LABOULBENIALES NEW FOR BELGIUM (1)

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Summary

In our continued search for Laboulbeniales we report five *Laboulbenia* species, all representing new records for Belgium. Brief descriptions, notes and photographs are given for *L. contorta*, *L. curtipes*, *L. patrata*, *L. paradoxa* and *L. uncinata*.

Samenvatting

In onze zoektocht naar Laboulbeniales rapporteren we hier vijf *Laboulbenia*-soorten, allemaal nieuw voor België. We geven korte beschrijvingen, aantekeningen en foto illustraties van *L. contorta*, *L. curtipes*, *L. patrata*, *L. paradoxa* en *L. uncinata.*

Keywords: Laboulbeniales, Belgium, *L. contorta*, *L. curtipes*, *L. patrata*, *L. paradoxa* and *L. uncinata.*

1. Introduction

Although the last decades we have been looking on and off for Laboulbeniomycetes, the probability to find new taxa in Belgium is still fairly high. This can easily be deduced from the distribution data of European Laboulbeniomycetes given by Santamaria & Pedersen (2021, Appendix 1, gives 10 countries, pg. 387) and Haelewaters & De Kesel (2021, Belgium and the Netherlands). At this point we still lack about 140 species, all reported from one or more neighboring countries with whom we share – at least partly – a similar climate and host availability (The Netherlands, Germany, the UK and Denmark). Based on this simple extrapolation we estimate the number of Laboulbeniomycetes in Belgium at about 280 species, i.e. the double of what we recorded so far. Broadly speaking, we are looking for at least 25 species in *Laboulbenia*, 15 in *Stigmatomyces*, 40 on aquatic host taxa, and 70 more on a variety of hosts that we never had the chance to screen. In order to find some of these taxa we re-checked part of our collection material and collected potential hosts in a number of new sites. This resulted in five new species records for Belgium and additional corrections to the *Catalogue* (De Kesel *et al*. 2020).

2. Materials & methods

Collecting of host insects was done using an insect pooter (mouth aspirator) or pitfall traps filled with water and 5% propane-1.2-diol (killing and conserving agent). Long-term preservation of insects was in 96% ethanol. Insects were screened for Laboulbeniales under a dissecting microscope (50×). Thalli were removed at the foot using an insect pin (Sphinx stainless steel nr. 000). They were then transferred to a tiny droplet of Hoyer's medium (30 g Arabic gum, 200 g chloral hydrate, 16 mL glycerol, 50 mL ddH_2O) on a microscope slide, and immediately arranged. Slides were closed with a cover slip carrying a drop of Amann solution (Benjamin 1971), then sealed with transparent nail varnish. Photographs and measurements were made using an Olympus BX51 light microscope with DIC optics and drawing tube, digital camera and analySIS® (Soft Imaging System GmbH). Measurements and scaling of drawings were checked using a 0.01 mm micrometer calibration slide. Image treatment and compositions were made using GIMP 2.8 software. Carabidae hosts were identified using Muilwijk *et al.* (2015), Elateridae with Lompe (2002). Hosts and microscope slides are deposited at the Herbarium of Meise Botanic Garden (BR). Fungal names correspond to Index Fungorum (2022). Terminology and more details concerning these methods can be found in Sterbeeckia 36 (De Kesel *et al.* 2020).

3. Results – new country records for Belgium

Laboulbenia contorta Thaxt. **Fig. 1. a-e**
Proc. Amer. Acad. Arts & Sci. 27: 42 (1892)

Brief description
Thallus with a double kink, one of about 90° just above cell II and a second at cells IV and V. Outer appendage long and bifurcating on its supra basal cell. Inner appendage long, simple, with 2 antheridia on its suprabasal cell (sometimes on its basal or its third cell). Perithecium with asymmetrical and ventrally bent apex, anterior lips much smaller than the posterior ones. Thalli up to 400 µm long.

Studied material:
On *Pterostichus vernalis* (Panzer, 1796) [Coleoptera, Carabidae]. BELGIUM, prov. Luxembourg, Chiny, grassland, in remnants of mowed grass and hay, 04/07/2020, 49°42'40,73''N - 5°21'03.92''E, alt. 349m. Coll. Gerstmans C., slide **CG516a** (cluster of thalli from right side of pronotum), CG516b (= *Laboulbenia flagellata* Speg. from the same host specimen).

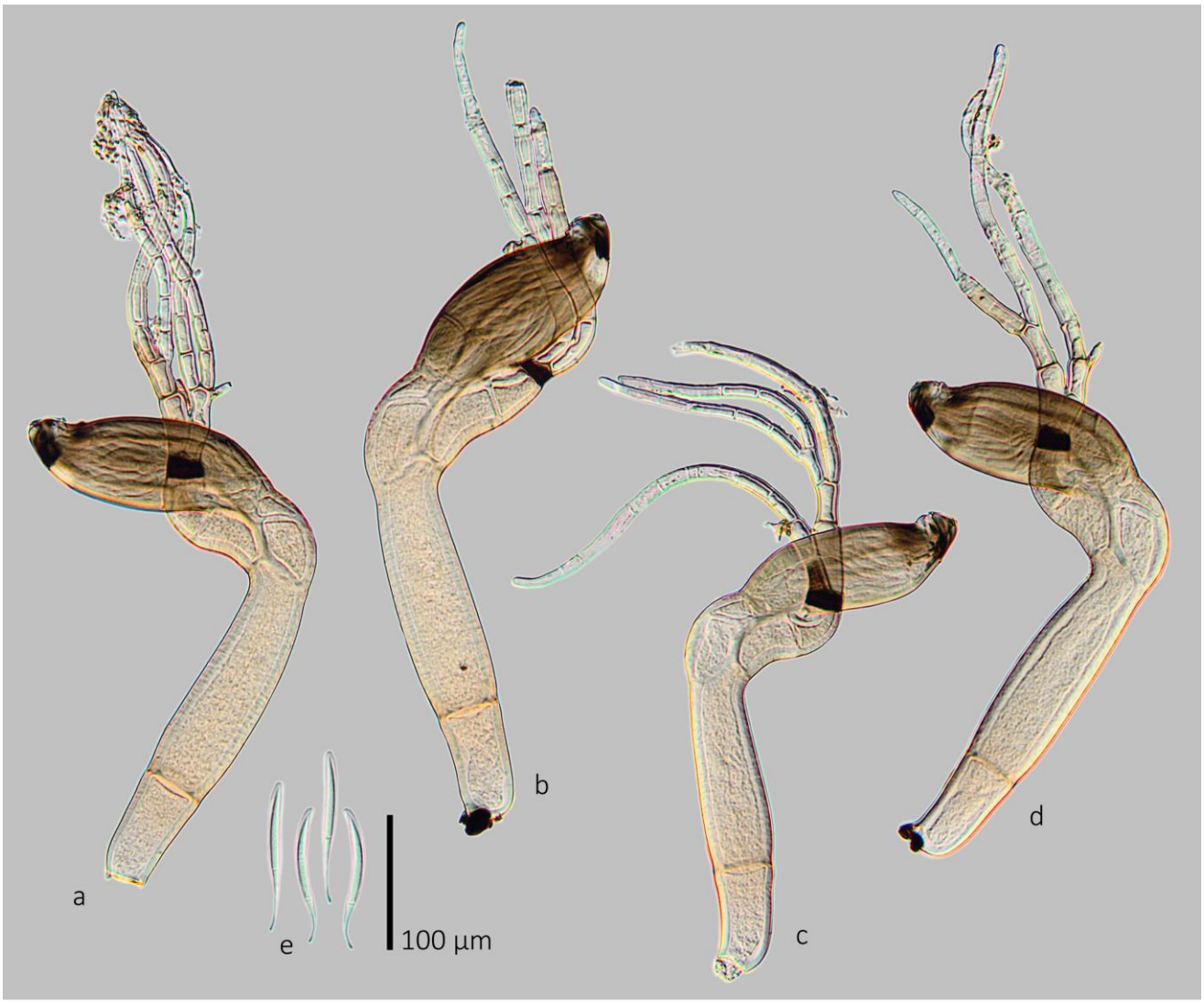

Fig. 1. *Laboulbenia contorta* from *Pterostichus vernalis* (Coleoptera, Carabidae), **a-d**. mature thalli, **e.** ascospores. All from herb. CG516a.

On *Pterostichus strenuus* (Panzer, 1796) [Coleoptera, Carabidae]. BELGIUM, prov. Vlaams Brabant, Meise, domein van Bouchout, 04/08/2008. Coll. De Kesel A., slide **ADK4657** (on pronotum (as *Laboulbenia kajanensis* Huldén in De Kesel *et al*. 2020).

Notes

Based on the orientation of its appendage system, *L. contorta* fits in an artificial group of species including for example *Laboulbenia uncinata* Thaxt., *Laboulbenia kajanensis* Huldén and *L. chionophila* Santam. As pointed out by Santamaria *et al.* (2020), this particular morphology seems to be related to a growth under the pronotum. Our material confirms this.

L. contorta is rare, only known from the US (Thaxter 1892) and much later also reported in Europe from Spain (Santamaria 1998) and Sweden (Huggert 2010, Santamaria & Pedersen 2021).

Laboulbenia curtipes Thaxt. **Fig. 2a**

Proc. Amer. Acad. Arts & Sci. 27: 40 (1892)

Brief description

Thallus with some features reminding *L. pedicellata*, but easily recognized by a relatively large and entirely free perithecium. The latter without large, dark ostiolar spots. Receptacle very small compared to the perithecium. Insertion cell small, situated at the very base of the perithecium. Cell IV and V of similar height. Appendage system short, basal cell of outer appendage (normally) with a dark septum.

Studied material:

On *Bembidion assimile* Gyllenhall, 1810 [Coleoptera, Carabidae]. BELGIUM, West-Vlaanderen, Woumen-Merkem (Diksmuide), De Blankaart, 1/9/1988, leg. C. Decleer, coll. A. De Kesel, slide **ADK6576** (thalli from tarsi of left meso tibia).

Notes

L. curtipes always grows on the distal parts of legs and tarsi of ground beetles (Carabidae) from the genus *Bembidion* (Santamaria 1998). The hosts we screened for *L. curtipes* (*Bembidion assimile)* were all infected with *L. murmanica* Huldén. *L. curtipes* is very rare in our material and - as expected - only found on tarsi. Under the dissecting microscope we initially found many thalli on the tarsi of *Bembidion assimile*, but after preparation most of these turned out to be young thalli of *L. murmanica* (Fig. 2b,c). This indicates that *L. murmanica* is morphologically stable

when growing on tarsi, and that *L. curtipes* is not some growth form of it. Our observation strengthens the statements of Majewski (1994), Santamaria (1998) and Santamaria & Pedersen (2021), i.e., that *L. curtipes* is not a growth form of *L. pedicellata* or *L. luxurians,* as suggested earlier by Scheloske (1969). The appendage system of *L. curtipes* is finer than the one from *L. murmanica* and fits the description given by Santamaria (1998, Fig. 9e & g).
L. curtipes was described from *Bembidion bimaculatum* (Kirby, 1837), a species only known from North America. The species is new for Belgium and previously reported from Spain (Santamaria 1998), Poland (Majewski 1994, 2008), Finland, Sweden, Russia (Huldén 1983), Bulgaria (Rossi *et al.* 2019a) and Denmark (Santamaria & Pedersen 2021).

Laboulbenia paradoxa Speg.. **Fig. 3. a-c**
Anal. Mus. nac. Hist. nat. B. Aires 27: 58 (1915)

Brief description
L. paradoxa is a very dark species with a fairly short, curved, pigmented and upward tapering outer appendage composed of 3-4 cells that are separated by slightly darker septa; its basal cell always trapezoid, with a very broad base, the terminal cell short or elongate, with pointed or round apex.

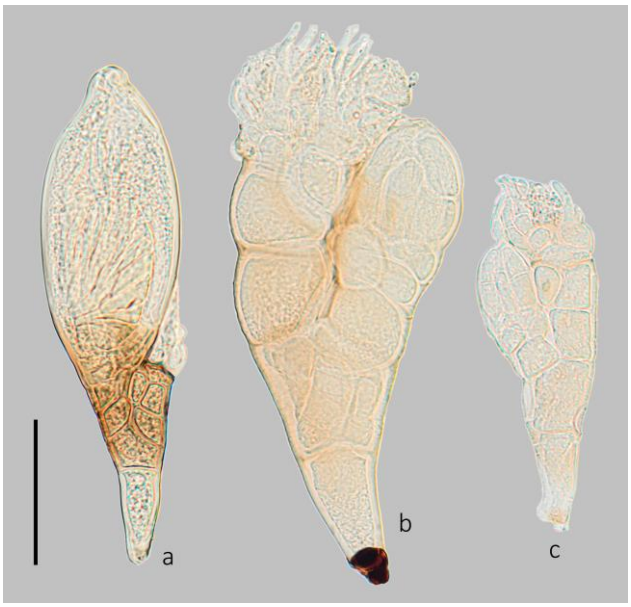

Fig. 2. *Laboulbenia spp*. from *Bembidion assimile* (Coleoptera, Carabidae), **a.** *Laboulbenia curtipes*, mature thallus from tarsi (herb. ADK6576), **b.** *Laboulbenia murmanica*, juvenile thallus from tars (herb. ADK6577b), **c.** *Laboulbenia murmanica*, juvenile thallus from tars (herb. ADK6578a). Scale bar 50 µm.

Fig. 3. *Laboulbenia paradoxa* from *Bembidion decorum* (Coleoptera, Carabidae), **a.** mature thallus showing released spores (herb. CG622b), **b.** mature thalli (herb. CG622a), **c.** juvenile thallus (herb. CG622a). Scale bar 50 µm.

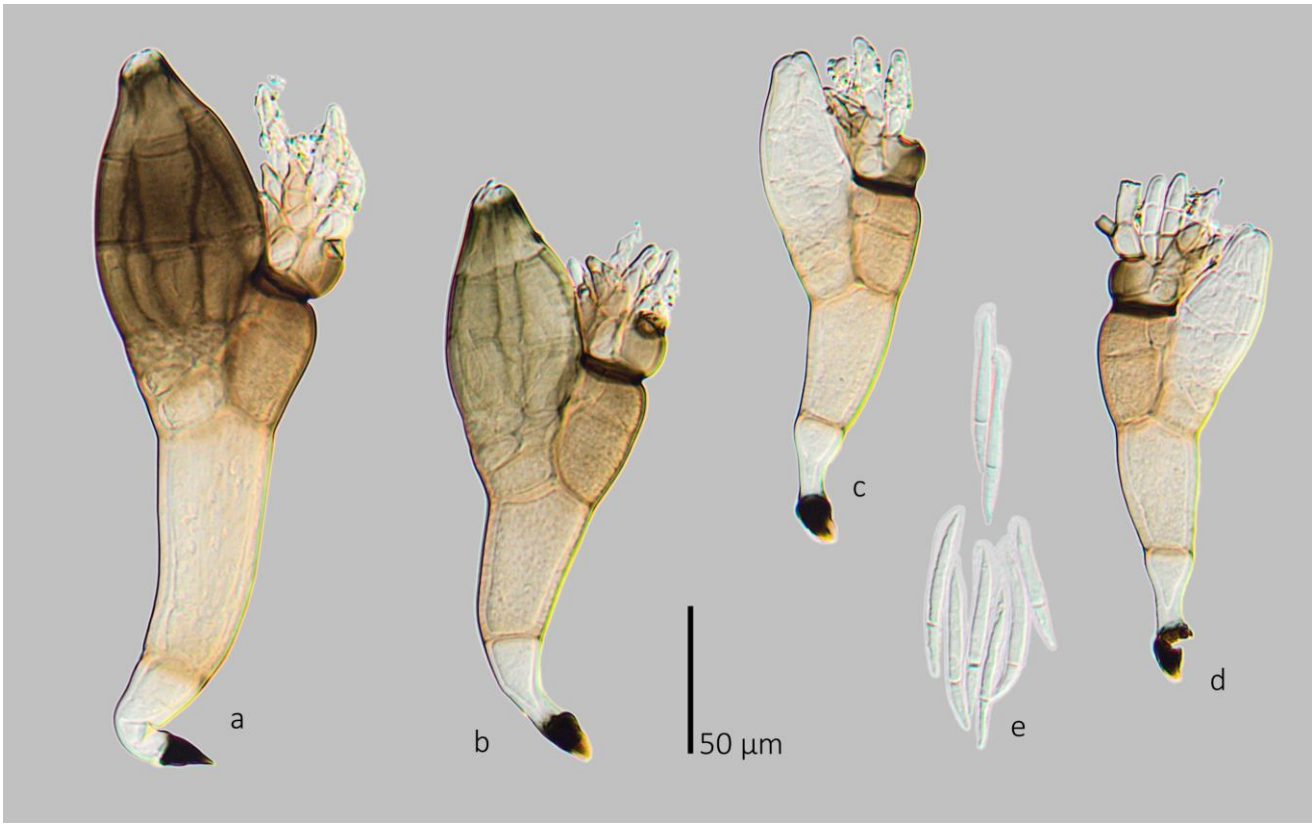

Fig. 4. *Laboulbenia patrata* from *Zorochros dermestoides* (Coleoptera, Elateridae), **a-b.** mature thalli from antenna, **c-d.** juvenile thalli, **e.** ascospores. All from herb. CG621. Scale bar 50 µm.

Inner appendage apparently without obvious antheridia, with small basal cell that later generates a robust, simple branch that extends beyond the perithecium. Perithecium dark, relatively small, less than half-free (black insertion cell above its middle), with subapical constriction, anterior lips very large, slightly darkened, posterior lips pale and much smaller; ostiolum turned in dorsal direction. Cell V smaller than cell IV, cells VI and VII often with unclear outline, very flattened.

Studied material:

On *Bembidion decorum* (Zenker, 1799) [Coleoptera, Carabidae].
BELGIUM, prov. Luxembourg, Chiny, river banks of the Semois, close to 'Rocher du Hat', 12/06/2022, 49°44'13.47''N - 5°19'00.52''E, alt. ca. 300m. Coll. Gerstmans C., slide **CG622a,b** (thalli from right front leg).

Notes: *L. paradoxa* is new for Belgium. It is a very rare species, described from Italy (Spegazzini 1915), reported again in this country by Colla (1934). The latter author doubts whether this taxon should be considered a form of *L. vulgaris* Peyr., a species co-occurring with *L. paradoxa* on *Bembidion spp.* Huldén (1985) reports *L. paradoxa* from Austria and accepts it as a species. His illustration (Huldén 1985, Fig. 20a, pg. 11) corresponds perfectly with our material. The hosts are typical for bare sand and gravel banks of mainly fast-flowing rivers and streams.

Laboulbenia patrata Speg.. **Fig. 4. a-e**
Anal. Mus. nac. Hist. nat. B. Aires 27: 59 (1915)

Brief description

Thallus resembling *L. pedicellata*, young thalli recognized by a short inner appendage, with isodiametric basal cell, supporting two simple branches each forming a pair of antheridia, the latter often darkened. In *L. pedicellata* the inner appendage has more branches and antheridia.

Studied material:

On *Zorochros dermestoides* (Herbst, 1806) [Coleoptera, Elateridae].
BELGIUM, prov. Luxembourg, Chiny, river banks of the Semois, close to 'Rocher du Hat', 12/06/2022, 49°44'13.47''N - 5°19'00.52''E, alt. ca. 300m. Coll. Gerstmans C., slide **CG621** (4 thalli from antenna).

Notes

Although click beetles represent a species-rich group, only very few Laboulbeniales are specialized in them. *L. patrata* is exclusively found on Elateridae (click beetles). Its riparian hosts, *Zorochros spp.* (mainly *Z. dermestoides*), are typically found along sandy or gravely banks of relatively fast flowing rivers and streams. In Belgium *Zorochros dermestoides* is quite rare.

L. patrata is new for Belgium and previously reported in France, Italy (type), Poland (Majewski 2008, 1994), Spain (Santamaria & Pedersen 2021), Czech Republic (Rossi *et al.* 2019b), Switzerland (Baumgartner 1923) and Ukraine (Siemaszko & Siemaszko 1933).

Fig. 5. *Laboulbenia uncinata* from *Harpalus affinis* (Coleoptera, Carabidae), **a-c.** mature thalli (herb. CG92c).

Laboulbenia uncinata Thaxt. **Fig. 5. a-c**
Proc. Amer. Acad. Arts & Sci. 35(9): 208 (1899) [1899-1900]

Brief description

Laboulbenia uncinata is a fairly large species (up to 350 µm in our material), pale, at most with a reddish tint, only blackened at its bent perithecial apex; outer appendage (partly also inner appendage) robust, frequently branched. Antheridia in small tufts, supported by 2-3 celled branches born on the basal cell of the inner appendage.

Studied material:

On *Harpalus affinis* (Schrank, 1781) [Coleoptera, Carabidae]. BELGIUM, prov. Namur, Anhée, rue Petit, jardin, 27-

28/04/2008, 50°18'38.18''N - 4°52'50.45''E, alt. ca. 90m. Coll. Gerstmans C., slide **CG92c** (3 thalli from prothorax edge), slide **CG92a,b** (co-infected with *Laboulbenia coneglianensis* Speg).

Notes

In absence of good material some authors find the position of *L. uncinata* unclear, i.e. either close to or identical to *L. flagellata* (Majewski 1994) or a growth form of *L. macrotheca* (Scheloske 1969, Balazuc 1974, Huggert 2010).

Our material of *L. uncinata* co-occurs with *Laboulbenia coneglianensis* Speg. The latter differs because of its deep pigmentation, bent thallus, almost free perithecium and long but much less branched outer appendage.

L. uncinata is new for Belgium and reported from Siberia (type), the USA and several European countries: Germany (Scheloske 1969), France (Balazuc 1974), Poland (Siemaszko & Siemaszko 1928, Majewski 1994), Spain (Santamaria *et al.* 2020, Santamaria & Pedersen 2021), Switzerland, Hungary (Majewski 1994) and Ukraine (Mishustin *et al.* 2022).

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