

Laboulbeniales (Ascomycota) on Carabidae (Insecta: Coleoptera) from the Galápagos Archipelago

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ABSTRACT. Five species of *Laboulbenia* are recorded from 15 species of Carabidae (Coleoptera) of the Galápagos Archipelago. Laboulbeniales were found on seven of the 15 larger Galápagos Islands.

L. sanjoaquina sp. nov. is described from *Platynus* (subgenus *Dyscolus*) species, endemic to San Cristobal, *L. galapagoensis* sp. nov. from *Tachys* species, occurring on several Galápagos Islands and *L. appendiculata* sp. nov. from a *Bradycellus* species, occurring on Pinzon. The genera *Selenophorus* and *Calosoma* are new host records of *L. flagellata*, this being the first definite record of a *Laboulbenia*-infested Carabini under natural conditions. In general, most of the Laboulbeniales have been observed in more humid conditions, mainly from coastal littoral habitats and the fern-sedge vegetation zone at higher elevation on Galápagos islands and volcanoes. *L. galapagoensis* and *L. appendiculata* are assumed to be dioecious. Together with *L. inflata*, three presumably dioecious species thus occur on the Galápagos Islands. Characters, a distribution map and an identification key are given for the *Laboulbenia inflata* species-group.

KEY WORDS: Carabidae, Laboulbeniales, Galápagos Islands.

INTRODUCTION

The Galápagos Archipelago is situated in the Pacific Ocean, 1000 km west of the Ecuadorian coast. Composed of many small and 15 larger islands, the archipelago is of volcanic origin. The age of the islands has been estimated between 0.7 (westernmost islands, e.g. Isla Isabela) and 3 million years (eastern islands such as San Cristobal) (SIMKIN 1984).

Despite the recent age and the strong isolation of Galápagos, some 40 species of Carabidae (Coleoptera), belonging to 15 genera, are at present known from the islands (DESENDER et al. 1992a; DESENDER, unpubl.). Most of these carabid species are endemic to the islands, while their biogeographical origin is assumed to be in western South America (DESENDER et al. 1992b).

During several recent expeditions, the junior author and co-workers sampled all main islands and volcanoes of the archipelago in order to study carabid beetles, herbivorous beetles and spiders (BAERT et al. 1994). As a consequence,

systematic, ecological and molecular studies are in progress for several carabid genera (e.g. DESENDER et al. 1990, 1991, 1992a,b, 1999; DESENDER & VERDYCK 2000).

One aim of the recent study is the examination of the Laboulbeniales (Ascomycota) infesting Carabidae from the Galápagos Archipelago in order to obtain information about species numbers and the degree of endemism as well as the rate of parasitism of the ground beetle communities and populations. Such ecological data are very scarce from tropical regions but are needed to answer open questions of host specificity and transfer mechanisms of the parasites.

We will (1) summarise the results of our studies of Laboulbeniales on Galápagos carabids, with a description of three new species (in annex), (2) discuss the distribution and host-parasite relationships and (3) present an identification key to the *Laboulbenia inflata* species group.

HOST-PARASITE RELATIONSHIPS BETWEEN GROUND BEETLES AND LABOULBENIALES

Laboulbeniales are parasitic fungi infesting insects, diplopods and mites. Ground beetles represent one of the

most frequently infested host groups. Sixteen genera of Laboulbeniales are known from Carabidae including *Laboulbenia* Montagne & Robin with several hundred ground beetle-infesting species.

Host specificity and mechanisms of parasite transfer of many taxa, especially those from tropical regions, are still unclear. There are few specific studies of these problems of population ecology. DE KESEL (1997) concluded from transmission experiments with a *Pogonus*-infesting *Laboulbenia* species that direct infections are much more frequent than soil-borne infections. This is due to the extremely low pick-up probability of spores left on the substrate. SCHELOSKE (1976a,b) demonstrated in hydrophilid beetles the transfer of Laboulbeniales during the copulation of the hosts. The parasitic fungi cover specific regions of the body (posterior margin of elytra in females, ventral side of mesothorax in males) in these hosts. Several carabid hosts bear Laboulbeniales on the same body areas, which suggests the transfer of parasites during copulation. In other cases, the fungi infest the mouth parts of their hosts (see below, e.g. *Calosoma linelli* Mutchler) and were obviously transferred during feeding on infested prey. Often co-occurring carabids have the same parasite species. ARNDT (unpubl.) recognized the same *Laboulbenia* species in three co-occurring ground beetles (two *Notiobia* species, one *Selenophorus* species) on fruit fall areas in a Venezuelan rain forest site. The parasite infested these genera, overlapping all members of a spermatophagous carabid community. This phenomenon leads to the problem of host specificity. While most known Laboulbeniales infest only one species, a species group or one genus, there are some carabid-infesting fungi that are extremely polyphagous. Whereas e.g. *Laboulbenia palmella* Thaxter is restricted to *Mormolyce phyllodes* Hagenbach in South East Asia, and *L. pheropsophi* Thaxter to the genus *Pheropsophus* Solier world-wide, *L. flagellata* Peyritsch, *L. polyphaga* Thaxter and *L. vulgaris* Peyritsch are world-wide occurring carabid parasites, each infesting more than 25 host genera. The basis for this range in host specificity is not clear. DE KESEL (1996, 1997) showed that *L. slackensis* Cépède & Picard, under natural conditions monophagous on *Pogonus* species, can infest several carabids under laboratory conditions, even members of the genus *Carabus*, which are not known as a regular hosts of any Laboulbeniales.

A close host specificity can give information on the history of infection as well as on biogeographical aspects. TAVARES (1985) showed a co-evolution between the parasitic genus *Rhachomyces* Thaxter and their hosts in the carabid tribe Trechini. Trechini species migrated into Europe several times pre- and postglacial. Every migration was combined with its specific *Laboulbenia*- or *Rhachomyces* infection. Later, cross infections occurred between species of Trechini of different migrations and *Bembidion* species. TAVARES (1985) also summarized the knowledge on Laboulbeniales of Carabidae from the Hawaii archipelago. On these islands, a considerable evo-

lution and radiation of colonizing ground beetles took place, but the number of Laboulbeniales species remained low. With the exception of *L. vulgaris*, all recent species appear to be descendants from a single ancestor that invaded the Hawaii archipelago with its original host species.

MATERIAL AND METHODS

During several recent expeditions to the Galápagos Islands, more than 25,000 specimens of Carabidae have been collected from all of the larger Galápagos Islands and examined for the presence of Laboulbeniales (Dept. Entomology, Royal Belgian Institute of Natural Sciences, Brussels: BAERT, DESENDER, MAELFAIT & VERDYCK, 1986, 1988, 1991, 1996, 1998, 2000; complemented with Galápagos carabid material sampled by I. and H. Schatz, University of Innsbruck, Austria and by S. B. Peck and co-workers, Carleton University, Ottawa, Canada). On the whole, 480 carabid beetles, belonging to 15 species appeared to be infected. From these specimens, subsamples from all different localities were investigated in more detail.

Most beetles have been preserved in ethanol. Parasitic thalli were carefully removed from the host surface with the help of an insect pin (size 3). The thalli were mounted on a microscope slide in a mixture of glycerol+lactid acid+acid fuch sine and ringed using finger nail polish. The fungi were studied using a phase contrast microscope (Carl Zeiss Jena) at magnifications up to 600x.

Morphological terms are used according to SANTAMARIA (1998).

RESULTS AND DISCUSSION

Laboulbeniales on Carabidae from Galápagos

Laboulbeniales were recorded on 15 carabid species belonging to seven genera (Tab. 1). Host specimens occurred on seven of the 15 larger islands (Fig. 1).

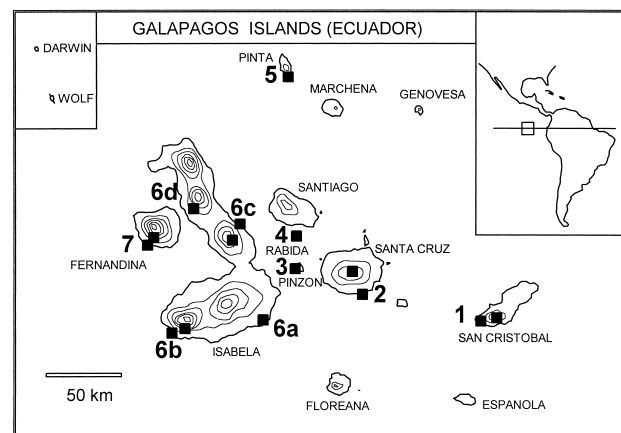


Fig. 1. – Sampling sites of Carabidae infested with Laboulbeniales in the Galápagos Archipelago: 1= San Cristobal, 2= Santa Cruz, 3= Pinzon, 4= Rabida, 5= Pinta, 6= Isabela: 6a= Volcan Sierra Negra, 6b= Volcan Cerro Azul, 6c= Volcan Alcedo, 6d= Volcan Darwin, 7= Fernandina.

TABLE 1

List of recorded *Laboulbenia* species, their host insects and infection rate. T – total number of collected host specimens in the sample. I – Number of infected specimens in sample.

Laboulbenia species	Host name	Island and volcano	exposition	m. a. sl.	vegetation zone	T	I
<i>appendiculata</i>	<i>Bradycellus insularis</i>	Pinzon	SE	380	dry arid zone	24	2
? <i>flagellata</i>	<i>Bembidion galapagoensis</i>	Isabela Volcan Alcedo	SE	1060	fern sedge zone	28	1
<i>flagellata</i>	<i>Calosoma linelli</i>	San Cristobal	W	675	fern sedge zone	7	2
<i>flagellata</i>	<i>Platynus albemarli</i>	Isabela Volcan Cerro Azul	WSW	680	fern sedge zone	1	1
<i>flagellata</i>	<i>Platynus darwini</i>	San Cristobal	W	700	fern sedge zone	5	1
<i>flagellata</i>	<i>Pterostichus calathoides</i>	San Cristobal	W	675	fern sedge zone	37	37
<i>flagellata</i>	<i>Pterostichus leleuporum</i>	Santa Cruz	S	875	fern sedge zone	18	10
<i>flagellata</i>	<i>Pterostichus leleuporum</i>	Santa Cruz	E	570	fern sedge zone	6	6
<i>flagellata</i>	<i>Selenophorus galapagoensis</i>	Pinta	SSE	2	litoral zone	2	1
<i>flagellata</i>	<i>Selenophorus galapagoensis</i>	Pinta	SSE	2	litoral zone	18	3
<i>flagellata</i>	<i>Selenophorus obscuricornis</i>	Fernandina	SW	400	dry arid zone	46	3
<i>flagellata</i>	<i>Selenophorus obscuricornis</i>	Isabela Volcan Alcedo	SE	600	dry arid zone	1	1
<i>flagellata</i>	<i>Selenophorus obscuricornis</i>	Isabela Volcan Darwin	SW	600	dry arid zone	24	3
<i>galapagoensis</i>	<i>Tachys erwini</i>	Fernandina	SW	5	litoral zone	24	5
<i>galapagoensis</i>	<i>Tachys erwini</i>	Pinta	SSE	2	litoral zone	22	5
<i>galapagoensis</i>	<i>Tachys nov. spec. 1</i>	San Cristobal	W	2	litoral zone	2	2
<i>galapagoensis</i>	<i>Tachys nov. spec. 2</i>	Isabela Volcan Alcedo	NE	2	litoral zone	3	1
<i>galapagoensis</i>	<i>Tachys vittiger</i>	Isabela Volcan Sierra Negra	SE	2	litoral zone	17	1
<i>galapagoensis</i>	<i>Tachys vittiger</i>	Rabida	NW	2	litoral zone	35	12
<i>galapagoensis</i>	<i>Tachys vittiger</i>	Santa Cruz	S	0	litoral zone	403	40
<i>inflata</i>	<i>Bradycellus insularis</i>	Isabela Volcan Alcedo	SE	1060	fern sedge zone	8	3
<i>inflata</i>	<i>Bradycellus insularis</i>	Pinzon	SE	380	dry arid zone	24	6
<i>inflata</i>	<i>Bradycellus spec.</i>	Santa Cruz	S	5	dry arid zone	2	2
<i>inflata</i>	<i>Bradycellus spec.</i>	Santa Cruz	S	20	dry arid zone	5	1
? <i>inflata</i>	<i>Bradycellus spec.</i>	Santa Cruz	S	5	dry arid zone	1	1
<i>sanjoaquina</i>	<i>Platynus chathamii</i>	San Cristobal	W	530	fern sedge zone	3	2
<i>sanjoaquina</i>	<i>Platynus chathamii</i>	San Cristobal	W	700	fern sedge zone	12	12
<i>sanjoaquina</i>	<i>Platynus darwini</i>	San Cristobal	W	700	fern sedge zone	5	5

One juvenile *Laboulbenia* specimen infested *Bembidion (Notaphus) galapagoensis* (Waterhouse) from Isla Isabela, Volcan Alcedo. This *Laboulbenia* species cannot be identified beyond doubt.

Laboulbenia flagellata Peyritsch

(Fig. 2)

Studied hosts and localities (+ sample codes, elevation and sampling date or period):

Calosoma linelli Mutchler, 1925, San Joaquin, Isla San Cristobal (A92/35, 675m, 21.02.92) (two specimens).

Pterostichus (Blennidius) calathoides (Waterhouse, 1845), San Joaquin, Isla San Cristobal (A92/33, 675m, 21.02.-01.03.92, A92/32, 530m, same period).

Pterostichus (Blennidius) leleuporum Reichardt, 1976, top of Isla Santa Cruz (A91/A20, 875m, 16.10.-15.11.92); Isla Santa Cruz, Los Gemelos (R00/01, 650m, 17.12.97).

Platynus (Dyscolus) albemarli (Van Dyke, 1953), Isla Isabela, Volcan Cerro Azul (P91/163, 680m, 21.-25.05.91) (only one female).

Platynus (Dyscolus) darwini (Van Dyke, 1953), San Joaquin, Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92). (Only one male, *L. flagellata* on the same host as *L. sanjoaquina* sp. nov.).

Selenophorus obscuricornis (Waterhouse, 1845), Isla Fernandina (B91/765, 400m, 04.05.91); Isla Isabela, Volcan Darwin (P92/189, 600m, 16.05.92); Isla Isabela, Volcan Alcedo (R00/06, 600m, 07.-10.04.99).

Selenophorus galapagoensis (Waterhouse, 1845), Isla Pinta (P92/43, 2m, 13.-23.03.92); Isla Pinta (B00/108A, 2m, 31.03.00).

L. flagellata occurs on the whole body surface of male and female beetles, often in groups of large numbers on *P. calathoides*, *P. leleuporum* and an infested male of *C. linelli*. The fungi grow very often pair-wise. The *Selenophorus* species are infested less numerously (scattered individuals, only one host specimen with 50 individuals of *Laboulbenia*; fungi mostly on elytra, very few individuals on ventral side and legs), whereas the *Platynus* species bear only scattered individuals on the elytra.

L. flagellata is on average longer and more slender on *Pterostichus* (full-grown: 220-280µm) than on *Selenophorus* and *Calosoma* species (140-200µm), but no other morphological differences were found.

L. flagellata is regarded as one of the most polyphagous and widespread species of the Laboulbeniales. MAJEWSKI (1994) mentioned that at least 80 gen-

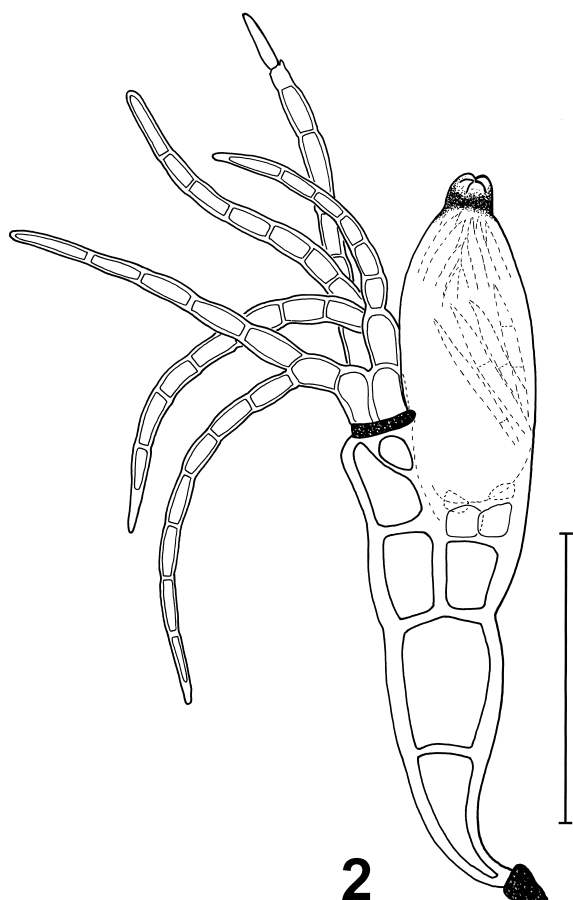


Fig. 2. – *Laboulbenia flagellata* Peyritsch from *Selenophorus galapagoensis* (Waterhouse). Bar 100µm.

era of Carabidae are hosts of this species. However, this number can vary because of different opinions on the generic state of several subgenera belonging to the large groups *Pterostichus* Bonelli (in widest sense) and *Platynus* Bonelli (in widest sense).

Selenophorus and *Calosoma* species are new host genera of this fungus. This is the first definite record of an infested Carabini under natural conditions; infection of *Carabus* spec. in laboratory experiments have already been described by DE KESEL (1997). However, *Calosoma linelli* is probably a secondary host in the sense of SCHELOSKE (1969). *C. linelli* co-occurs in the fern-sedge zone at higher elevations of Isla San Cristobal with the much more frequently-parasitised species *Platynus calathoides* as well as with *P. darwini* (cf. Tab. 2). Head and mouthparts of one of the *Calosoma* specimens were heavily infested.

Pterostichus leleuporum is the most frequent host species in Santa Cruz. It is endemic to the relatively more humid highlands of Isla Santa Cruz (fern-sedge zone). We could not find fungi on *Pterostichus williamsi*, the other endemic species of the same genus, occurring in the dry arid zone at low elevation on the same island.

All infested specimens of *Selenophorus galapagoensis* are from the littoral zone of Isla Pinta, one of the northernmost islands. Although this carabid species occurs on several other islands in the archipelago, parasites could not be found elsewhere. *Selenophorus obscuricornis*, the second infested species of this genus, lives in the dry arid zone of the central and western islands, although it is also found at higher elevation, especially on the younger volcanoes of Isabela and Fernandina. However, this species

TABLE 2

Carabid communities from three different localities (two habitat types) with recorded parasitic *Laboulbenia* species and infection rate. T – total number of collected carabid specimens in the sample. I – Number of infested specimens in the sample.

Carabid community	T	Infesting <i>Laboulbenia</i> species	I
Santa Cruz, South exposition, 5-20m a.s.l., dry arid zone, 21.3.98			
<i>Bradycellus</i> spec.	8	<i>L. inflata</i>	4
<i>Selenophorus obscuricornis</i>	330	-	0
<i>Calosoma granatense</i>	34	-	0
<i>Pentagonica flavipes</i>	2	-	0
Isabela Volcan Cerro Azul, WSW exposition, 680m a.s.l., fern sedge zone, 21.-25.05.91			
<i>Platynus albemarli</i>	1	<i>L. flagellata</i>	1
<i>Scarites williamsi</i>	7	-	0
<i>Bembidion galapagoensis</i>	9	-	0
<i>Bradycellus insularis</i>	1	-	0
<i>Pterostichus insularis</i>	3	-	0
San Cristobal, West exposition, 530-700m a.s.l., fern sedge zone, 20.02.-01.03.92			
<i>Pterostichus calathoides</i>	96	<i>L. flagellata</i>	95
<i>Calosoma linelli</i>	7	<i>L. flagellata</i>	2
<i>Platynus darwini</i>	6	<i>L. flagellata</i> + <i>L. sanjoaquina</i>	6
<i>Platynus chathamii</i>	18	<i>L. sanjoaquina</i>	17
<i>Scarites galapagoensis</i>	5	-	0

is only abundant in the 'dry arid zone' during periods with unusual rainfall (e.g. during El Niño events).

In conclusion, *L. flagellata* occurs in the Galápagos Archipelago from the littoral over the dry arid zone to the fern-sedge zone in the highlands, on eight different host ground beetle species (four genera). A large majority of infected beetles were found in humid habitats. All of the infected species are endemic to the archipelago. Five of these carabid species are limited to the higher and more humid parts of a single island (three species from San Cristobal, one from Santa Cruz and one from Isabela). Interestingly, we did not find any Laboulbeniales on carabids of the southernmost islands Floreana and Española. These islands are indeed known to be strongly isolated from the other Galápagos Islands by sea-currents, which mostly go westwards from San Cristobal towards Santa Cruz and Isabela, and then bend northwards. The same currents explain how *L. flagellata* could have reached the latter islands (see above).

***Laboulbenia sanjoaquina* sp. nov.**

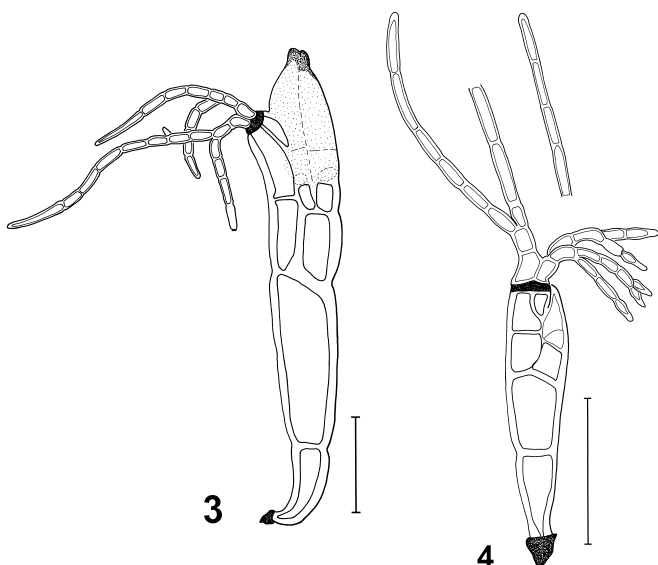
(Figs 3, 4)

Studied hosts and localities:

Platynus (Dyscolus) chathamii (Van Dyke, 1953), several infested specimens from San Joaquin near the top of Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92; A92/30, 530m).

Platynus (Dyscolus) darwini (Van Dyke, 1953), several specimens, Isla San Cristobal, San Joaquin, same date (A92/38, 700m). One female also infected with *L. flagellata*.

Hosts often infested with more than 100 *L. sanjoaquina* on dorsal and ventral sides of body. Usually females are infested on dorsal side (thorax, elytra), and males on ventral side (sternites, trochanter), suggesting regular infestation during copulation. Antennae and legs are mostly free from parasites. Several *L. sanjoaquina* of *P. darwini* are secondarily infested with nematode cysts. The hosts co-occur in the fern-sedge zone of Isla San Cristobal.



Figs 3, 4. – *Laboulbenia sanjoaquina* sp. nov. from *Platynus (Dyscolus) chathamii* (Van Dyke). Fig. 3. Fully-grown specimen. Fig. 4. Juvenile specimen. Bars 100µm.

***Laboulbenia galapagoensis* sp. nov.**

(Figs 5, 6)

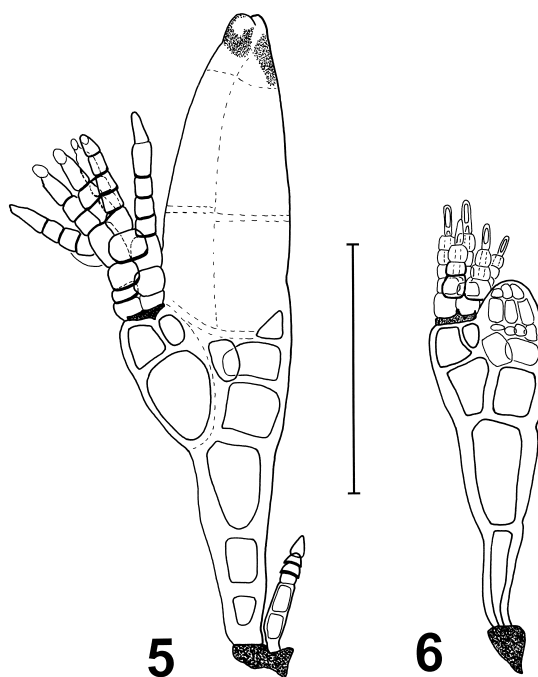
Tachys vittiger LeConte, 1851, female from Isla Santa Cruz (B91/890, 0m, 28.-29.05.91); Isla Rabida (P92/90, 2m, 03.03.92); Isla Isabela, Volcan Sierra Negra (B91/884, 2m, 26.05.91).

Tachys erwini Reichardt, 1976, Isla Fernandina (P91/115, 5m, 03.05.91); Isla Pinta (P92/55, 2m, 19.03.92).

Tachys nov. sp. 1, Isla San Cristobal (P96/27, 2m, 16.03.96).

Tachys nov. sp. 2, Isla Isabela, Volcan Alcedo (P96/77, 2m, 01.-03.04.96).

About 30 host specimens were examined. Hosts bear usually 1-2, very rarely up to five *L. galapagoensis*. The parasites occur most frequently on elytra or margin of thorax, rarely on sternites and legs of males and females.



Figs 5, 6. – *Laboulbenia galapagoensis* sp. nov. from *Tachys vittiger* LeConte. Fig. 5. Fully-grown presumed-female thallus and small male. Fig. 6. Juvenile presumed-female thallus. Bar 100µm.

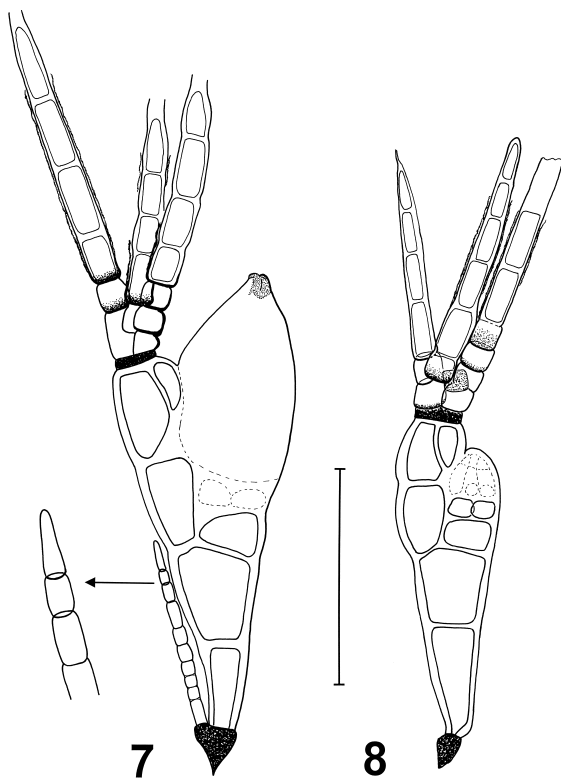
***Laboulbenia appendiculata* sp. nov.**

(Figs 7, 8)

Studied hosts and distribution:

Bradycellus insularis Reichardt, 1976, four specimens from Isla Pinzon (P91/255, 380m, 27.06.91).

From a group of seven co-occurring host specimens, six were infested by *L. inflata* (see below) and two by this new species on elytra and sternites. Two specimens of *L. appendiculata* occurred together with one specimen of *L. inflata* on one of the hosts.



Figs 7, 8. – *Laboulbenia appendiculata* sp. nov. from *Bradycellus* sp. Fig. 7. Fully-grown presumed female thallus and small male. Fig. 8. Juvenile presumed-female thallus. Bar 100µm.

Laboulbenia inflata Thaxter

Studied hosts and distribution:

Bradycellus insularis Reichardt, 1976, 3 specimens, Isla Isabela, Volcan Alcedo (P91/250, 1060m, 22.06.91); 6 specimens from Isla Pinzon, P91/255, 27.06.91. *L. inflata* occurred together with *L. appendiculata* sp. nov. on one male host specimen of *Bradycellus insularis*.

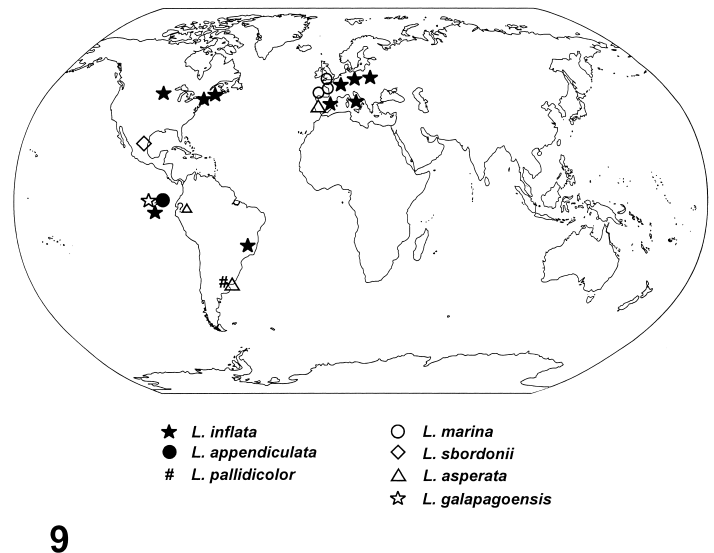
Bradycellus sp., 4 specimens from Santa Cruz (B98/049A, 21.3.98, B98/065A, 22.3.98, 2 specimens, B98/096A, 30.3.98, 5-20 m).

13 infested hosts were examined, each with one, rarely three, parasites. *L. inflata* occurred on elytra, sternites, thorax and tarsi.

Distribution and host-parasite relationships of the *Laboulbenia inflata* species group

Three of the *Laboulbenia* species occurring on the Galápagos archipelago are presumably dioecious, which is an extreme exception in this genus. Moreover, we suggest that these species (*L. inflata*, *L. appendiculata*, and *L. galapagoensis*) together with four other taxa form a monophyletic species group of *Laboulbenia* (see Annex for morphological details and arguments of the monophyly of this group).

L. inflata is regarded as the most primitive representative of the group; it also has the widest distribution. The known records of the *L. inflata*-group show a wide distribution in the New World and in Europe (Fig. 9). However, they also show a very scattered distribution due to inadequate collecting.



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Fig. 9. – Distribution map of the species of the *Laboulbenia inflata*-group.

The *L. inflata*-group infests two groups of hosts (see key for details of host genera): Stenolophini, a small group of the subfamily Harpalinae with world-wide distribution, and the tachyine-trechine-complex of the subfamily Trechinae. The host species of the latter group seem to prefer littoral habitats, whereas species of the former also occur in riparian, but not necessarily saline, habitats. Host specificity of the *L. inflata*-group is high, most species infecting only one ecologically-restricted host species or a group of related hosts in a geographically-restricted area. This may indicate host-parasite co-evolution. In the case of the *Tachys* species from Galápagos Islands, the species group-specificity apparently arose on the continent. A comparable situation was described for *Laboulbenia* species infesting Carabidae on Hawaii (TAVARES 1985, THAXTER 1908).

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ANNEX

Description of new *Laboulbenia* species and taxonomic remarks***Laboulbenia sanjoaquina* sp. nov.**

(Figs 3, 4)

Thallus gracilis, 500-650µm longus. Cellulae receptaculi elongatae, flavo-fusca; cellula V parva. Cellula insertionis atra. Appendix externa elata semel ramosa, appendix interna brevis in plurima antheridia desinens. Perithecium ovatum semi-liberum, fuliginosum. Parasitus Platynus (Dyscolus) (Coleoptera, Carabidae, Agonini). Typus EA02/33 (coll. Arndt, Anhalt University).

Description: Mature thallus slender, from foot to perithecial tip 500-650µm. Thallus pale olive-brown, perithecium dark brown, preostiolar spots black, the posterior spot occupying most of the respective lips. Insertion cell black, opaque; both appendages of pale colour.

Cells I-IV very slender, each 2.5-3.8 times longer than wide; cell V narrow oval, about half as long as cell IV. Septum IV-V straight to slightly curved. Insertion cell slightly constricting the posterior margin of thallus, situated towards the middle of posterior margin of perithecial wall, but separated from it.

Basal cells of appendages of similar size. Outer appendage consisting of two branches arising from the suprabasal cell, exceeding the perithecial apex, up to 320µm long. Inner appendage divided twice, rarely more above the basal cell, with antheridia at the tip of branches (Fig. 4), forming sterile branches exceeding the perithecial apex in mature thalli (Fig. 3). Cell VI more than twice as long as wide, trapezoid in section; cell VII longer than wide, square to trapezoid.

Perithecium relatively narrow, at the level of insertion cell 64-80µm wide, adnate to cells IV and V for 2/3 of its length. Perithecium is about 1/3 of length of the thallus. Apex asymmetrical, with prominent rounded posterior lips. Ascospores 81-90µm.

Holotype: on *Platynus (Dyscolus) chathamii* (Van Dyke), female from San Joaquin near the top of Isla San Cristobal (A92/38, 700m, 20.02.-01.03.92, collection number EA02/33).

Paratypes: same host species, several infested specimens from same date and locality; same host species, several infested specimens from Isla San Cristobal, same date (A92/30, 530m, EA02/31-32, EA02/72-74). *Platynus (Dyscolus) darwini* (Van Dyke), several specimens, Isla San Cristobal, San Joaquin, same date (A92/38, 700m, EA02/19-25).

Remarks. *L. sanjoaquina* is similar to *L. collae* Majewski from a platynine host in Europe and the group of *L. pseudomasei* Thaxter and related species from pterostichine hosts. *L. collae* has a smaller inner appendage basal cell and an insertion cell adnate to the side of the perithecium in contrast to *L. sanjoaquina* with basal appendage cells of similar size and an insertion cell which is separated from the perithecium. *L. pseudomasei* has a smaller inner appendage basal cell and phialides occurring laterally along the appendage branches, rather than forming terminal clusters as in *L. sanjoaquina*.

***Laboulbenia galapagoensis* sp. nov.**

(Figs 5, 6)

Dioecious. Mas. Hyalinus, elongatus, gracilis, e 6 cellulis confectus, 32-40µm longus. Receptaculum cellularum superpositarum constans. Antheridium unum simplex apicale.

Femina. Thallus 176-250 µm longus, melleo pro parte maxima. Cellula I divisa aut duplo-triplo longior quam latior; II sesqui longior quam latior; III obovata; cellula IV cellulam V amplitudine parum superat. Perithecium circiter duplo-triplo longius quam latius. Cellula insertionis atra. Appendices perithecio breviores, fuscis septis separatae. Parasitus Tachys (Coleoptera, Carabidae, Tachyini). Holotypus EA02/26 (coll. Arndt, Anhalt University).

Description: Presumably dioecious. Male and female thalli often occurring in pair, attached at their respective feet (Fig. 5). Both thalli including appendages pale yellowish except the black, opaque insertion cell and black preostiolar spots, the latter more or less merging with the paler colour of the perithecial walls by preapical shading.

Male thallus consisting of one row of apparently six cells, 32-40µm or slightly longer than cell I of female thallus. Basal cell extends into the opaque region of foot, cells 2 and 3 longer than wide and longer than following cells; cell 4 wider than long, cells 5, 6 cap-shaped, septa between cells 4-6 black, ultimate cell (presumed antheridium) triangular to flask-shaped.

Mature presumed female thallus comparably short and stout, from foot to perithecial tip 176-250µm long, only in rare cases more slender with a length up to 296µm.

Cell I often (=60% of examined specimens) divided in two small cells; cells I (if undivided) and II of similar length, cell II more or less wedge-shaped; cell III large, rounded, less than 1.5 times longer than wide, much larger than cell IV; cell V comparably large, not much smaller than cell IV; septum IV-V more or less straight, not turned inward. Insertion cell thin, at posterior margin of thallus strongly constricted; insertion cell attached to the base of perithecium.

Basal cells of appendages of similar size, outer basal cell only slightly larger. Both appendages of similar structure, dividing into several branchlets from suprabasal and following cells above; branches robust, short, with a maximum length of 90µm, not exceeding the middle third of the perithecium. Cells of basal and mesal parts of the branches with black septa (Figs 5, 6).

Perithecium relatively large, about half of thallus length. Perithecium with maximum width of 50-65µm. Apex nearly symmetrical with prominent rounded posterior lips. Cell VI about as long as wide, cell VII wider than long.

Holotype: on *Tachys vittiger* LeConte, female from Isla Santa Cruz (B91/890, 0m, 28.-29.05.91, collection number EA02/26).

Paratypes: same host species, date and locality (EA02/28-29, 39-40); Isla Rabida (P92/90, 2m, 03.03.92, EA02/36-38); Isla Isabela, Volcan Sierra Negra (B91/884, 2m, 26.05.91, EA02/63). *Tachys erwini* Reichardt, Isla Fernandina (P91/115, 5m, 03.05.91, EA02/17-18); Isla Pinta (P92/55, 2m, 19.03.92,

EA02/39). *Tachys* nov. sp. 1, Isla San Cristobal (P96/27, 2m, 16.03.96, EA 02/62). *Tachys* nov. sp. 2, Isla Isabela, Volcan Alcedo (P96/77, 2m, 01.-03.04.96, EA02/27).

Remarks. *L. galapagoensis* belongs to the *Laboulbenia inflata*-group (see below). The shape of the appendages and the character combination [small cell IV+ pale colour +often divided cell I] distinguish it from all other species known to us.

All known *Tachys* species from Galápagos are infected by this *Laboulbenia* species, suggesting coevolution at the level of host genus. All recorded hosts are littoral species from mangrove and salt marshes. *Tachys* nov. sp. 2 has a more or less marine way of life. The biology of this species (as far as it is known) resembles that of *Aepopsis robini* (Laboulbène), which is infested by *Laboulbenia marina* Picard, a representative of the same species group.

One of the host species of *L. galapagoensis*, *Tachys vittiger*, is also distributed on the mainland. It is known from the coastal region of Ecuador, and from Guatemala northwards up to British Columbia (Canada). We found one infested *T. vittiger* with one specimen of *Laboulbenia* from mainland Ecuador (Prov. Guayas, Bahia, Agangue, 18-30.VIII.1964, N. & J. Leleup leg.). However, the fungus is in poor condition and cannot be determined beyond doubt. It is most similar to the widespread *Laboulbenia asperata* Thaxter.

***Laboulbenia appendiculata* sp. nov.**

(Figs 7, 8)

Dioecious. Mas. Hyalinus, elongatus, gracilis, e 10 cellulis confectus, 70-85 µm longus. Receptaculum cellularum superpositarum constans. Antheridium unum simplex apicale.

Femina. Thallus 178-220 µm longus, melleo colore pro parte maxima. Cellula I duplo-triplo longior quam latior; II paulo longior quam latior; cellula III cellulam IV amplitudine adaequat, cellulam V superat. Perithecium ovatum semiliberum, circiter sesqui-duplo longius quam latius. Cellula insertionis atra. Appendices longior quam perithecii, cum septa e basi nigrum. Parasitus Bradycellus insularis (Coleoptera, Carabidae, Harpalini). Holotypus EA02/65 (coll. Arndt, Anhalt University).

Description: Presumably dioecious. Male and presumed female thalli often occurring in pairs, attached at their respective feet (Fig. 7). Both thalli pale yellowish; only preostiole spots, insertion cell and basal part of first cells of appendages dark brown to black.

Male thallus slender, consisting of one row of 10 cells of different length, from foot to apex 70-85µm long. The basal cell longer than following cells; cells of middle and apical part with dark septa constricting the male thallus; apical cell (presumed antheridium) slender and flask-shaped.

Mature presumed female thallus relatively short and stout, from foot to perithecial tip 178-220µm. Cell I two to three times longer than wide; cell II slightly longer than wide, widened upward; cells III and IV of similar size, about two times longer than wide; cell V more than half as long as cell IV, septum IV-V more or less straight, slightly oblique. Insertion cell thick, opaque, the posterior margin of thallus strongly constricting; insertion cell separated from the perithecial wall, situated above the middle of perithecium.

Inner and outer appendages of similar structure, quite robust. Basal cell of outer appendage two times longer and slightly wider than basal cell of inner appendage. All examined specimens with three branchlets at least in the juvenile specimens; outer appendage consisting of two branches arising from the basal cell; inner appendage mostly simple. First three or four cells of appendages with blackish septa and basal region partly darkened. Often new branches arising within deteriorating walls of old branches (Figs 7, 8). All branchlets exceeding the tip of the perithecium, branchlets of inner appendage with maximum length of 140µm, branchlets of outer appendage with maximum length of 180µm.

Cell VI wider than long, triangular to rhomboidal in optical section. Perithecium large, about half of thallus length. Perithecium with maximum width of 48-56µm. Apex nearly symmetrical with prominent rounded lips.

Holotype: on *Bradycellus insularis* Reichardt, male from Isla Pinzon (P91/255, 380m, 27.06.91, collection number EA02/65).

One juvenile (same host, same collection number) and three further adult specimens from another *Bradycellus insularis*, female, same date and locality (EA02/68).

Remarks. *L. appendiculata* belongs to the *Laboulbenia inflata*-group. Besides *L. inflata*, this is the only known species of the group infesting Stenolophini. It is, however, clearly different from *L. inflata* because of its pale colour, large cell V, broadly rounded perithecium with narrow apex, and cell VI that is wider than tall. Also it is paired with a long, thin supposed male thallus (Fig. 7). Appendages may arise in older branches and resemble those of *L. marina* Picard. However, they are much longer in *L. appendiculata*. *L. appendiculata* could be more closely related to *L. asperata*, a species of Tachyini/Trechini, than to *L. inflata* (see SANTAMARIA 1999).

***Laboulbenia inflata* Thaxter**

Remarks. The specimens of *L. inflata* from the Galápagos Archipelago are very similar to specimens from North America and Western Europe described e.g. by SANTAMARIA (1998).

Taxonomic characters, phylogenetic relationships and identification key of the presumable dioecious *Laboulbenia inflata* species group

Dioecism is an extreme exception in the genus *Laboulbenia* Montagne & Robin. It was first described for *L. formicarum* Thaxter, an ant-infesting species from North America (BENJAMIN & SHANOR 1950a, b). This is one of the smallest and most peculiar species of *Laboulbenia*. With a total length of only 70-80µm, it has a comparably large perithecium, which is longer than the remaining part of the female thallus. The appendages are about as long as the rest of the female thallus; the outer appendage is simple, the inner one consists of two branches. The first three cells of both appendages show constricted, dark septa. The male thallus is similar in structure to the female thallus in *L. formicarum*.

More recently, *L. asperata* Thaxter, *L. inflata* Thaxter, and *L. marina* Picard have been shown to be dioecious (SANTAMARIA 1996, 1998, 1999). Dioecism was not described explicitly for *L. sbordonii* W. Rossi & Cesari. However, its characters and the

illustrated thalli (ROSSI & CESARI ROSSI 1977) leave little doubt that *L. sbordonii* belongs to this group. The new species *L. galapagoensis* and *L. appendiculata* from Galápagos are probably dioecious species because of the characters shared with *L. asperata* and *L. inflata*. All these *Laboulbenia* species are parasites of Carabidae.

A perithecium-bearing thallus paired with a small filiform thallus was also described for *L. lecoareri* (Balazuc) Huldén. However, the structure of these thalli (BALAZUC 1974: 305) differs from those of the above-mentioned species group by the extreme reduction in size of the basal cell of the inner appendage, the branches of which are extremely small or even absent. Most probably the other seven species form a monophyletic group (*L. inflata*-group) within the large genus *Laboulbenia*. We regard dioecism as a synapomorphic character of this group. A second autapomorphic character of the group is the organisation of the male thallus, which differs strictly from that of *L. formicarum*. The male thallus consists of a simple

series of superposed cells, similar to the male thalli of some other dioecious genera of Laboulbeniales (BENJAMIN 1995, SANTAMARIA 1996). The preapical cells have black basal septa. Male and female thalli occur together with their feet attached to the host side-by-side. A further apomorphic character of the *L. inflata*-group is the black basal septa of the lower cells of their appendages. Simple long appendages or two simple branches respectively, as in *L. inflata*, are the supposed plesiomorphic state of appendages. The two new species from Galápagos as well as *L. marina* from a marine carabid species have derived appendages. The host of *L. marina* and the hosts of the new species from Galápagos live in coastal habitats.

The *L. inflata*-group may have been derived from a monoecious ancestor with similar appendages and upper receptacle. *Laboulbenia tachyis* Thaxter might appear to be such a species; however, no evidence has been published that antheridia are borne on the perithecial thallus in *L. tachyis*.

Identification key to the species of the *Laboulbenia inflata*-group

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| <p>1 Appendages of mature female thalli much shorter than perithecium. Hosts in littoral habitats2
 - Appendages exceeding the perithecium distinctly3</p> <p>2 Outer appendage simple, very wide. Cell V equal to or larger than cell IV, cells III and VI subequal in size. Male thallus consisting of 6 cells. Host: <i>Aepopsis robini</i> (Laboulbène) (subfamily Trechinae).<i>L. marina</i> Picard
 - Outer appendage consisting of more than one branch arising from the second or third cell (Figs 5, 6). Cell V not larger than cell IV, cell III much larger than cells IV-VI. Male thallus consists of 6 cells. Host: <i>Tachys</i> Stephens (subfamily Trechinae)<i>L. galapagoensis</i> sp. nov.</p> <p>3 Insertion cell transparent, reddish and outer basal wall cells of perithecium roughened. Male thallus consists of 5 cells. Host: <i>Tachys</i> Stephens (subfamily Trechinae)<i>L. asperata</i> Thaxter</p> | <p>- Insertion cell dark, opaque and/or outer basal wall cells smooth4</p> <p>4 Appendages long and filiform, as long as female thallus or longer. Cells of appendages pale, not darkened in basal part. Male thallus not longer than cell I of female, with less than 10 cells5
 - Appendages robust, shorter than female thallus with basally darkened cells in basal part of appendages (Figs 7, 8). Male thallus longer than cell I+II of female thallus, consisting of 10 cells. Host: <i>Bradycellus</i> Erichson<i>L. appendiculata</i> sp. nov.</p> <p>5 Female thalli unicoloured pale, slender, total length 280-463µm. Host: <i>Mexaphaenops intermedius</i> Barr, restricted to Central America (subfamily Trechinae)<i>L. sbordonii</i> W. Rossi & Cesari
 - Female thalli bicoloured, perithecium brown, basal part pale, less slender, maximum length 266µm, average length 220µm. Male thallus consists of 6-7 cells. Hosts: <i>Bradycellus</i> Erichson, <i>Acupalpus</i> Latreille, <i>Stenolophus</i> Latreille (Stenolophini, subfamily Harpalinae)<i>L. inflata</i> Thaxter</p> |
|---|--|