Notes on *Laboulbenia coneglanensis* (Ascomycetes, Laboulbeniales) in Japan

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Accepted for publication 29 September 1998

Laboulbenia coneglanensis isolated from Japanese harpaline carabids is described and photographed. It is compared morphologically with *L. flagellata* from Japan and Europe, which is similar in appearance. The most important difference between the two species is in antheridial character and host range. Other characters such as coloration, perithecial shape, especially the shape of the perithecial apex, and size of ascospores are also useful for distinction. *Laboulbenia ophoni* var. *dilatata* described by Maire (1920) is regarded as a synonym of *L. coneglanensis*.

Key Words—Anisodactylus; Ascomycetes; Carabidae; Harpalus; Laboulbeniales.

Laboulbenia coneglanensis Speg. was described by Spegazzini (1914), based on specimens collected on Harpalus (Pseudoophonus) rufipes (DeGeer) (=Ophonus pubescens Müller) in Italy. This fungus has been reported in Europe several times (Santamaria et al., 1991) and is frequently found on various species of harpaline carabids, especially of the genus Harpalus (Coleoptera, Carabidae, Harpalini). Laboulbenia flagellata Peyritsch (1873) was described as being on Bembidion and Patrobus, as well as on Agonum (as Anchomenus), all belonging to the family Carabidae, but the first host is regarded as doubtful for this taxon (Tavares, 1985).

As the thalli of *L. coneglanensis* and *L. flagellata* are much alike in appearance, some students have thought the two species to be conspecific (Balazuc, 1974; Majewski, 1994). However, distinct morphological differences separate them, and the two species do not occur on the same hosts in Japan. As part of a study of the carabidicolous Laboulbeniales of Japan, *L. coneglanensis* is described and compared with some characteristics of *L. flagellata* in the present paper.

Laboulbenia coneglanensis Spegazzini, Redia 10: 47. 1914. Type: On Harpalus (Pseudoophonus) rufipes (DeGeer) (Carabidae, Harpalini), Italy.

Figs. 1-3, 10-15, 17-20

Laboulbenia grisea Speg., Redia 10: 48. 1914.

Laboulbenia psittacea Speg., Redia 10: 48. 1914.

Laboulbenia ophoni Thaxter var. dilatata Maire, Bull. Soc. Hist. Nat. Afr. N. 11: 149, 1920.

Laboulbenia flagellata Peyritsch, in Sugiyama, Ginkgoana 2: 51. 1973.

Laboulbenia flagellata Peyritsch, in Majewski, Trans. Mycol. Soc. Japan 29: 40. 1988.

Laboulbenia macrotheca Thaxter, in Santamaria et al., Treb. Inst. Bot. Barcelona 14: 29. 1991 (in part). Laboulbenia flagellata Peyritsch, in Majewski, Pol. Bot. Stud. 7: 98. 1994 (in part).

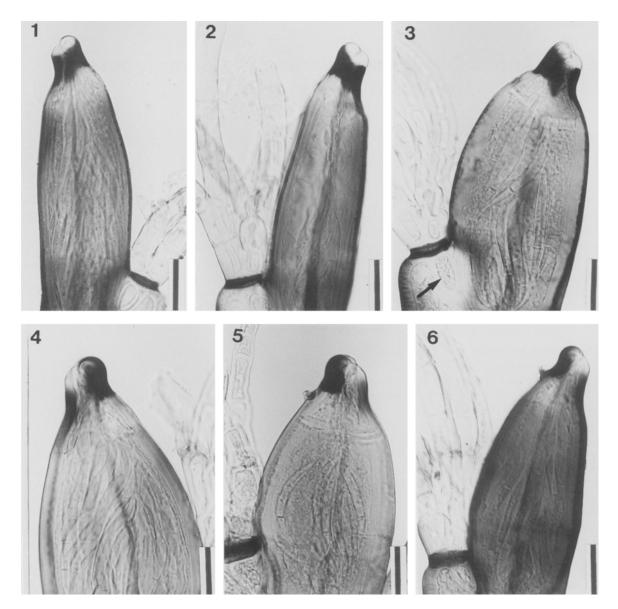
Measurements Total length to tip of perithecium $300-710~\mu\text{m}$; perithecium $105-180\times42-70~\mu\text{m}$; outer appendage $400-700~\mu\text{m}$ (up to $1,000~\mu\text{m}$) long; antheridia $12-15\times5-7~\mu\text{m}$; ascospore $47-68\times4.5-5~\mu\text{m}$ (excluding cell wall).

Thallus color In determining thallus color, specimens were observed under oblique illumination (incident light) by using a stereoscopic microscope at low magnification. Thalli of L. coneglanensis are at first yellowish or almost colorless except for the black zone below the ostiole, the black insertion cell, and the black foot, but gradually come to show a deep coloration, especially in the portion of the perithecia. Mature perithecia have a range of color from yellowish brown to blackish brown. Sometimes a dark olive or gray tinge is also present. The apical portion around the ostiole is quite hyaline in contrast to the blackened zone below (Figs. 1-3). Receptacles are usually almost colorless even when the perithecia are fully mature. Sometimes they are partially or wholly pigmented as darkly as the perithecia, depending on the stage of thallus development. Sometimes cell V is so hyaline that it is conspicuous among other cells (Fig. 3; also see Spegazzini, 1914, pl. IV, 32-c and pl. V, 34-b as L. psittacea). Appendages are also yellowish in color or almost colorless, but sometimes become brown.

Laboulbenia flagellata has a somewhat different pigmentation pattern from that of *L. coneglanensis*. The blackened area in the apical portion of the perithecium extends to the tip on the posterior side, whereas the anterior side of the apex is mostly unblackened (Figs. 4–6). The colorless area around the ostiole is not as clearly delimited as in *L. coneglanensis*.

Perithecia and ascospores Mature perithecia of *L. coneglanensis* are almost cylindrical (Figs. 10–12; cf. figures in Spegazzini, 1914), but become stout in short thalli (Figs. 13, 14). In *L. flagellata* thalli, on the other

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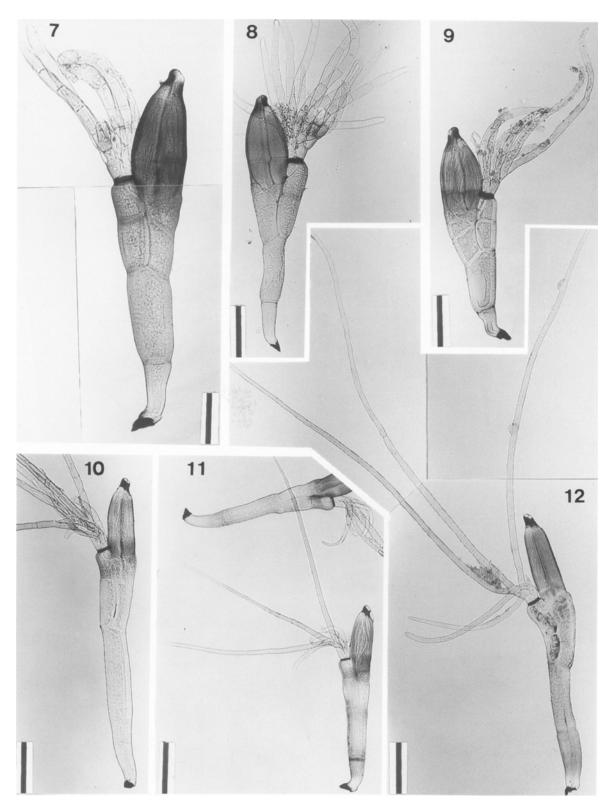
Figs. 1–6. Perithecia of *Laboulbenia* spp., showing differences in shape and intensity of color.
Bar = 25 μm. 1–3. *L. coneglanensis*. Quite hyaline around ostiole, where it is almost completely bordered with blackened zone.
1. On *Harpalus griseus*, KT-1368. 2. On *H. niigatanus*, KT-1384. 3. On *H. discrepans*, KT-527. Arrow indicates hyaline cell V. 4–6. *L. flagellata*. Not quite hyaline around ostiole. Blackened zone extends to posterior tip so that posterior lip cells become almost entirely dark. 4. On *Platynus assimilis*, France, KT-934. 5. On *Agonum moestum*, Italy, KT-996. 6. On *A. piceum*, Hokkaido, Japan, KT-1382.

hand, lateral sides of perithecia are frequently more convex (Figs. 7–9) than those in *L. coneglanensis* (Figs. 10–12). The most distinctive characteristic of the perithecium in *L. coneglanensis* is the more cylindrical shape of the apex, which is encircled completely by the blackened zone (Figs. 1-3; also see Spegazzini, 1914, pl. IV, figs. 32-a, 32-c and pl. V, 33-e as *L. grisea*, 34-a as *L. psittacea*).

The average size of ascospores of *L. coneglanensis* is smaller than that of *L. flagellata* (cf. Figs. 15, 16). Judging from my specimens on *Agonum dolens* (Sahlberg) from Japan and on *Platynus assimilis* (Paykull) from

France, the ascospores of *L. flagellata* are $64\text{--}78\times5\text{--}6~\mu\text{m}$. Thaxter (1896) measured ascospores of *L. flagellata* (as *L. elongata*) as $60\text{--}100\times5\text{--}8~\mu\text{m}$, whereas a somewhat smaller size was given by Majewski (1994, $55\text{--}60\times4\text{--}5~\mu\text{m}$) for the ascospores of *L. flagellata*, in which he synonymized *L. coneglanensis* with *L. flagellata*.

Appendages and antheridia The outer appendages become very long and taper as the thalli lengthen, but the inner appendages are comparatively short in most thalli of *L. coneglanensis* (Figs. 11, 12). Neither of the appendages has blackened septa. The outer basal cell is



Figs. 7–12. Thalli of *Laboulbenia* spp.

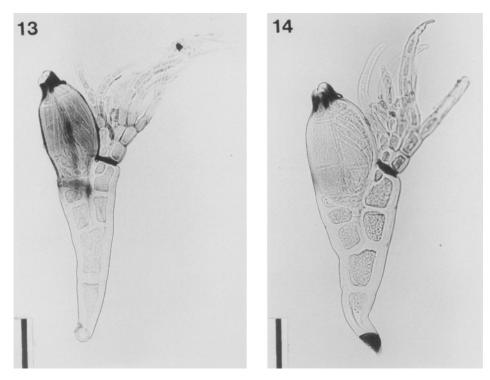
Bar=50 μm (for Fig. 7); 100 μm (for remaining figures). 7–9. *L. flagellata*. 7. On *Agonum piceum*, KT-1382. 8. On *A. dolens*, Hokkaido, Japan, KT-1354. 9. On *Platynus assimilis*, KT-934. 10–12. *L. coneglanensis*. 10. On *Harpalus eous*, KT-989. 11. On *H. sinicus*, KT-1366. 12. On *H. vicarius*, KT-1346.

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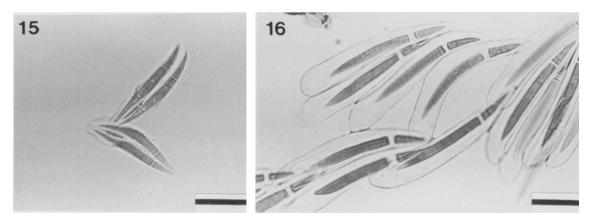
short-cylindrical and the inner basal cell is shorter or almost cubical in shape. The inner appendage consists of right and left axes and usually has two antheridia near the base in each axis; they are persistent, sessile, and borne singly, never in clusters (Figs. 19, 20). The antheridia of *L. flagellata* are also persistent, but they are either sessile or stalked, slightly larger ($18-20\times6-8~\mu m$ in my specimens) than those of *L. coneglanensis*, and usually occur in pairs or in a tuft with a 1-celled stalk (Figs. 21, 22). The outer appendages of *L. flagellata* are shorter ($300-480~\mu m$ long in my specimens) than those

of L. coneglanensis.

Receptacles The receptacle cell II of *L. coneglanensis* is cylindrical and usually 2–3 times longer than cell I (Figs. 10–12), but similar in length to cell I in short thalli (Figs. 13, 14). Cell III and cell VI are cylindrical and usually parallel in disposition (Figs. 17, 18), though cell VI is sometimes situated slightly lower than cell III. The oblique septum between cell IV and cell V is very short and does not reach cell III; cell V is wedge-shaped and much smaller than cell IV (Figs. 17, 18). The insertion cell is adnate to the basal portion of the perithecium, whereas



Figs. 13, 14. Short thalli of *Laboulbenia coneglanensis*. Bar=50 μm. 13. On *Harpalus sinicus*, KT-1058. 14. On *H. tinctulus*, KT-276.



Figs. 15, 16. Ascospores of Laboulbenia spp.
Stained by acetocarmine. Bar = 25 μm. 15. L. coneglanensis on Harpalus sinicus, KT-1332. 16. L. flagellata on Agonum dolens, KT-1339. Each spore is enveloped by thick gelatinous substance.

some tall thalli have insertion cells located at the perithecial base and slightly free from the perithecium (Fig. 12). The free insertion cells are more frequently observed in thalli on *Harpalus capito*. In comparison with most thalli on *Harpalus* spp., thalli on *Anisodactylus sadoensis* have receptacles with thicker cell walls (cf. Figs. 17, 18).

Host range In Japan, *L. coneglanensis* is found only on *Harpalus* spp. and *Anisodactylus* spp., both belonging to the tribe Harpalini (Carabidae), from which I have never obtained any forms of *L. flagellata*. The Japanese hosts of *L. flagellata* tend to be concentrated in Platynini and Pterostichini of the Carabidae. The following are the Japanese hosts of *L. coneglanensis*:

Anisodactylus punctatipennis Morawitz: I have not examined any specimens on this host species, which is, however, undoubtedly to be regarded as one of the hosts of *L. coneglanensis* (Sugiyama, 1973).

Anisodactylus sadoensis Schauberger: KT-358, 468, 597, Saijo, Higashi-Hiroshima, Hiroshima Pref.; KT-518, 519, Mt. Azumayama, Hiwa, Hiroshima Pref.; KT-1186, 1329, 1331, Otagawa River, Hiroshima, Hiroshima Pref.; KT-1372, Yuhuin, Oita Pref.

Harpalus (Cephalomorphus) capito Morawitz: KT-457, Horomui, Iwamizawa, Hokkaido; KT-830, Ishikari, Hokkaido; KT-821, Noda, Chiba Pref.

Harpalus (Pseudoophonus) vicarius Harold: KT-344, Nopporo, Ebetsu, Hokkaido; KT-526, 858, 944, Ikushunbetsu, Mikasa, Hokkaido; KT-1346 (sent from M. Ishitani), Mogamigawa River, Hunagata, Yamagata Pref.; KT-1370 (sent from K. Kurosa), Nishikawa, Yamagata

Pref.; KT-1333, Hosomidani, Yoshiwa, Hiroshima Pref. Harpalus (P.) roninus Bates: KT-1335 (sent from M. Nishida), Madarajima, Saga Pref.

Harpalus (P.) jureceki (Jedlička): KT-1367 (sent from K. Kurosa) Kita-Amarume, Yamagata Pref.; KT-1369 (sent from K. Kurosa), Tobishima, Yamagata Pref.; KT-822, Noda, Chiba Pref.

Harpalus (P.) griseus (Panzer): KT-824, Ishikari, Hokkaido; KT-1059, 1368, Otagawa River, Hiroshima, Hiroshima Pref. This host species also occurs in Europe (Spegazzini, 1914).

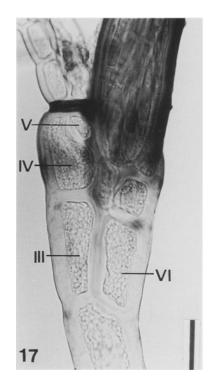
Harpalus (P.) eous Tschitschérine: KT-680, 829, 1358, Ishikari, Hokkaido; KT-989, Otagawa River, Hiroshima, Hiroshima Pref.

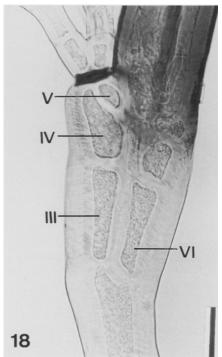
Harpalus (P.) pseudoophonoides Schauberger: KT-1056, Yasu-Huruichi, Hiroshima, Hiroshima Pref.

Harpalus (P.) tridens Morawitz: KT-130, 826, Mt. Moiwayama, Sapporo, Hokkaido; KT-1345 (sent from M. Ishitani), Mogamigawa River, Nagai, Yamagata Pref.

Harpalus (P.) sinicus Hope: KT-681, 828, 1057, 1058, 1332 (sent from M. Mori), Ishikari, Hokkaido; KT-1344 (sent from M. Ishitani), Mogamigawa River, Nagai, Yamagata Pref.; KT-1366 (sent from K. Kurosa), Amarume, Yamagata Pref.; KT-823, Noda, Chiba Pref.; KT-865, Yoshinogawa River, Yoshino, Nara Pref.; KT-1328, Otagawa River, Hiroshima, Hiroshima Pref.; KT-667, Ishigakijima, Okinawa Pref.

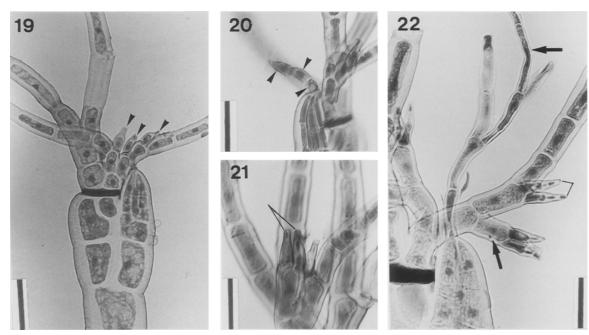
Harpalus (P.) niigatanus Schauberger: KT-1383, Bibi, Tomakomai, Hokkaido; KT-1384, Mt. Moiwayama, Sapporo, Hokkaido; KT-1388, Hashirajima, Yamaguchi





Figs. 17, 18. Middle portions of *Laboulbenia coneglanensis* thalli, showing receptacles with difference in cell wall thickness. Bar = 25 μm. 17. On *Harpalus tridens*, KT-1345. 18. On *Anisodactylus sadoensis*, KT-1331.

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Figs. 19–22. Antheridia of *Laboulbenia* spp.
Stained by acetocarmine. Bar=25 μm. 19, 20. *Laboulbenia coneglanensis*. 19. On *Harpalus sinicus*, KT-1332. Each arrowhead indicates sessile antheridium. 20. On *A. sadoensis*, KT-1331. Each arrowhead indicates sessile antheridium. 21, 22. *Laboulbenia flagellata*. 21. On *Agonum dolens*, KT-1348. Pair of sessile antheridia is indicated by V-line. 22. On *Platynus assimilis*, France, KT-1336. Upper arrow indicates branching trichogyne. Lower arrow indicates 1-celled stalk bearing tuft of antheridia. V-line indicates pair of sessile antheridia.

Pref.

Harpalus (P.) simplicidens Schauberger: KT-1387 (sent from Y. Nakamura), Yamakita, Kanagawa Pref.

Harpalus (Acardistus) platynotus Bates: KT-222, Shizunai, Hokkaido; KT-541 (sent from M. Mori), Toride, Ibaraki Pref.

Harpalus (Harpalus) tinctulus Bates: KT-276, Ugakyo, Hiroshima, Hiroshima Pref.; KT-466, Mt. Togosan, Yuki, Hiroshima Pref.; KT-1187, 1350, 1371, Otagawa River, Hiroshima, Hiroshima Pref.; KT-467, Bungo-Mori, Oita Pref.; KT-1349 (sent from K. Kurosa), Kuroishi, Kumamoto Pref.

Harpalus (H.) discrepans Morawitz: KT-527, 528, Mt. Azumayama, Hiwa, Hiroshima Pref.

Discussion

My observations indicate that *L. coneglanensis* is a distinct species, though it appears to be closely related to *L. flagellata*. The more cylindrical perithecial apex, ringed with a blackened zone below the hyaline tip, solitary sessile antheridia that are smaller in size, and smaller ascospores distinguish *L. coneglanensis* from *L. flagellata*. The hosts of *L. coneglanensis* seem to be restricted to the tribe Harpalini, though there are a few doubtful records of other groups of hosts such as Pterostichini and Platynini (Siemaszko and Siemaszko, 1932; Colla, 1934). The latter two tribes are widely considered to be host groups of *L. flagellata*.

Maire (1920) described *L. ophoni* var. *dilatata* on *H. rufipes* from Algeria. However, this host carabid had already been reported as the original host of *L. coneglanensis* by Spegazzini (1914). Moreover, Maire's figure of his variety and its spore size given shows clearly his material to be *L. coneglanensis* itself (Santamaria et al., 1991).

Thaxter (1908) referred the fungus found on *H. roninus* from Japan to *Laboulbenia madagascarensis* Thaxter, the thalli of which are somewhat similar to those of *L. coneglanensis*, except for the dark-edged outer appendage of the former fungus. Antheridial characters of *L. madagascarensis* are unknown.

Lee (1986) described a new species, Laboulbenia kwangjuensis Y.-B. Lee, on H. roninus and H. sinicus from South Korea and recently Terada (1998) recorded this fungus species on H. vicarius from Japan. Laboulbenia kwangjuensis is not closely related to L. coneglanensis, in which the former fungus has the crest-like appendages, the ellipsoidal perithecia with spirally arranged outer wall cells, and the branched stalks bearing clusters of antheridia.

Acknowledgements——I thank Dr. Isabelle I. Tavares, University of California, Berkeley, for reviewing this manuscript and offering valuable suggestions. I also thank Dr. W, Rossi, Dr. J. Balazuc, Dr. K. Kurosa, Dr. M. Ishitani, M. Nishida, Y. Nakamura and M. Mori, who kindly offered me some interesting hosts.

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