# TWO INTERESTING SPECIES OF *RICKIA* (LABOULBENIALES) FROM COASTAL HABITATS IN BELGIUM AND THE NETHERLANDS

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# **Summary**

This paper presents new records of *Rickia* (Laboulbeniales) from hosts collected in organic litter along the high-water mark of the North Sea beach. Samples were taken in Belgium and the Netherlands. *Rickia proteini* was found on *Proteinus* sp. (Coleoptera, Staphylinidae); it is a quite uncommon species and a first record for Belgium. *Rickia laboulbenioides* is a newly described species, found on the millipede *Cylindroiulus latestriatus* (Diplopoda, Julida, Blaniulidae). Descriptions and illustrations are given for both taxa.

# **Samenvatting**

Deze bijdrage geeft nieuwe Belgische en Nederlandse vondsten van *Rickia*'s (Laboulbeniales) die parasiteren op geleedpotigen afkomstig uit het aanspoelsel langsheen de hoogwaterlijn (Noordzeestrand). *Rickia proteini* werd gevonden op *Proteinus* sp. (Coleoptera, Staphylinidae). Het is een relatief zeldzame soort en een eerste melding voor België. *Rickia laboulbenioides* is een nieuwe soort; ze werd gevonden op de miljoenpoot *Cylindroiulus latestriatus* (Diplopoda, Julida, Blaniulidae). Beschrijvingen en illustraties worden gegeven voor beide soorten.

Keywords: Laboulbeniales, Rickia, Coleoptera, Diplopoda, Julidae, millipedes, halobiont.

#### Introduction

Coastal habitats are home to a variety of halobiont arthropods, i.e. adapted or tolerant towards higher levels of salt and regular flooding. In Belgium and the Netherlands several species of *Laboulbenia* have been reported from this particular type of habitat (Meijer 1975, De Kesel 1998, Haelewaters *et al.* 2012). In an attempt to find more records from Belgium and the Netherlands, the litter and debris along the foreshore was sampled and investigated. Two interesting species of *Rickia* were found, one on a millipede and another on a staphylinid beetle.

### Materials and methods

Insects were obtained from organic litter found on the North Sea beach. Two sites were investigated, one in Belgium, the other in the Netherlands. In each site 40-80 liters of seaweed debris was collected along the high-water mark. This material was carefully transferred to a bag and transported to the laboratory. The samples were placed in a large Berlese-Tullgren funnel for 24 hours (Domingo-Quero & Alonso-Zaraga 2010). Insects were collected and stored in 90% ethanol. Screening for infection and removal of thalli was done at 50x magnification using an Olympus SZ61 stereo microscope. Thalli were mounted in Amann's medium (Benjamin 1971) and slides were sealed with transparent nail varnish. Both hosts and microscope slides are deposited at the National Herbarium of (BR). Photographs, drawings measurements were made using an Olympus BX51 light microscope with drawing tube, digital camera and AnalySIS (Soft Imaging System GmbH).

# 1. Rickia laboulbenioides De Kesel, sp. nov.

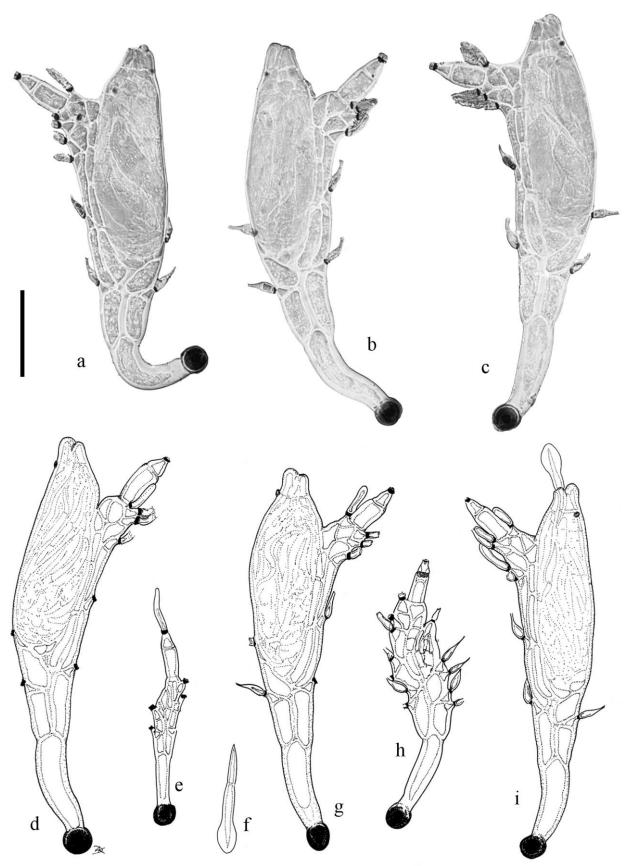
Fig. 1a-i.

MycoBank: MB 805476

Diagnosis: differs from Rickia uncigeri Scheloske and R. dendroiuli W. Rossi by a reduced receptacle with two receptacle cells in the anterior series and 4(-5) cells in the posterior series. Receptacle cells of the median series are lacking under the perithecium. Lowest three receptacle cells of the median series very narrow and flattened against the posterior side of the perithecium. Second and third cell of the posterior series also very narrow and flattened against the mediane series. Receptacle cells of anterior and posterior series with only one, rarely two, smaller apical cells, the latter producing each a single and flask-shaped antheridium. Perithecial apex asymmetrical, the anterior lips bigger and taller than the posterior ones. Foot almost circular, in both adult and young thalli black, with a pale concentric zone.

**Etymology**: the epithet refers to the superficial resemblance with *Laboulbenia*.

**Type material**: Netherlands, Prov. Zeeland, Nieuwe Sluis (51°24'21,14"N - 3°30'19,08"E), on front legs of *Cylindroiulus latestriatus* (Julida, Blaniulidae) collected from litter of the high-tide mark, 25.iv.2013, leg. A. De Kesel, slides: De Kesel 5533b (HOLOTYPE, BR-MYCO 174750-53), De Kesel 5533a (ISOTYPE, BR-MYCO 174749-52).



**Figure 1**. *Rickia laboulbenioides* De Kesel, thalli from second pair of front legs of *Cylindroiulus latestriatus*, with photographs **a**, **b** (= **holotype**), **c** of mature thalli (De Kesel 5533b) and drawings **d**. mature thallus (De Kesel 5533a), **e**. immature thallus (De Kesel 5533e), **f**. spore (De Kesel 5533a), **g**. mature thallus (De Kesel 5533a), **h**. immature thallus with perithecial primordium (De Kesel 5533c) and **i**. mature thallus with emerging spore at the apex (De Kesel 5533a). Scale bar =  $50\mu m$ .

Additional material: Belgium, Prov. Westestuary Vlaanderen. Knokke-Heist, Zwin (51°21'59,76"N 3°21'53,57"E), on front legs of Cylindroiulus latestriatus (Julida, Blaniulidae) collected from litter at the high-tide mark, 17.v.2012, leg. A. De Kesel, slides: De Kesel 5157a (BR-MYCO 174748-51), De Kesel 5157b (BR-MYCO 174747-50), De Kesel 5157c (BR-MYCO 174746-49), De Kesel 5157d (BR-MYCO 174745-48), De Kesel 5157e (BR-MYCO 174744-47).

**Description**: thallus hyaline, rarely yellowish, 194-236 um long. Receptacle unbranched, triseriate; basal cell slender,  $55-70.8 \times 15.8-17.1 \mu m$ , foot never entirely blackened, even in older thalli. Ventral (anterior) series composed of two receptacle cells, slightly higher than broad, each with one (very rarely two) small apical cells. Median series composed of 4(-5) receptacle cells, the lower three very narrow and elongate. Dorsal (posterior) series composed of (3-)4 receptacle cells, the lower two narrow and elongate. Distal cell of median and posterior series, triangular to isodiametric, forming with the basal cell of the primary appendage a 30.5-40.3 µm long conical body, its upper margin forming an angle of 60-90° with the posterior perithecial wall. Basal cell of the primary appendage  $28-36.6 \times 10.1-17.7 \mu m$ , with opaque septum at the apex. Appendages short, 20-35µm long, with opaque septum at the base, often broken off, sparsely formed in the most upper part of the receptacle. Antheridia 14.7- $19.2 \times 3.7$ -5.9 µm, solitary, usually formed in the lower part of the receptacle, always with constricted dark basal septum. Perithecium 96.7-128.8  $\times$  28.4-39.4  $\mu m$ , elongate, symmetric, the anterior margin almost entirely free, the posterior margin 1/3 free from the receptacle, with poorly defined neck and asymmetrical apex, the anterior ostiolar lips taller and bigger than the posterior ones. Spores hyaline, 57-66 × 4-5 µm, slime sheath abruptly widened at the base of the larger cell.

# **Ecology**

Rickia laboulbenioides grows most often on the hosts' second pair of front legs. At full maturity thalli are glass-like and easily overlooked. The parasite has less than 5% prevalence (6 hosts on 120) in spring. Thallus density (number of thalli per host) is relatively low in the investigated sites and season.

The host *Cylindroiulus latestriatus* is a pioneer of sandy habitats, either natural (dunes) or synanthropic (gardens, parks, even compost). It is common in Belgium and the Netherlands and mostly observed in the dunes (Kime 2004, Berg et al. 2008). In this habitat it is the dominant species, i.e. with high densities. The species shows a clear preference for open habitats with sandy soils. It most often occurs under branches, litter, wood, stones. It may be a thermophilic species (Berg et al. 2008). Given the nature of the sites where we collected it, we assume both host and parasite are at least halotolerant.

#### Discussion

From more than 2000 described species of Laboulbeniales, only seven are specialized to parasitize millipedes (Diplopoda). These species belong to four genera: *Rickia* Cavara, *Troglomyces* S. Colla, *Diplopodomyces* W. Rossi & Balazuc, and *Triainomyces* W. Rossi & A. Weir (Rossi & Weir 1998). The latter three genera are monotypic and exclusively reported from Diplopoda. *Rickia*, however, counts over 140 species, infesting a wide array of hosts (Acarina, Coleoptera, Hymenoptera, Blattaria, Isoptera, Orthoptera, Julida) from all over the world (Tavares 1985).

There is very little information on the distribution of Laboulbeniales from Diplopoda. *Troglomyces manfrediae* S. Colla and *Diplopodomyces callipodos* W. Rossi & Balazuc are only known from Europe (Italy and France respecyively), while *Triainomyces hollowayanus* W. Rossi & A. Weir was described from New Zealand. The very diverse genus *Rickia* comprises four species parasitizing Diplopoda, these are *Rickia dendroiuli* W. Rossi, *R. pachyiuli* M. Bechet & I. Bechet, *R. uncigeri* Scheloske, and *R. siddhartha* Balazuc.

The latter so far has only been collected in Sri Lanka. It is very long-stalked and clearly different from *R. laboulbenioides*. The other three species are only known from Europe (Bechet & Bechet 1986, Colla 1932, Rossi & Balazuc 1977, Rossi & Weir 1998, Scheloske 1969, Majewski 1994).

Rickia uncigeri differs from R. laboulbenioides by a more developed receptacle; it comprises more and much wider cells in the mediane and posterior series. Rickia uncigeri also has a reddish pigmented thallus, with 3-4(-5) antheridia developing on the posterior and anterior series of receptacle cells. The perithecium of R. uncigeri is mostly free on the posterior side and has a symmetrical ostiolum.

Rickia pachyiuli differs from R. laboulbenioides by an even more developed receptacle, having 8-9 cells in the anterior series and 12-14 in the posterior series. The thallus has a more clavate outline. It can develop a second perithecium and reaches lengths of 400 μm or more. R. pachyiuli has a perithecium with symmetrical apex, completely free anterior side and half-free posterior side.

In spite of being taller, i.e. up to 590 µm, *Rickia dendroiuli* seems the closest to *R. laboulbenioides*. It is easily separated from it by the presence of 3-4 subperithecial receptacle cells in the median series. In *Rickia laboulbenioides* these cells are always absent, hence its superficial resemblance to the receptacle of a *Laboulbenia*.

# 2. Rickia proteini T. Majewski

Acta Mycologica 19: 191 (1986)

Iconography: Majewski 1986 (fig.4), Majewski 1988 (fig. 47), Majewski 1994 (Pl 98, fig. 5-16), Duverger 1995 (Pl. vi, fig. 24). **Fig. 2a-c.** 

Thallus hyaline, 136-150 µm long, fusiform to broadly fusiform. Receptacle unbranched, triseriate, singlelayered; stalk cell short, obtriangular, 12-16 × 13-18 µm, foot entirely blackened. Ventral (anterior) series composed of four isodiametric to slightly flattened receptacle cells, the upper three apically forming one or two small triangular cells, each of them bearing a single antheridium or an appendage. Median series composed of 8-10 isodiametric to slightly flattened receptacle cells, the lower two (three) situated below the base of the perithecium. Dorsal (posterior) series composed of (6)7-8 isodiametric to slightly flattened receptacle cells, each apically forming one (two) triangular cells that bear a single antheridium or an appendage. Distal or subdistal cell of the posterior series carries the basal cell of the primary appendage, the latter triangular and with darkened septum at the apex. Appendages short, 10-15µm long, with darkened and constricted septum at the base. Antheridia up to  $13\mu m$  long, solitary, with constricted dark basal septum. Perithecium 77-82  $\times$  32-37  $\mu m,$  broadly ellipsoid, symmetrical, with narrowing neck, the anterior margin almost entirely free, the posterior margin 3/4-2/3 adnate to the receptacle; apex symmetrical and slightly truncate. Spores hyaline, 30- $33 \times 2.7$ -3.2 µm, slime sheath not abruptly widened.

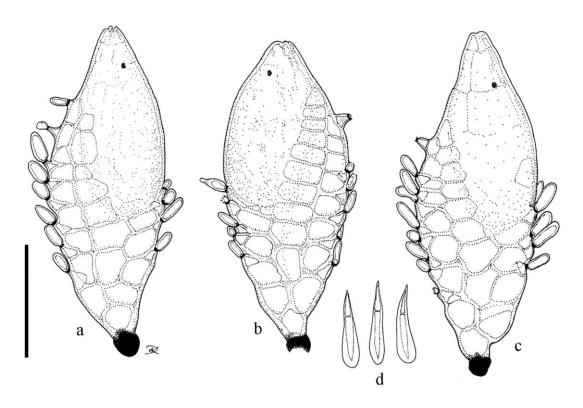
### Studied material:

BELGIUM, Prov. West-Vlaanderen, Knokke-Heist, Zwin estuary (51°21'59,76"N - 3°21'53,57"E), on *Proteinus sp.* (Coleoptera, Staphylinidae) collected from litter at the high-tide mark, 28.iv.2012, leg. A. De Kesel, slides: De Kesel 5146a (abdomen, BR-MYCO 174743-46), De Kesel 5146b (elytra, BR-MYCO 174742-45).

## Discussion

The type specimens of *Rickia proteini* and *Euphoriomyces huggertii* T. Majewski were recorded on *Proteinus brachypterus* (Fabr.) (Majewski 1994). *Euphoriomyces huggertii* also occurs on other Staphylinidae and is so far not recorded in the area (Belgium, Netherlands).

Rickia proteini only occurs on hosts of the genus Proteinus Latreille (Coleoptera, Staphylinidae). It is reported from Poland (Majewski 1986, 1994), France (Duverger 1995), Norway (Majewski 2008), and Japan (Majewski 1988). The hosts are generally found in decomposing plant material (phytodetriticolous), near water or in relatively damp places. A number of records in Majewski (2003, 2008) come from hosts collected from fruiting bodies of macromycetes. Infections occur on various parts of the body, albeit most often on elytra and the abdominal tergites.



**Figure 2**. *Rickia proteini* T. Majewski from *Proteinus sp.*, with **a.** mature thallus from elytron (De Kesel 5146b), **b.** mature thallus from upper abdomen (De Kesel 5146a), **c.** mature thallus from elytron (De Kesel 5146b) and **d**. spores (De Kesel 5146a). Scale bar =  $50\mu m$ .

# References

- BENJAMIN R.K. (1971) Introduction and supplement to Roland Thaxter's contribution towards a monograph of the Laboulbeniaceae. *Bibliotheca Mycologica* **80**: 1-155.
- BECHET M. & BECHET I. (1986) *Rickia pachyiuli* n. sp. (Ascomycetes, Laboulbeniales) parazita pe *Pachyiulus hungaricus* (Karsch.) (Diplopoda, Iulidae). Contributii Botanice, Cluj-Napoca: 31-34
- BERG M.P., SOESBERGEN M., TEMPELMAN D. & WIJNHOVEN H. (2008) Verspreidingsatlas Nederlandse landpissebedden, duizendpoten en miljoenpoten (Isopoda, Chilopoda, Diplopoda). EIS-Nederland, Leiden & Vrije Universiteit-Afdeling Dierecologie, Amsterdam.
- COLLA S. (1932) *Troglomyces Manfredii* n. gen. et n. sp.: Nuova Laboulbeniacea sopra un Miriapode. *Nuovo Giornale Botanico Italiano* **39**(3): 450-453.
- DE KESEL A. (1998) Identificatie en gastheerspectrum van het genus *Laboulbenia* in België (Ascomycetes, Laboulbeniales). *Sterbeeckia* **18**: 13-31.
- DOMINGO-QUERO T & ALONSO-ZARAZAGA M. (2010)

   Soil and litter sampling, including MSS Chapter
  9. In: Eymann J, Degreef J, Häuser C, Monje JC,
  Samyn Y, VandenSpiegel D (eds). Manual on
  Field Recording Techniques and Protocols for All
  Taxa Biodiversity Inventories. ABC Taxa
  8(1):173-212.
- DUVERGER C. (1995) Laboulbéniales (Fungi, Ascomycètes) parasites de Staphylinidae (Coleoptera) du sud-ouest de la France conservées dans la collection de l'auteur. *Bull. Soc. linn. Bordeaux* **23**(4): 147-175.
- HAELEWATERS D., NUYTINCK J. & DE KESEL A. (2012)
  Laboulbeniales (Fungi, Ascomycota) in the Nederlands: an introduction. *Natuurhistorisch Maandblad* 101(5): 88-93.
- KIME R.D. (2004) The Belgian Millipede Fauna (Diplopoda). *Bull. K. Belg. Inst. Nat. Wet. Entomologie* **74**: 35-68.
- MAJEWSKI T. (1986) Rare and new Laboulbeniales from Poland. VIII. *Acta Mycologica* **19**(2): 183-192.
- MAJEWSKI T. (1988) Some Laboulbeniales (Ascomycotina) collected in Japan I. Species from Shizuoka Prefecture. *Trans.Mycol. Soc. Japan* **29**: 33-54.
- MAJEWSKI T. (1994) The Laboulbeniales of Poland. *Polish Botanical Studies* **7**: 1-466.
- MAJEWSKI T. (2003) Distribution and ecology of Laboulbeniales (Fungi, Ascomycetes) in the Białowieża Forest and its western foreland. Phytocoenosis 15 (S.N.), Supplementum Cartographiae Geobotanicae 16: 1–144.
- MAJEWSKI T. (2008) Atlas of the geographical distribution of Fungi in Poland. Fascicle 4.

- Laboulbeniales. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
- MEIJER J. (1975) Carabid (Coleoptera, Carabidae) migration studied with Laboulbeniales (Ascomycetes) as biological tags. *Oecologia* **19**: 99-103.
- ROSSI W. & BALAZUC J. (1977) Laboulbéniales parasites de myriapodes. *Revue de Mycologie* **41**: 525-535.
- ROSSI W. & WEIR A. (1998) *Triainomyces*, a new genus of Laboulbeniales on the pill-millipede *Procyliosoma tuberculatum* from New Zealand. *Mycologia* **90**(2): 282-289.
- SCHELOSKE H.W. (1969) Beiträge zur Biologie, Ökologie und Systematik der Laboulbeniales (Ascomycetes) unter besonderer Berücksichtigung des Parasit-Wirt-Verhältnisses. Parasitol. Schriftenreihe 19: 1-176.
- TAVARES I.I. (1985) Laboulbeniales (Fungi, Ascomycetes). *Mycologia Memoir* **9**: 1-627.