Preliminary observations of the ectoparasitic fungus *Rickia laboulbenioides* De Kesel (Laboulbeniales) in Britain

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Abstract

The ectoparasitic fungus *Rickia laboulbenioides* De Kesel (order Laboulbeniales) is known to infect various species of *Cylindroiulus* millipedes in Western Europe. Its occurrence in Britain has been long known, but there are few documented observations. In this paper British records are collated and five host species of *Cylindroiulus* are reported. A preliminary distribution map shows *R. laboulbenioides* to occur throughout England, Wales and into southern Scotland. Its occurrence is determined by the presence of suitable host millipedes rather than by habitat type. Of reference collection samples examined 12% were found to contain at least one infected specimen (with a prevalence of 15.5% in samples showing infection). Observations have been made throughout the year, with a late spring peak possibly reflecting millipede and/or field recorder activity.

Keywords: Laboulbeniales, Rickia laboulbenioides, Cylindroiulus, Britain, distribution.

Introduction

The order Laboulbeniales (Fungi, Ascomycota) are obligate ectoparasitic fungi that live externally upon the cuticle of their associated arthropod host. Typically they are limited to one, or a few closely related, species. They produce translucent thalli that grow directly from sticky ascospores that are dispersed by

physical contact, often during copulation (De Kesel, 1997; Haelewaters *et al.*, 2012; Santamaria *et al.*, 2014). As a rule host species must have large and stable populations with overlapping generations (to allow transmission between adults) and inhabit moist environments (Santamaria *et al.*, 2014). The fungus seems to have little or no effect on the reproduction or survival of their host (Haelewaters *et al.*, 2012). Although mostly known from a wide range of insect orders (e.g. Wier, 1996; De Kesel, 1997), recent work has revealed many species associated with millipedes (e.g. Santamaria *et al.*, 2014; Santamaria *et al.*, 2016). On millipedes, the thalli are often found towards the anterior of the body; on fore legs, body rings, gonopods, antennae, mandibles, etc (De Kesel, Haelewaters & Gerstmans, 2013; Santamaria *et al.*, 2016).

The occurrence of Laboulbeniales fungi on *Cylindroiulus* millipedes has been known for many years in the British Isles. Blower (1985; pg. 36) noted that "species of the '*luscus*' group of *Cylindroiulus* millipedes are fairly frequently attacked by ectoparasitic fungi of the Laboulbeniales". Irwin (1989) reports an un-identified Laboulbeniales growing on specimens of *C. britannicus* (Verhoeff) and put out a plea for more material in an attempt to discover which species occur in Britain. As far as the author is aware this never happened. A preliminary host-parasite list of British Laboulbeniales (mainly insect orders) was



Figure 1: *Rickia laboulbenioides* reproduced from Jeekel (2001).

published by Weir (1996), which erroneously listed *Rickia dendroiuli* Rossi as the species infecting *Cylindroiulus punctatus* (Leach) (based on information in Rossi & Balazuc, 1977). It is now known that *R. dendroiuli* is associated with *Cylindroiulus (Dendroiulus) latzeli* (Berlese), a millipede endemic to Italy (Santamaria *et al.*, 2016; Kime & Enghoff, 2017).

In the Netherlands, Jeekel (2001) reported the occurrence of a widely recorded, but un-described *Rickia* species occurring on *C. latestriatus* (Curtis) (which he referred to as *R. cylindroiuli* spec. ined.). Jeekel included a detailed line-drawing (reproduced in Fig. 1), which quite clearly shows the species now known as *R. laboulbenioides* and comments "I am pretty sure it will turn up in the UK before long". It was over a decade later before this fungus was formally described and named as *Rickia laboulbenioides* De Kesel (*in* De Kesel *et al.*, 2013) from infected *C. latestriatus* collected in the Netherlands and Belgium. Examination of museum collections (Santamaria *et al.*, 2016) has shown *R. laboulbenioides* to be widely distributed across western Europe with records from Denmark (on *C. latestriatus* and *C. punctatus*) to the Iberian Peninsula (on *C. perforatus* Verhoeff and *C. dahli* Demange). However, there were no confirmed records of *R. laboulbenioides* for Britain or Ireland until Santamaria *et al.* (2016) identified the species from images taken by Malcolm Storey (2009) of an infected *C. punctatus* recorded at Bucklebury Common, Berkshire, in 2001 (see Appendix I for record details).

Inspired by Enghoff & Reboleira's (2015) plea to 'Look out for ectoparasitic fungi on millipedes' and Santamaria *et al.*'s (2016) 'Hidden biodiversity revealed by collections-based research' this paper attempts to collate modern observations of *Rickia laboulbenioides* in Britain in order to ascertain its host millipede associations, distribution, habitat preferences, phenology and prevalence.

Sources of observations

Observations of *Rickia laboulbenioides* in Britain have been collated from a number of sources:

- **Published literature**: Observations have been extracted from recently published sources.
- Author's personal collection: 53 tubes of *Cylindroiulus* species mainly from widely scattered localities across Oxfordshire (VC23 and VC22), including 9 tubes of *C. britannicus*, 4 of *C. latestriatus*, 9 of *C. parisiorum* (Brölemann & Verhoeff) and 15 of *C. punctatus*, were examined.
- **BMIG's basic and research collections** (Harper, 2007): 65 tubes of *Cylindroiulus* species mainly collected from widely scattered localities in south Wales, including 14 tubes of *C. britannicus*, 15 of *C. latestriatus* and 19 of *C. punctatus*, were examined.
- Author's personal observations: Recently, millipede specimens have been collected in the field from various locations in the UK and examined for signs of infection.
- On-line resources: Records have been gleaned from images posted online, such as BMIG's *Isopods and Myriapods of Britain and Ireland* group (www.facebook.com/groups/ 407075766387553) and *Insects and other Invertebrates of Britain and Europe* group (www.facebook.com/groups/invertid) and Biological Record Centre's *iRecord* website (www.brc.ac.uk/irecord).
- **Historical records**: Records (listed as *Rickia dendroiuli* Rossi) have been extracted from *Fungal Records Database of Britain and Ireland* (via the NBN Atlas; NBN, 2017).

Specimens of potential millipede hosts (when available for examination) were preserved in 70% ethanol and were examined using a low-power binocular (dissecting) microscope for the presence of thalli of Laboulbeniales fungi. When found, identification was confirmed by transferring thalli to a temporary slide and viewing with transmitted light. A few observations are based solely on photographic images, where although thalli could be clearly seen, key microscopic characters needed to provide positive identification of the fungus may not be visible.

Observations of *Rickia laboulbenioides*

Thirty nine occurrences of *Rickia laboulbenioides* utilising five British host millipede species, *Cylindroiulus britannicus* (Verhoeff), *C. latestriatus* (Curtis), *C. punctatus* (Leach), *C. pyrenaicus* (Brölemann) and *C. sagittarius* (Brölemann), have been collated. Three observations have been previously published; Gregory *et al.* (2018) (a new species host of *C. pyrenaicus*), Gregory & Owen (2019) (a new species host of *C. sagittarius*) and Storey (2019) (a new species host of *C. britannicus*). Eight records have been derived through examination of voucher specimens (author's and BMIG's collections). A number of recent field records have been made by the author and additional field observations have been derived from images posted online. Five records have been extracted from the *Fungal Records Database of Britain and Ireland* (via NBN, 2017). These occurrences are listed in Appendix I and are outlined below.

On occasions thalli can be numerous on the anterior legs, making them look conspicuously 'frilly' even at a casual glance (e.g. Fig. 2B & 2C). However, sometimes only a few thalli may be present, which can be difficult to observe, even in preserved material, and are easily overlooked.

Cylindroiulus britannicus (Verhoeff)

There are eight confirmed British observations (seven sites) of this host being utilised (Appendix I). The first confirmed occurrence of *C. britannicus* as a host species for *R. laboulbenioides* was reported and figured by Storey (2019) based on a male specimen collected from deciduous woodland in the New Forest, southern England in December 2017. However, it is very likely that the un-identified Laboulbeniales infecting *C. britannicus* reported by Irwin (1989) is also *R. laboulbenioides*. Examination of preserved voucher material has revealed two additional sites. One is from the BMIG reference collection (tube CC) from beneath flood debris beside the river Usk in south Wales in 2004 (6.3% prevalence, Table 1) and the other from the author's personal collection from scrubby grassland in western Scotland in 2006 (14.3% prevalence, Table 1). Recently there have been five additional field observations; a domestic garden, an ornamental garden, a deciduous woodland and a sedge bed (*Carex* sp.) scattered across southern England and from inside a glasshouse in an ornamental garden in southwest Scotland.

Cylindroiulus latestriatus (Curtis)

The eleven confirmed observations from six sites listed herein (Appendix I) represent the first recorded British occurrences of *C. latestriatus* as a host species for *R. laboulbenioides*. This not unexpected since this fungus was originally described from this millipede host in the Netherlands and Belgium by De Kesel *et al.* (2013) and was reported to occur widely on this host in the Netherlands by Jeekel (2001).

The earliest confirmed British records are from the author's personal collection collected from Oxfordshire in 1992 and 1993 (but not identified until 2017). Of four tubes (total 17 specimens), three tubes were found to contain infected specimens. Examination by the author (in 2019) of pitfall trap samples collected by J.M. Campbell between 2000 and 2002 from an Oxfordshire golf course (on sandy soils) revealed small numbers of infected specimens mainly between May and July (Appendix I). In Britain *C. latestriatus* is mainly coastal (Lee, 2006) and this is a rare millipede in Oxfordshire (Gregory & Campbell, 1996) with a handful of known records from relict acid grassland/heathland or churchyards on sandy soils. Thus, it is of note that the prevalence of *R. laboulbenioides* on this species appears to be relatively high within the county.

Examination of the BMIG basic collection revealed an additional site (10.8% prevalence, Table 1) from sand dunes at Crymlyn Burrows, south Wales in 2006. Recently, two additional field observations have been made, from upper salt marsh in Lancashire (specimen shown in Fig. 2A) and from coastal grassland in the Lothians (south-east Scotland).



Figure 2: Cylindroiulus species bearing thalli of Rickia laboulbenioides on anterior legs (arrowed).A) C. latestriatus male (specimen examined by author), Morecambe Bay, West Lancashire;

B) *C. punctatus* male, Yew Tree Tarn, Westmorland; C) Same specimen, close up of badly infected anterior legs (some of the numerous thalli arrowed), ventral view (images © Nicola Garnham).

Cylindroiulus punctatus (Leach)

This is the most widely recorded host millipede with thirteen confirmed British observations from twelve sites (Appendix I). The earliest observation is of an infected female collected in a pitfall trap (J.M. Campbell, leg.) set beside a stream in Besselsleigh Wood, Oxfordshire in 2000 (the sample examined by the author in 2019). The hygrophilous woodlouse *Ligidium hypnorum* (Cuvier) and the centipede *Lithobius muticus* (C.L. Koch) were also recorded in the sample. Both are typically associated with ancient woodland in Oxfordshire (Gregory & Campbell, 1995; 1996). The 14 tubes of this species

held in the author's personal collection (of different localities) showed no evidence of infection. The specimen recorded from Bucklebury Common in 2001 (photographed in Storey, 2019) was collected from under dumped rotting wood in oak *Quercus* sp. and birch *Betula* sp. woodland (M. Storey, pers. comm.).

Examination of the BMIG research collection adds two localities (tubes KH and LB) from south Devon collected in 2005 (with 46.7% and 6.7% prevalence respectively, Table 1). Recently, eight additional records have been added. Five were collected from woodland: from Westmorland, northern England (the specimen shown in Fig. 2B-C); from Kirkcudbrightshire, south-west Scotland; and Oxfordshire, Berkshire and Hampshire in southern England. The other three observations are from domestic or ornamental gardens on Guernsey, Channel Islands, Cornwall, south-west England and the Isle of Arran, western Scotland.

The *Fungal Records Database of Britain and Ireland* (accessed via NBN, 2017) gives additional records of *R. laboulbenioides* (under the name *R. dendroiuli*, as the records pre-date the description of *R. laboulbenioides*) infecting '*C. punctatus*'. However, there is some doubt about the reliability of the identification of the millipede hosts (M. Storey, pers. comm.) and consequently these have been listed as *Cylindroiulus sp.* in Appendix I.

Cylindroiulus pyrenaicus (Brölemann)

Two observations (from a single site) are known, a new host millipede species for *R. laboulbenioides* (Gregory *et al.*, 2018). Several specimens of this millipede collected in May 2017 by Christian Owen from deciduous woodland in the Kenfig Valley, south Wales were found to be infected. This millipede is native to the Pyrenees and Montagne Noire (Kime & Enghoff, 2017) and is believed to be an accidental introduction into Britain.

Cylindroiulus sagittarius (Brölemann)

There is one observation in Britain reported by Gregory & Owen (2019), a new host millipede species for *R. laboulbenioides*. A male specimen collected by Christian Owen in December 2017 from deciduous woodland in the Sirhowy Valley, south Wales was found to be infected. This millipede is native to the Western Pyrenees and Cantabrian Mountains (Kime & Enghoff, 2017) and is believed to be an accidental introduction into Britain.

Discussion

Host species

In Britain *R. laboulbenioides* is able to utilise a range *Cylindroiulus* species with five millipede hosts confirmed: *C. britannicus, C. latestriatus, C. punctatus, C. pyrenaicus* and *C. sagittarius*. This includes several closely related species falling within Blower's (1985) *luscus* Meinert, 1868 group. The first three species have very widespread distributions in the UK (Lee, 2006). The other two are believed to be accidental introductions in the UK that are restricted, albeit locally numerous, within single river catchments in south Wales (Gregory *et al.*, 2018; Gregory & Owen, 2019). It is possible that additional host species, such as *C. truncorum* (Silvestri) and *C. parisiorum* (Brölemann & Verhoeff) may be utilised. However, specimens of the latter species held in the author's collection (9 tubes; 42 specimens) have been examined (twice) for *R. laboulbenioides* without success.

Distribution

The known distribution of *R. laboulbenioides* in the British Isles (Fig. 3, based on records in Appendix I) extends from the Channel Islands (located about 30 miles (48 km) west of Normandy, France), through England and Wales, and continues at least as far north as southern Scotland (Isle of Arran and

The Lothians). However, it is apparent that this distribution reflects the activities of a few key recorders and *R. laboulbenioides* is almost certainly overlooked and under-recorded in Britain. The absence of records from eastern England may be due to the drier climate experienced there, but equally may be due to the lack of active observers. Given the overall abundance of *C. punctatus* throughout the UK, and the widespread occurrence of *C. britannicus* and *C. latestriatus* (Lee, 2006), then it is possible that *R. laboulbenioides* will prove to be considerably more widespread than indicated by this preliminary distribution map. However, at a few sites in Oxfordshire, the author has examined over a 100 specimens of *C. britannicus* and *C. punctatus* over the course of several years and *R. laboulbenioides* has not been found, suggesting its distribution may be patchy.



Figure 3: Preliminary distribution map for *Rickia laboulbenioides* in Britain.
Plotted at 10km (hectad) resolution using records listed in Appendix I. Records date from 1992 to September 2020. Host species: ● *Cylindroiulus britannicus*, ● *C. latestriatus*, ● *C. punctatus*, ● *C. pyrenaicus*, ● *C. sagittarius*, ● unknown *Cylindroiulus* sp.

Habitat

Rickia laboulbenioides shows no obvious habitat preferences with records from both semi-natural and synanthropic habitats. Although the majority of infected millipedes have been collected from deciduous woodland (Appendix I) this probably reflects the abundance and habitat preferences of two of its widespread host millipedes, *C. punctatus* and *C. britannicus*, which both favour rotting dead wood (Lee, 2006). Other habitats in which *R. laboulbenioides* has been recorded include acidic grassland, salt marsh and coastal sand-dunes (which are habitats favoured by *C. latestriatus*), a scrubby south facing sea cliff and gardens. It is concluded that the presence of host millipede species is more important than the habitat in which they occur. However, there are likely to be environmental factors at play, such as moisture, which affect the successful development of this fungus on its host millipedes.

Phenology

Plotting the number of British records of *R. laboulbenioides* against month of observation (28 records, Appendix I) suggests that thalli are present on host millipedes throughout the year, but there is a peak of observations in late spring (April to June) (Fig. 4). However, for the few species of Laboulbeniales that have been studied (mainly on Coleoptera) the life cycle of an individual thallus in completed within a few weeks (e.g. De Kesel, 1997). Since transmission of ascospores (at least for epigeal species) is primarily through physical contact between adults of host species, particularly during copulation (De Kesel, 1997; Haelewaters *et al.* 2012; Santamaria *et al.*, 2014), then by implication host millipedes bearing mature thalli need to be present all year round. Thus, this spring peak in observations of *R. laboulbenioides* probably reflects the surface activity of millipedes and/or millipede recorders, which both become more elusive during the hotter and drier months of late summer.



Figure 4: Number of records of *Rickia laboulbenioides* per month (data from Appendix I).

Prevalence

Combined together, the author's and BMIG's collections of *C. britannicus*, *C. latestriatus* and *C. punctatus* include 76 tubes (mostly containing less than ten specimens). Of these, nine tubes (12%) were found to contain at least one specimen bearing thalli of *R. laboulbenioides*. Only five tubes with infected material contained 10 or more specimens (Table 1). For these tubes the prevalence of host

infection within the sample varied considerably. Four samples varied between 6.3% and 14.3%, whereas the remaining sample (Bolberry Down, BMIG Collection, tube KH) showed 46.7% prevalence (seven infected specimens from 15 in sample). For the pooled data (all five tubes combined) this equates to 15.5% prevalence. This is higher than the 5% prevalence reported by De Kesel *et al.* (2013) for *C. latestriatus* (where there were six infected specimens of 120 examined). However, the samples examined here (Table 1) contain relatively few specimens and may not be representative of the larger population as a whole.

Source	Host species	Total no. specimens	Specimens infected	% infected
Author's Collection	C. britannicus	14∂₽	1∂1♀	14.3
BMIG Collection, Tube CC	C. britannicus	16∂♀	1 🖒	6.3
BMIG Collection, Basic	C. latestriatus	37 ♂♀	2♂ 2♀	10.8
BMIG Collection, Tube KH	C. punctatus	15∂♀	4∂ 3♀	46.7
BMIG Collection, Tube LB	C. punctatus	15∂₽	18	6.7
	Pooled data:	97	15	15.5%

Table 1: Prevalence of infection in samples examined that contain more than 10 specimens

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Appendix I: Observations of *Rickia laboulbenioides* in Britain of which the author is aware up to October 2020

Observations based on published records, examination of reference collections, author's personal observations, records submitted to the author and records held by *Fungal Records Database of Britain and Ireland* (FRDBI; NBN, 2017). * Indicates specimen(s) examined by the author.

IMBI - image originally posted on *Isopods and Myriapods of Britain and Ireland* group (<u>www.facebook.com/groups/407075766387553</u>); IOIBE - image originally posted on *Insects and other Invertebrates of Britain and Europe* group (<u>www.facebook.com/groups/invertid</u>); iRecord - image originally posted on *iRecord* (<u>www.brc.ac.uk/irecord</u>); OBRC/JMC – Oxfordshire Biological Records Centre/John Campbell leg.

Species/Locality	Number specimens	Grid Reference	VC	Date	Habitat	Source		
Cylindroiulus britannicus								
River Usk, Abergavenny	18	SO242157	35	13.iii.2004	Under flood debris	BMIG Collection, tube CC*		
Auchalton Meadows SWT	1819	NS335036	75	08.iv.2006	Scrubby grassland	Author's Collection *		
New Forest	18	SU410023	11	06.x.2017	Deciduous woodland	Storey (2019)		
Upham Village	18	SU538208	11	01.i.2019	Domestic garden	Personal Observation*		
Cally Gardens, Vinery	1♀	NX604549	73	27.iv.2019	Inside greenhouse	Personal Observation*		
Rack Marsh	1♀ 1♂	SU451692 SU452693	22	31.v.2019 30.x.2019	Deciduous woodland Carex Sedge bed	iRecord, image Personal Observation*		
Lamorran House Gardens	18	SW843331	2	09.ix.2020	Ornamental garden	Personal Observation*		
Cylindroiulus latestriatus								
Frilford Heath SSSI	18 18	SU443985 SU438983	22	05.v.1992 08.ii.1993	Acidic grassland	Author's Collection*		
Hook Norton Cutting	1012	SP358315	23	17.vi.1993	Scrubby grassland	Author's Collection*		
Tadmarton Golf Course	10 14 10 10 10	SP 39 35	23	May 2000 May 2001 June 2001 July 2001 Jan-Feb 2002	Golf course on sandy heathland	OBRC/JMC pitfall traps*		
Crymlyn Burrows	2♂2♀	SS715933	41	09.v.2004	Sand dunes	BMIG Collection, Basic*		
Morecambe Bay	18	SD479687	60	28.xii.2018	Upper salt marsh	IOIBE, images*		
Skateraw Harbour	1♀	NT738759	82	13.v.2019	Coastal grassland	Personal Observation*		

Cylindroiulus punctatus								
Besselsleigh Common Wood	19 1ð19	SP449014	22	Jan 2000 June 2001	Deciduous woodland	OBRC/JMC pitfall traps*		
Bucklebury Common	18	SU547687	22	09.vi.2001	Deciduous woodland	Storey (2009), FRDBI		
Bolberry Down	4∂ 3♀	SX689383	3	13.v.2005	Scrubby sea cliff	BMIG Collection, tube KH*		
Wood, N. of Slapton	18	SX829456	3	14.v.2005	Deciduous woodland	BMIG Collection, tube LB*		
Yew Tree Tarn	18	NY321003	69	26.i.2019	Mixed woodland	IOIBE, images *		
Kirroughtree Forest	18	NX455645	73	28.iv.2019	Deciduous woodland	Personal Observation*		
Sole Common	1C	SU412706	22	11.v.2019	Deciduous woodland	iRecord, images		
St Sampsons, Guernsey	19	WV320808	113	20.v.2019	Domestic garden	IMBI, images		
Margnaheglish, Arran	19	NS043323	100	31.vii.2019	Domestic garden	Specimen sent to author *		
Kingwood Common	18	SU69-82-	23	16.iii.2020	Deciduous woodland	Personal Observation*		
Hilsea Lines, Portsmouth	18	SU658044	11	28.vii.2020	Mixed Woodland	iRecord, images *		
Lamorran House Gardens	18	SW843331	2	09.ix.2020	Ornamental garden	Personal Observation*		
Cylindroiulus pyrenaicus								
Craig yr Aber	1♂1♀ 1♂ 1♂	SS855850	41	01.v.2017 30.xi.2017 30.xi.2017	Deciduous woodland	Gregory <i>et al.</i> (2018) Personal Observation* Telfer, M.G., pers. comm.		
Cylindroiulus sagittarius								
Sirhowy Valley	18	ST177943	35	03.xii.2017	Deciduous woodland	Gregory & Owen (2019)		
Unidentified Cylindroiulus sp.								
Esher Common	unknown	TQ12-62-	17	1994 1995	Not recorded	FRDBI (A. Weir, det.)		
Slapton Ley	unknown	SX8-4-	3	1995	Not recorded	FRDBI (A. Weir, det.)		
New Forest	unknown	SU410012	11	03.iv.2005	Deciduous woodland	FRDBI (M. Storey, det.)		
New Forest	unknown	SU412014	11	12.iv.2005	Deciduous woodland	FRDBI (M. Storey. det.)		