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New or interesting Laboulbeniales (Ascomycetes) on Japanese chlaeniine beetles (Coleoptera, Carabidae)

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Four species of *Laboulbenia* were isolated from Japanese chlaeniines and are reported. *Laboulbenia habui* is described as a new species; it is possibly related to *L. fasciculata*. *Laboulbenia habui* is characterized by the proliferation and division of cell V, resulting in the formation of an obliquely superposed series of cells from which short, cylindrical cells arise in a double row, whereas there is a single row of cubical cells in *L. fasciculata*. *Laboulbenia humilis* is new for the Japanese mycoflora; antheridia of this fungus were observed for the first time; shape of the perithecial apex, persistent antheridia and the spore size suggest that *L. humilis* is not closely related to *L. exigua* and its related taxa. For *L. proliferans* and *L. fasciculata*, new chlaeniine hosts are listed and some supplemental notes are given.

Key Words——chlaeniine beetles; Japan; Laboulbeniales; new species.

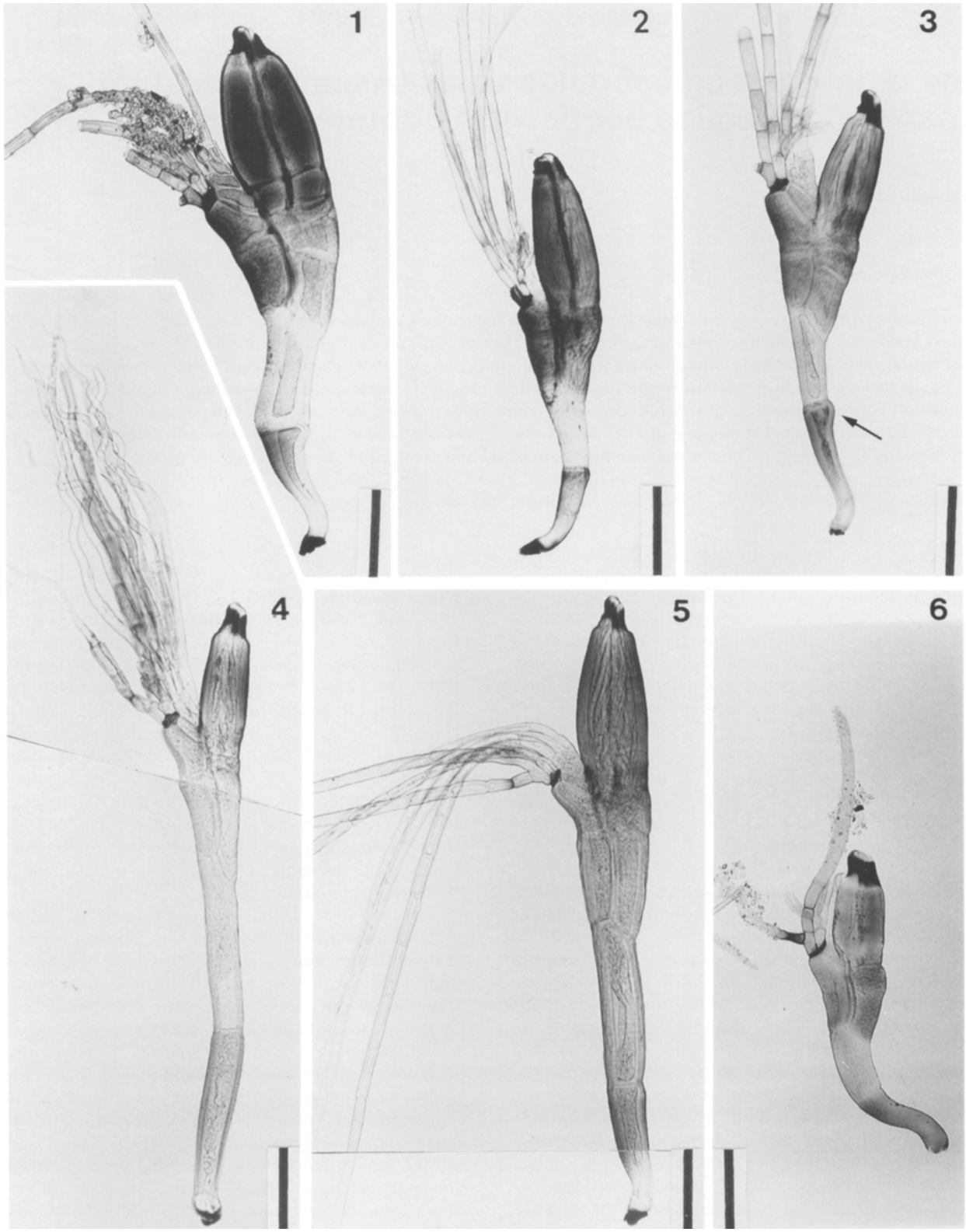
In the course of a study of the carabidicolous Laboulbeniales of Japan, a group of chlaeniicolous species of *Laboulbenia* was recently reported (Terada, 1995). However, still more species exist on Japanese chlaeniine beetles: for example, *L. proliferans*, *L. fasciculata* and *L. humilis*. The present paper provides some supplemental data on the above species as well as a description of an interesting new species.

Laboulbenia proliferans Thaxter, Proc. Amer. Acad. Arts Sci. **28**: 168. 1893; Mem. Amer. Acad. Arts Sci. **12**: 348. 1896; Mem. Amer. Acad. Arts Sci. **13**: 331. 1908; Sugiyama, Ginkgoana **2**: 60. 1973. On *Craspedophorus tropicus* (Hope) (Syn. *Eudema tropicum* Hope), Sierra Leone; *Chlaenius auricollis* Gory, Syria; *Dolichus* sp. ?, Japan. (Thaxter did not definitely designate the type.) Figs. 1–12

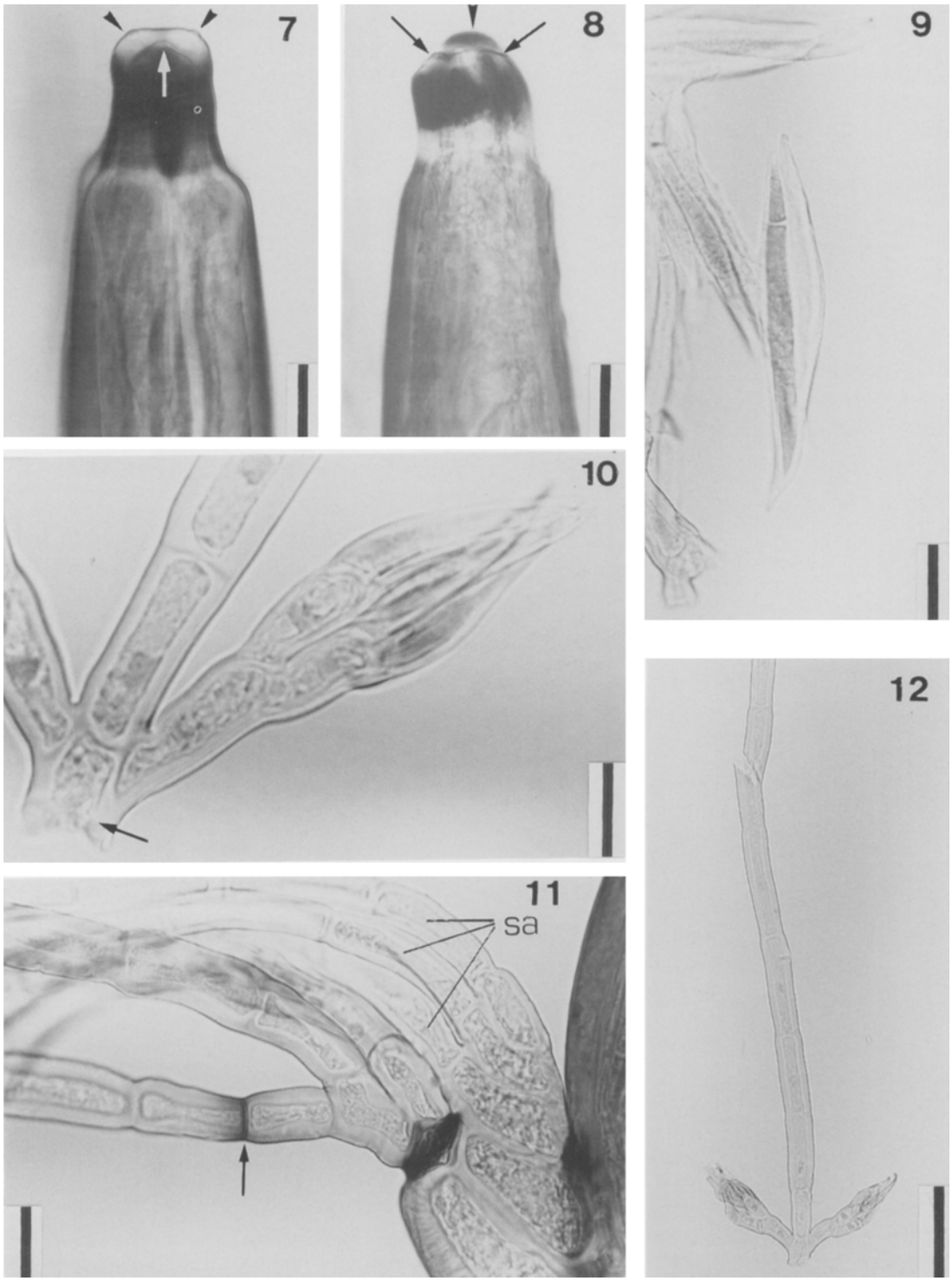
Specimens examined: On *Eochlaenius suvorovi* Semenov-Tian-Shanskij. No. 437, 4-I-1976, Watarase-gawa, Kita-Kawabe, Saitama. On *Lithochlaenius noguchii* (Bates). No. 1226, 19-VIII-1995, Yoneshiro-gawa, Futatsui, Akita. On *Chlaenius* (*Chlaenites*) *spolitatus motschulskyi* Andrewes. No. 557, 13-VIII-1974, Abiko, Chiba. On *Chlaenius* (*Ilaenchus*) *bimaculatus lynx* Chaudoir. No. 885, 8-VI-1978, Ohara, Iriomote-jima, Okinawa. On *Chlaenius* (*Pachydinodes*) *tetragonoderus* Chaudoir. No. 900, 11-VI-1978, Kabira, Ishigaki-jima, Okinawa. On *Chlaenius* (*Chlaeniostenus*) *circumdatus* Brullé. No. 353, 8-VIII-1974, Ishiyama, Otsu, Shiga; No. 409, 12-VI-1975, No. 1215, 29-VIII-1994, Nanatsuka, Shobara, Hiroshima. On *Chlaenius* (*Chinelaus*) *pallipes* Gebler. No. 463, 4-I-1976, Watarase-gawa, Kita-Kawabe, Saitama; Nos. 1227, 1228, 1229, 19-VIII-1995, Yoneshiro-gawa, Futatsui, Akita. On *Chlaenius* (*Agrochlaenius*) *circumductus* Morawitz. No. 151, 26-IV-1970, No. 416, 15-VII-1975, No. 945, 7-VIII-1976, Nopporo, Ebetsu, Hokkaido; No. 1230, 19-VIII-1995, Yoneshiro-gawa, Kita-Kawabe, Akita. On *Chlaenius variicornis* Morawitz. No. 390, Yasu-Huruichi, Hiroshima; No. 1157, 20-VIII-1993, No. 1204, 25-VIII-1992, No. 1206, 31-VII-1993, Ota-gawa, Hiroshima; No. 1167, 6-VI-1993, Nishizono, Izumo, Shimane. On *Chlaenius* (*Spirochlaenius*) *micans* (Fabricius). No. 1164, 1-VIII-1993, Ota-gawa, Hiroshima.

Measurements: Total length from foot to tip of perithecium 420–1125 μm long; perithecia 200–330 × 80–155 μm; appendages 850 μm (longest); antheridia ca. 25 μm long; ascospores 82.5–92.5 × 6.5–7.5 μm (60–70 × 5.5 μm in Thaxter, 1896).

This is one of the most common species in the genus *Laboulbenia*, mostly parasitic on chlaeniines, and it has a relatively large thallus which sometimes reaches over 1 mm in height. The color of this fungus is at first pale brownish yellow and later becomes deep amber, sometimes suffused with dark olive-brown or gray-brown (amber-brown, often tinged with olive in Thaxter, 1896). Such color variation may depend on thallus development. Proliferation and division of cell V, which are important characters in this fungus, result in a formation of an obliquely superposed series of cells between the insertion cell and the perithecial base (Fig. 11). From each cell of the series, a long secondary appendage, which is simple or branched once at the base, is formed. Basal cells of the secondary appendages are elongated and arranged in a single row. The primary appendage is composed of outer and inner axes situated on a disk-like, blackish insertion cell. The outer axis (outer appendage) is branched once at the base and is composed of two long, simple branches, the outer branch being slightly constricted

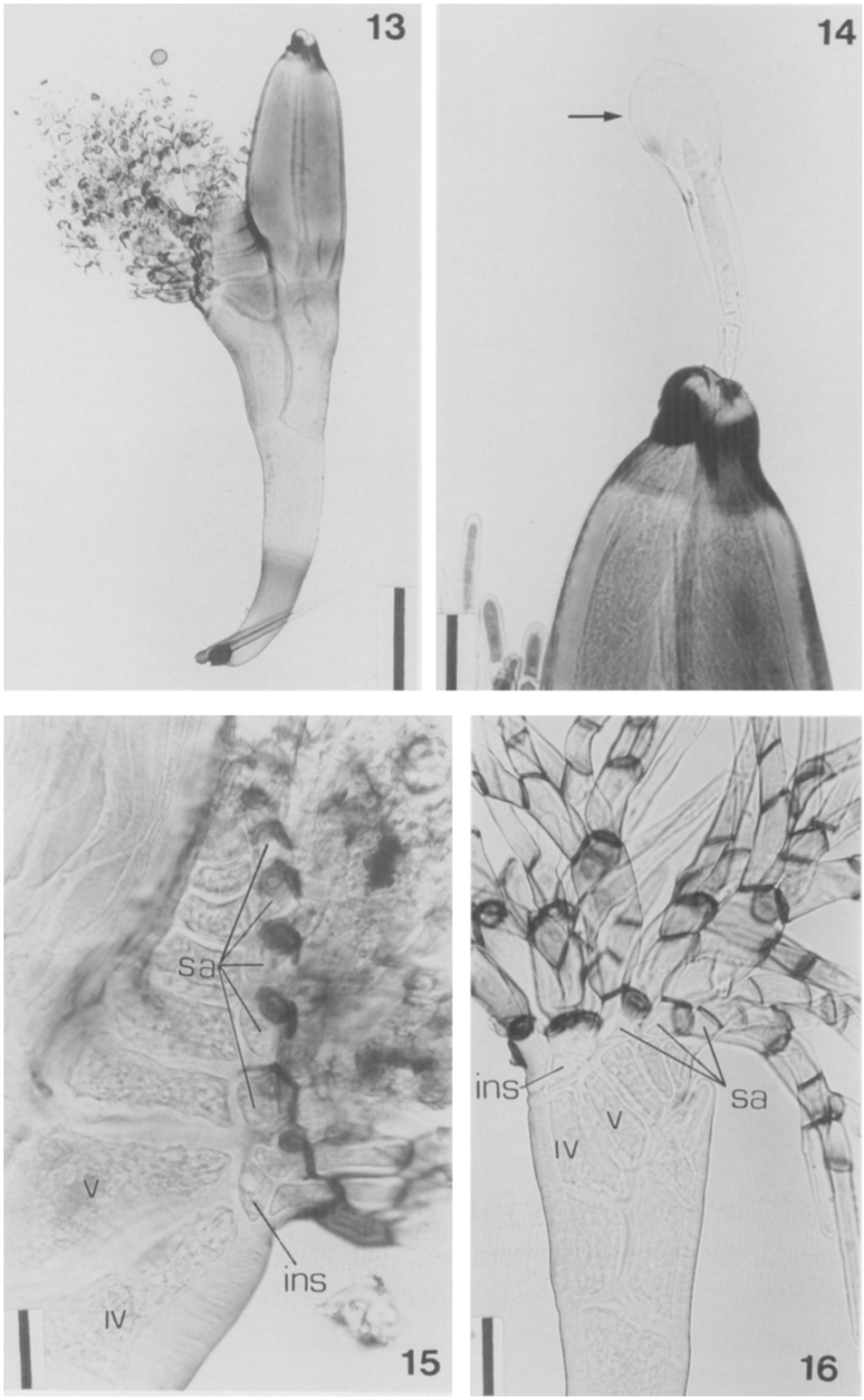


Figs. 1–6. *Laboulbenia proliferans*.
1. Mature thallus with geniculate receptacle. On *Chlaenius pallipes*. No. 463. 2. Mature thallus with broad-tipped perithecium. On *Chlaenius pallipes*. No. 1229. 3. Mature thallus with blunt projection on apical anterior side of cell I (arrow). On *Chlaenius pallipes*. No. 1228. 4. Slender form on *Lithochlaenius noguchii*. No. 1226. 5. Typical form on *Chlaenius circumductus*. No. 1230. 6. Mature thallus with reduced proliferation from cell V. On *Eochlaenius suvorovi*. No. 437. Each bar = 100 μm.



Figs. 7-12. *Laboulbenia proliferans*.

7. Upper portion of mature perithecium showing horizontal posterior apex and more or less rounded anterior apex (arrow). Each arrowhead indicates a minute papilla on posterior apex. No. 1226. Bar=20 μm. 8. Upper portion of mature perithecium showing anterior apex (arrowhead) extending above posterior apex (lateral arrows). No. 1227. Bar=20 μm. 9. Ascospores. No. 1167. Bar=20 μm. 10. Short, dichotomous branchlet with tufted antheridia. Arrow indicates basal cell of inner appendage. No. 1215. Bar=10 μm. 11. Distal portion of upper receptacle showing obliquely superposed extra cells from cell V. Each line indicates cylindrical basal cell of secondary appendages (sa). Arrow indicates blackish septum near base of outermost branch of outer appendage. No. 1230. Bar=20 μm. 12. Long, simple inner appendage with antheridial branchlets on both sides at base. No. 1215. Bar=50 μm.



Figs. 13–16. *Laboulbenia fasciculata*.

13. Typical form on *Chlaenius inops*. No. 410. Bar = 100 μm. 14. Upper portion of mature perithecium showing discharge of ascospore. Arrow indicates gelatinous envelope covering the ascospore. No. 1217. Bar = 20 μm. 15. Distal portion of upper receptacle showing obliquely superposed extra cells from cell V. Pale-colored, triangular insertion cell (ins) is situated on cells IV-V area. Each line indicates small, cubical basal cell of secondary appendage (sa). No. 1202. Bar = 20 μm. 16. Young thallus showing appendages, upper receptacle at left, and immature perithecium at right. Colorless, triangular insertion cell (ins) is situated on cells IV-V area. Each line indicates cubical basal cell of secondary appendage (sa). No. 1232. Bar = 20 μm.

at the blackish suprabasal septum. The inner axis (inner appendage) is also long and simple, and bears antheridial branchlets on both sides of the basal part (Fig. 12). Persistent, flask-shaped antheridia arise in tufts from the short, dichotomous branchlets (Fig. 10). Such antheridial branchlets are sometimes produced also from the secondary appendages. The apical part of the perithecium is strongly narrowed and projecting; the posterior margin of the apical part is almost flat, bearing a minute papilla on each side; the apical anterior part is situated slightly lower than the posterior part (Fig. 7), or else more or less projected over the posterior margin (Fig. 8). Ascospores are wider than those of the *L. exigua* group (Fig. 9; cf. Terada, 1995).

Morphological variations observed in Japanese material seem not to be so important taxonomically. The most common forms of this fungus in Japan are found on *Chlaenius circumductus* and *C. variicornis* (Fig. 5). Individuals taken from *Lithochlaenius noguchii* have a more slender form (Fig. 4). Some individuals on *Chlaenius pallipes* have a more or less noticeable swelling on the anterior upper side of cell I (Fig. 3), as Thaxter (1908) pointed out; some other individuals on the same host species have perithecium with broad apex (Fig. 2) or geniculate lower receptacle (Fig. 1). Some of the individuals on *Eochlaenius suvorovi* have only one secondary appendage (Fig. 6). A similar form was illustrated by Thaxter (1896, pl. XVII, fig. 8). This fungus is known from various parts of the world, although no records have been published from the New World. The first records of Japanese material were reported by Thaxter (1893, 1908) who listed four carabids as the host: *Dolichus* sp. ?, *Chlaenius* sp., *C. micans* (Fabr.) (as *C. biguttatus* Motsch.), and *C. pallipes* Gebl. The true names of the former two carabids are still unknown. An additional record was reported by Sugiyama (1973) who added one carabid, *Chlaenius variicornis*, as a host of this fungus.

Laboulbenia fasciculata Peyritsch, Sitzungsber. Kaiserl. Akad. Wiss., Math.-naturwiss. Kl., Apt. 1 (Wien) **68**: 248. 1873; Thaxter, Mem. Amer. Acad. Arts Sci. **13**: 330. 1908; Sugiyama, Ginkgoana, **2**: 49. 1973; Majewski, Trans. Mycol. Soc. Japan **29**: 39. 1988. Type: On *Chlaenius* (*Chlaeniellus*) *vestitus* (Paykull), Europe. Syn. *Laboulbenia brachiata* Thaxter, Proc. Amer. Acad. Arts Sci. **25**: 11. 1890; Mem. Amer. Acad. Arts Sci. **12**: 349. 1896. Figs. 13-22

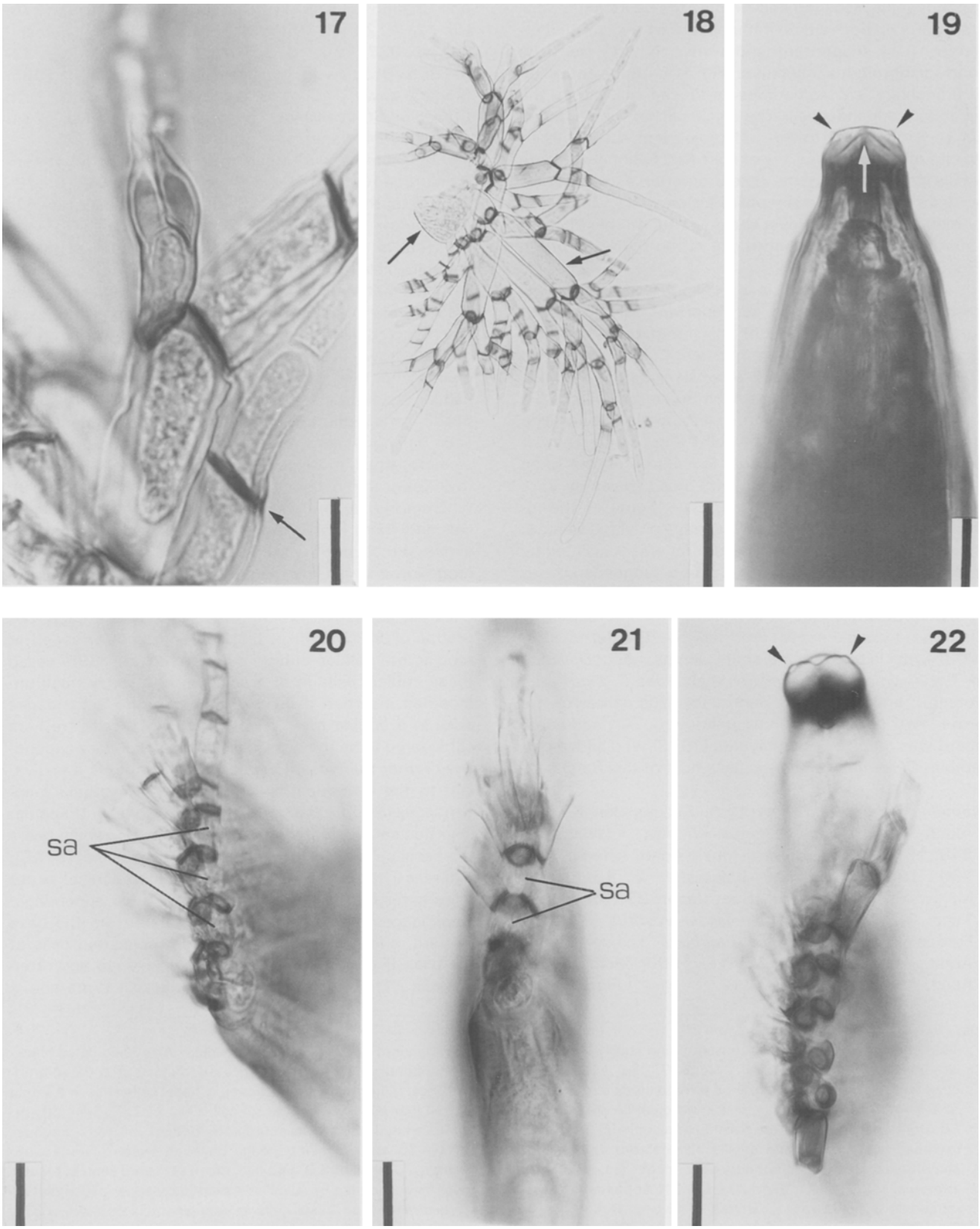
Specimens examined: On *Chlaenius* (*Chlaeniellus*) *inops* Chaudoir. Nos. 410, 417, 838, 839, 12-VI-1975, No. 1217, 29-VIII-1994, Nanatsuka, Shobara, Hiroshima; No. 837, 12-X-1975, Toride, Ibaragi; No. 1202, 22-V-1995, Ota-gawa, Hiroshima; No. 1232, 26-VII-1995, Mogami-gawa, Kubota, Yonezawa, Yamagata. On *Chlaenius* (*Chlaeniellus*) *prostenus* Bates. No. 1234, 29-VIII-1994, Nanatsuka, Shobara, Hiroshima.

Measurements: Total length from foot to tip of perithecium 410-500 μm long; perithecia 170-210 $\times$ 70-75 μm; antheridia 12.5-15 μm long; ascospores 62-65 $\times$ 5 μm.

This is one of the earliest-known species in the Laboulbeniales, parasitic on several different groups of carabids, but originally found on a chlaeniine beetle. The color of this fungus is at first pale brownish yellow, later becoming darker, and at last reaching gray-brown (smoky brown in Thaxter, 1896). The most distinctive character of this fungus is the proliferation and division of cell V, resulting in the formation of an obliquely superposed series of cells bearing secondary appendages, each having a series of black septa (Fig. 13). In *L. fasciculata*, small, more or less cubical cells arise in a single row from the oblique series of cells (Figs. 15, 16), whereas in *L. proliferans* these cells are long and cylindrical, and there are no series of black septa. Posterior views of thalli of *L. fasciculata* show the cubical cells arranged in a single row (Figs. 20-22). Each cubical cell bears two or three branches. In Figs. 20-22, the branches are almost all broken off except at the base, but groups of 2-3, ring-like, blackish septa are arranged at regular intervals on cubical cells. Thaxter (1896) called these cubical cells in *L. brachiata* "numerous small unmodified insertion cells,.. from which the appendages arise in a double row." However, these cells are here considered to be the basal cells of the secondary appendages rather than insertion cells because they are equivalent to the cylindrical basal cells of the secondary appendages in *L. proliferans*. A small, colorless, triangular insertion cell is situated on the cells IV-V area and it bears the basal cells of the primary appendages (Figs. 15, 16), whereas Thaxter (1908) said that the insertion cell is not directly related to the basal cell of the inner appendage. Appendages are branched several times from the base upward, and are composed of large, elongated cells at the base (Fig. 18); most septa are blackened and often oblique. The trichogyne also has blackish septa and is

Figs. 17-22. *Laboulbenia fasciculata*.

17. Lower portion of appendages showing short, 1-celled branchlet with paired, short-necked antheridia. Arrow indicates blackish, oblique septum of branch. No. 837. Bar = 10 μm. 18. Mass of appendages showing divaricated, distally attenuated branches with black septa. Arrow on left indicates extra cells of upper receptacle from which short,cubical basal cells arise in a single row. Arrow on right indicates extraordinarily elongated suprabasal cell just above cubical basal cell. No. 1232. Bar = 50 μm. 19. Upper portion of mature perithecium showing horizontal posterior apex and more or less acute anterior apex (arrow). Each arrowhead indicates minute papilla on posterior apex. No. 1232. Bar = 20 μm. 20. Appendage bases in postero-lateral view showing basal cells of secondary appendages (sa) arranged in a single row. Groups of 2-3, ring-like, black septa are seen at regular intervals. No. 1232. Bar = 20 μm. 21. Appendage bases in posterior view showing basal cells of secondary appendages (sa) arranged in a single row. Groups of two or three blackish septa are arranged on basal cells at regular intervals. No. 1232. Bar = 20 μm. 22. Appendage bases in posterior view showing groups of 2-3, ring-like black septa arranged at regular intervals on basal cells arranged in a single row. Each arrowhead indicates minute papilla on bilobed posterior apex of perithecium. No. 1232. Bar = 20 μm.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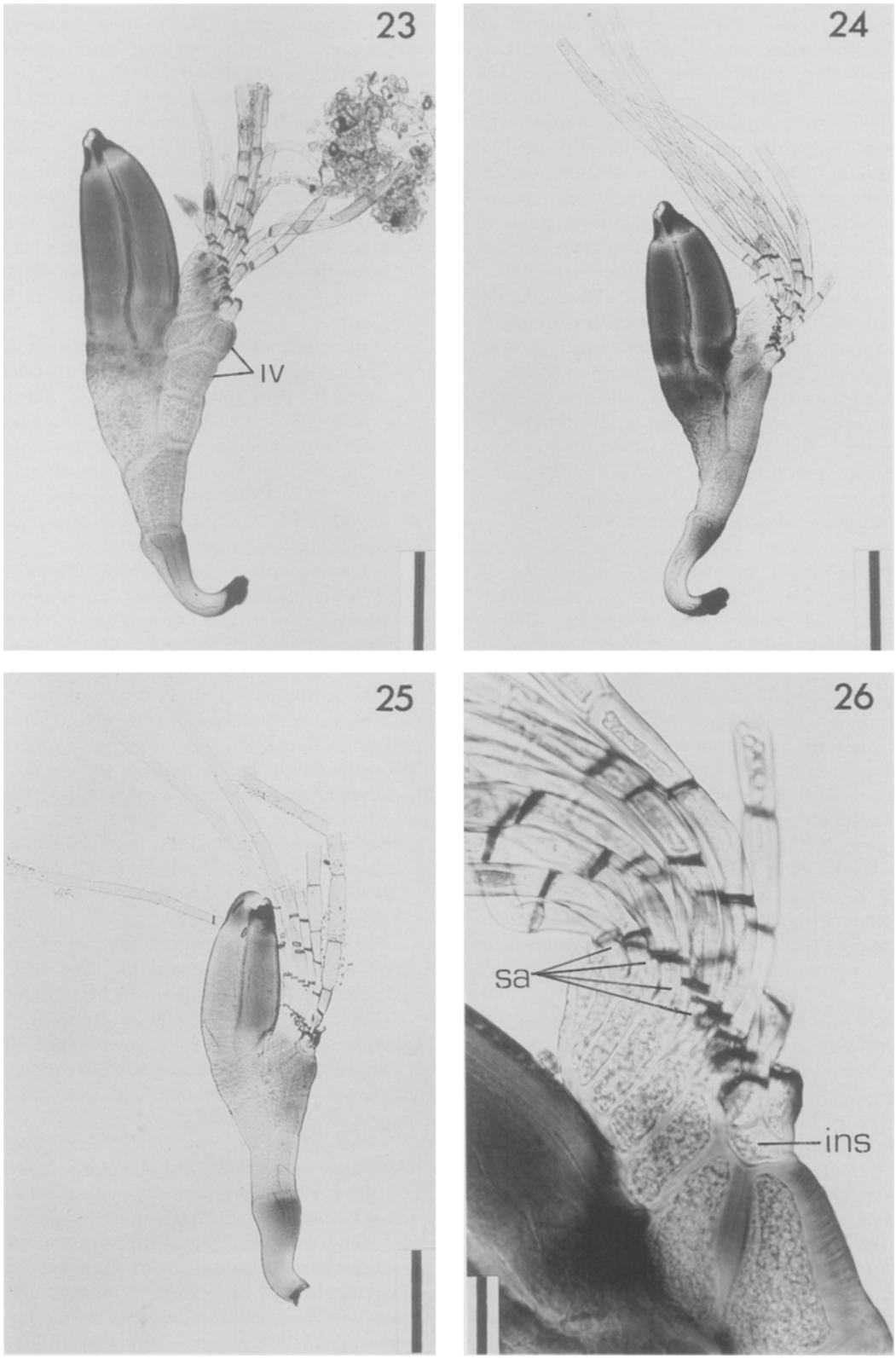


Figs. 23–26. *Laboulbenia habui*.
23. Mature thallus with cell IV divided into two superposed cells. On *Chlaenius kurosawai*. No. 1205. Bar = 100 μm. 24. Typical form on *Chlaenius kurosawai*. No. 1205. Bar = 100 μm. 25. Mature thallus with broad-tipped perithecium. On *Chlaenius ocreatus*. No. 834. Bar = 100 μm. 26. Distal portion of upper receptacle showing small, colorless insertion cell (ins) situated on cells IV-V area, and obliquely superposed extra cells, from which many secondary appendages arise. Basal cells of secondary appendages (sa) indicated by lines. No. 1205. Bar = 20 μm.

branched once or twice. Persistent, flask-shaped antheridia, each with a short neck, arise in pairs on short, 1-celled branchlets (Fig. 17). In the morphology of the perithecial apex (Fig. 19) and spore shape (Fig. 14), this fungus is similar to *L. proliferans*. Sometimes the posterior part of the perithecial apex is distinctly bilobed (Fig. 22). Thaxter (1908) suggested a possible relation between *L. variabilis* Thaxt. and *L. fasciculata*. However, in *L. variabilis*, the upper receptacle cells divide to form an upper layer of small cells producing black-septate appendages and the short antheridial branchlet forms a conical cluster of antheridia arising laterally and terminally on the branchlet. Thus the two species are completely different from each other (see Thaxter, 1896, pl. XXI, figs. 1-3, 5, 6).

Laboulbenia fasciculata may be world-wide in distribution, although no records have been reported from the Australian region. The first record of this fungus from *Chlaenius inops* was reported by Majewski (1988). This host species is very like *C. vestitus* in appearance, from which *L. fasciculata* was originally described.

Laboulbenia habui Terada, sp. nov. Figs. 23-31

Thallus fuscus, 390-510 μm longus. Cellula insertionis subhyalina. Cellula I prope basin curvata. Cellula II cellulam I subaequilonga. Cellula III oblonga, quam cellula II paulo brevior. Cellula IV cellulam III subaequilonga. Cellula V subtriangularis, seriem cellulae oblique superpositae formans. Appendices secundariae e serie obliqua cellulae orientes. Appendices primariae in cellula insertionis incolorata brevi sitae. Appendices e basi semel aut bis ramosae, plus minusve constrictae ad septa denigrata et plerumque obliqua. Antheridia persistentia, ampulliformia, longicollia, caespitosa, 27-30 μm longa, in ramulis dichotomis vel trichotomis sita. Perithecium 180-210 $\times$ 87-95 μm; pars apicalis parum angustata et projecta. Cellula VI quam cellula III paulo major et inferior. Ascosporae hyalinae, subfusiformes, 1-septatae, 57-68 $\times$ 6 μm. Holotypus: Terada No. 1205A (K. Terada Herbarium).

Thallus 390-510 μm long from foot to tip of perithecium, suffused with yellowish brown or yellowish gray brown, more deeply suffused in perithecium and upper part of cell I. Foot and subapical part of perithecium blackish. Insertion cell subhyaline. Cell I pale in color at base, abruptly curved near base; cell II subequal to cell I in height, with unequal lateral sides because lower septum of cell VI is oblique and reaches lower level than lower septum of cell III; cell III short-oblong in optical section, shorter than cell II; cell IV subequal to cell III in height, rarely divided into two superposed cells (Fig. 23); cell V subtriangular, producing an obliquely superposed series of cells, decreasing in size from below upward between insertion cell and perithecial base (Fig. 26). From oblique series of cells, short-cylindrical basal cells of long secondary appendages arising in double row (Figs. 26, 29). Primary appendage (outer and inner) situated on small, colorless, triangular insertion cell (Fig. 27). Each of appendages branched once or twice from base, more or less constricted at septa; lower septa blackened and often oblique (Fig. 27). Antheridia persistent, flask-shaped, tufted, 27-30 μm long, situated on dichotomous or trichotomous branchlets (Figs. 27, 28). Perithecium 180-210 $\times$ 87-95 μm; upper 4/5 of perithecium free; apical part of perithecium narrowed and projecting as in *L. fasciculata* (Figs. 23, 24), or sometimes broad (Fig. 25); posterior margin of apical part almost flat, bearing minute papillae (Fig. 31); anterior apex situated slightly lower than posterior apex (Fig. 31) or more or less projected over posterior margin (Fig. 25). Cell VI larger than cell III, situated beside cell III. Ascospores hyaline, subfusiform, 1-septate (septum subterminal), 57-68 $\times$ 6 μm (Fig. 30).

Etymology—After A. Habu, an entomologist.

Specimens examined: On elytra of *Chlaenius ocreatus* Bates. No. 354, 2-VIII-1974, Daisenji, Tottori; Nos. 834, 835, 25-VII-1977, Nanatsuka, Shobara, Hiroshima. On elytra of *Chlaenius kurosawai* Kasahara. Nos. 356, 1233, 6-VIII-1974, Ishiyama, Otsu, Shiga; No. 833, 12-X-1975, Ogonzan, Hiroshima; No. 836, 28-IX-1974, Toride, Ibaraki; No. 1205 (incl. Holotype), 6-VI-1993, Nishizono, Izumo, Shimane.

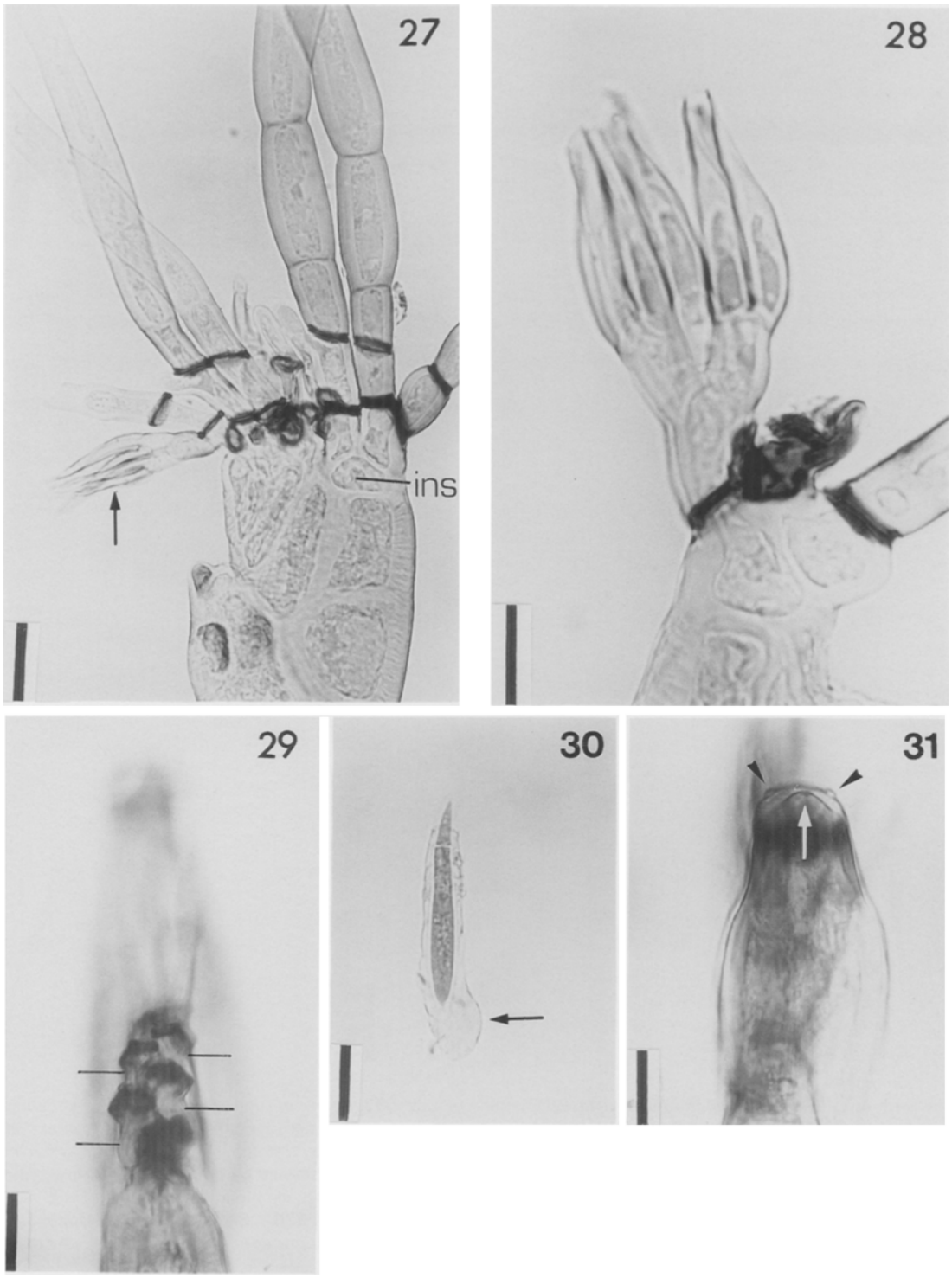
This new species is very like *L. fasciculata* in appearance. The blackened septa of the appendages and the colorless insertion cell show a close relation to *L. fasciculata*, from which it is, however, distinguished by the more slender basal-suprabasal cells in the appendages and the tufted antheridia with longer necks. The basal cells of the secondary appendages of *L. habui* are short-cylindrical and alternately arranged in a double row (Fig. 29), whereas in *L. fasciculata*, those cells are more or less cubical and arranged in a single row (Fig. 21).

Laboulbenia humilis Thaxter, Proc. Amer. Acad. Arts Sci. **38**: 42. 1902; Mem. Amer. Acad. Arts Sci. **13**: 334. 1908. Type: On *Chlaenius cyaniceps* Bates, Hong Kong. Figs. 32-37

Specimens examined: On elytra of *Chlaenius* (*Pachydinodes*) *tetragonoderus* Chaudoir. Nos. 879, 881, 8-VI-1978, Ohara, Iriomote-jima, Okinawa.

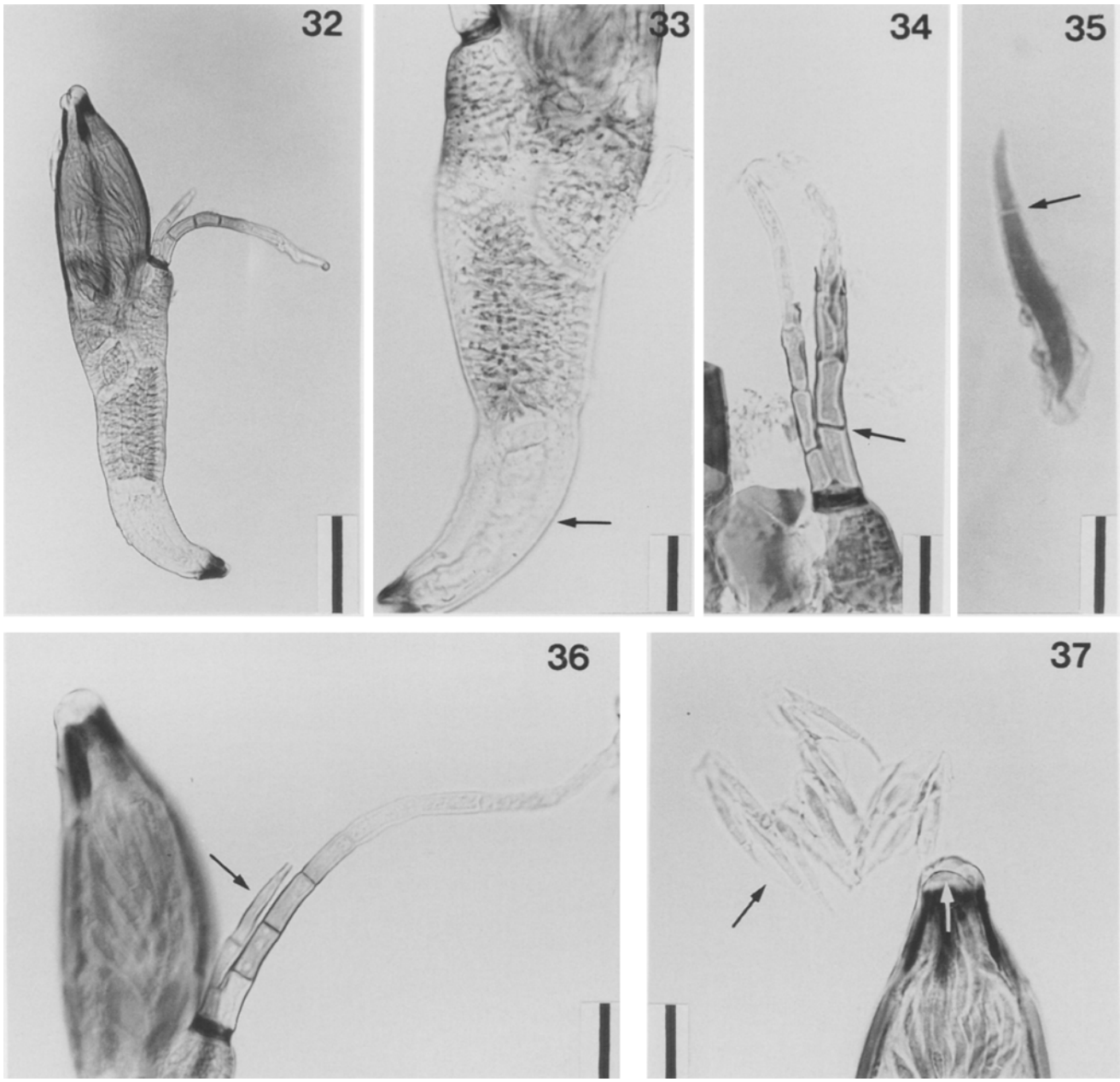
Measurements: Total length from foot to tip of perithecium 250-290 μm long; perithecia 105-120 $\times$ 45-50 μm; outer appendages 120-160 μm long; antheridia 20-22 μm long; ascospores 28-33 $\times$ 3 μm (about 50 $\times$ 5 μm in Thaxter, 1902).

This is a rare species. Two chlaeniine hosts, *Chlaenius monogrammus* Laf. and *C. cyaniceps* Bates, both from Hong Kong, were originally listed for this fungus. No additional information has been published so far. Olive-brown perithecia and amber-colored, streaked or spotted receptacles, with a contrasting pale cell I, make this fungus very unique (Fig. 32). Thaxter (1908) described the color of this fungus as "nearly uniform dirty olivaceous." However, Japanese material does not agree with his description. Perhaps Thaxter's material was old, because the color of the thallus generally becomes in dingy with age. The main feature of this fungus is the remarkable pattern of the receptacle (Fig. 33). Thaxter (1908) described the pattern of this fungus as "striate-punctate," but the fungal surface is



Figs. 27–31. *Laboulbenia habui*.

27. Young thallus showing appendages and upper receptacle. Colorless, triangular insertion cell (ins) is situated on cells IV-V area and bears outer and inner appendages. Arrow indicates tufted antheridia on short, dichotomous branchlet. More or less oblique, black septa of branch are seen. No. 835. Bar = 20 μm. 28. Short, trichotomous branchlet with tufted antheridia arising from abnormally developed young thallus. No. 835. Bar = 10 μm. 29. Appendage bases showing basal cells (lines) alternately arranged in a double row. No. 356. Bar = 20 μm. 30. Ascospore. Arrow indicates outer gelatinous envelope. No. 1205. Bar = 20 μm. 31. Upper portion of perithecium showing horizontal posterior apex and rounded anterior apex (arrow). Each arrowhead indicates minute papilla on posterior apex. No. 836. Bar = 20 μm.



Figs. 32-37. *Laboulbenia humilis.*
32. Typical form on *Chlaenius tetragonoderus.* No. 881. Bar=50 μm. 33. Lower portion of mature thallus showing pattern of pigment spots in wall of receptacle. Arrow indicates cell I essentially lacking this pigment pattern. No. 881. Bar=20 μm. 34. Simple inner and outer appendages. Distal portion of inner appendage is replaced by a sterile, simple branch. Arrow indicates oblique septum of outer appendage base. No. 879. Bar=20 μm. 35. Ascospore stained by cotton blue. Arrow indicates spore septum. No. 881. Bar=10 μm. 36. Upper portion of mature thallus showing long, simple outer appendage with very fragile upper half, and 3-celled inner appendage. Arrow indicates slender antheridium. No. 879. Bar=20 μm. 37. Upper portion of mature perithecium showing bluntly rounded posterior apex, and smaller, rounded anterior apex (arrow on right). Arrow on left indicates spore mass. No. 881. Bar=20 μm.

smooth. The outer appendage measures up to 120-160 μm in length, which is about two times longer than Thaxter's original measurement. This appendage is always curved outward and seems to be fragile enough to be broken off frequently at the midpoint (Fig. 36); the basal three septa are distinct; others above are very pale; no blackening or constriction is seen. Thaxter (1908) described the subbasal cell (of the outer appendage) as "separated by a more or less distinctly oblique septum." A similar oblique septum is shown in Fig. 34. However, this septum is at right angles to the lateral sides of the appendage in most individuals (Fig. 36). The inner appendage is usually 3-celled; the terminal cell is a narrow, flask-shaped antheridium, which is usually persistent

(Fig. 36) but occasionally replaced by a short sterile branch (Fig. 34). Thaxter (1908) said of the inner appendage: "the small basal cell producing two pale, short, few-celled, simple branches." However, no such individuals have been found in Japanese material. Ascospores are much smaller than those observed by Thaxter (1902) and stouter than those of *L. exigua* (Fig. 35; cf. Terada, 1995, fig. 7). The perithecial apex is not bilobed in antero-posterior view (Fig. 37). These observations indicate that *L. humilis* is not closely related to *L. exigua* as Thaxter suggested (Thaxter, 1908).

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