *sensu lato* (as *Cyrtona* [*sic!*]), are *L. cyrtomatis* Balazuc (Senegal), *L. matilei* Balazuc (Central African Republic), and *L. tsacasii* Balazuc (KwaZulu-Natal Province, South Africa) (Balazuc 1980). Besides having a very different perithecial neck and tip, the last-named three species also differ from *L. curtonoti* in having different appendages and a “normal” basal cell (cell I), bearing the “usual” black foot at the base, not penetrating the host.



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