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Ammonia fungi of Iriomote Island in the southern Ryukyus, Japan and a new ammonia fungus, *Hebeloma luchuense*

Toshimitsu Fukiharu[1] and Tsuguo Hongo[2]

[1] Natural History Museum and Institute, Chiba, Aoba-cho 955-2, Chuo-ku, Chiba-shi, Chiba 260, Japan
[2] Ogaya 3-3-7, Otsu-shi, Shiga 520-21, Japan

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Species composition and fruiting season of ammonia fungi were investigated in Iriomote Island of the southern Ryukyus, Okinawa Prefecture, Japan. *Castanopsis* and *Pinus* forests were surveyed and 10 species of ammonia fungi were collected, including one new agaric species, *Hebeloma luchuense* sp. nov. This new fungus is characterized by having a rooting, squamulose-scaly stipe and cortinate veil and forms ectomycorrhizae with *Castanopsis cuspidata* var. *sieboldii*. Although the general mushroom season in the *Castanopsis* forest in Iriomote island was very short and restricted to summer, ammonia fungi were observed to fruit throughout the year in urea-treated plots.

Key Words——ammonia fungi; *Castanopsis* forest; ectomycorrhiza; *Hebeloma luchuense*; rooting stipe.

Application of urea to soil stimulates the appearance of fungi termed ammonia fungi (Sagara, 1975, 1992). Sagara (1975) investigated the ammonia fungi in the three main islands of Japan, Hokkaido, Honshu and Kyushu. Information on ammonia fungi from the area south of Kushu, however, is very scarce, so floristic and ecological studies of ammonia fungi in this area is very important. Within ammonia fungi, three species of *Hebeloma* have been recorded (Sagara, 1975, 1992): *H. spoliatum* (Fr.) Karst., *H. vinosophyllum* Hongo, and *H. radicosoides* ined. In addition to them two more *Hebeloma* species, *H. radicosum* (Bull.: Fr.) Ricken and *H. aminophilum* Hilton & Miller, have been reported to have similar ecology with ammonia fungi. The former occurs from underground mammalian nests (Sagara, 1989) and the latter grows on the ground near decaying animal matter, such as kangaroo (Miller and Hilton, 1986). In this study, another apparently unknown species of *Hebeloma* with a rooting stipe was collected from urea-treated soil in a *Castanopsis* forest and is described here as a new species.

Materials and Methods

Two types of vegetation were investigated, a *Castanopsis* forest dominated by *C. cuspidata* var. *sieboldii* (Makino) Nakai, and a *Pinus* plantation of *P. luchuensis* Mayr (about 30 years old). To obtain ammonia fungi, urea (granular form fertilizer; N 46%) was applied to the ground surface in amounts of 86, 171, 343 and 686 g/m^2 (nitrogen amounts were 40, 80, 160 and 320 N g/m^2, respectively). Plots of 1×0.5 m were chosen on level ground at an altitude of about 50 m in each forest; two experimental plots were made for each urea amount in each forest, and the species occurring there were recorded during two seasons (Fig. 1). In the *Castanopsis* forest, urea was applied on 16 Aug. 1982 and 26 Jan. 1983, then the urea-treated plots were visited four times over a period of about one year. Surveys were conducted in summer (17 Aug.–25 Sep. 1982), winter (1 Jan.–31 Jan. 1983), spring (14 Mar.–22 Apr. 1983) and the following summer (6 Sep.–2 Oct. 1983). In the *Pinus* plantation, urea was applied on 23 Aug. 1982 and 9 Jan. 1983, and surveys were conducted in summer (24 Aug.–2 Oct. 1982), winter (1 Jan.–4 Feb. 1983), spring (14 Mar.–30 Mar. 1983) and the following summer (8 Sep.–2 Oct. 1983). The plots were examined at intervals of 2

Table 1. Ammonia fungi of *Castanopsis* and *Pinus* forests in Iriomote Island in the southern Ryukyus, Japan.

Species	Type of forest	
	Castanopsis	*Pinus*
Deuteromycetes		
Cladorrhinum foecundissimum	○	○
Ascomycetes		
Ascobolus denudatus		○
Ascobolus hansenii	○	
Basidiomycetes		
Coprinus cinereus	○	
Coprinus phlyctidosporus	○	○
Coprinus sp.	○	
Crucispora rhombisperma		○
Alnicola lactariolens	○	○
Hebeloma radicosoides	○	
Hebeloma luchuense	○	

○: occurrence comfirmed, Blank: no occurrence.

days to 1 week during the investigation periods. Maximum and minimum soil temperatures were measured about 5 cm below the ground surface near the plots in *Castanopsis* forest on every field observation. Precipitation data were obtained from Ishigaki Meteorological Station, located about 50 km from the study plots (Japan Meteorological Agency, 1982, 1983). A supplementary investigation in the *Castanopsis* forest was conducted in 1992. Urea was applied to four 2×2 m plots at a concentration of 400 g (184 N g)/m^2 on 24 Feb. 1992, and the plots were observed on 7-8 Sep. 1992. Fungi and mycorrhizal roots collected were dried at 60°C or stored in FAA (formalin-acetic acid-alchol), and observed microscopically. Anatomical observations and measurements were made in lactophenol mounts. Color notations used in the species description in parentheses are from Kornerup and Wanscher (1978). Holotype of *H. luchuense* and representative specimens of the collected fungi are deposited at the Natural History Museum and Institute, Chiba (CBM) with accession number.

Results and Discussion

Collected fungi and their fruiting behavior

The following 10 species were collected: *Ascobolus denudatus* Fr., *Ascobolus hansenii* Paulsen & Dissing, *Cladorrhinum foecundissimum* Sacc. & March, *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray, *Coprinus phlyctidosporus* Romagnesi, *Coprinus* sp. (spores of this species were similar to those of *C. neolagopus* Hongo & Sagara, but larger, $10 \times 6\ \mu$m, and more rounded), *Crucispora rhombisperma* (Hongo) Horak, *Alnicola lactariolens* Clémençon & Hongo, *Hebeloma radicosoides*, and *H. luchuense* Fukiharu & Hongo (described below).

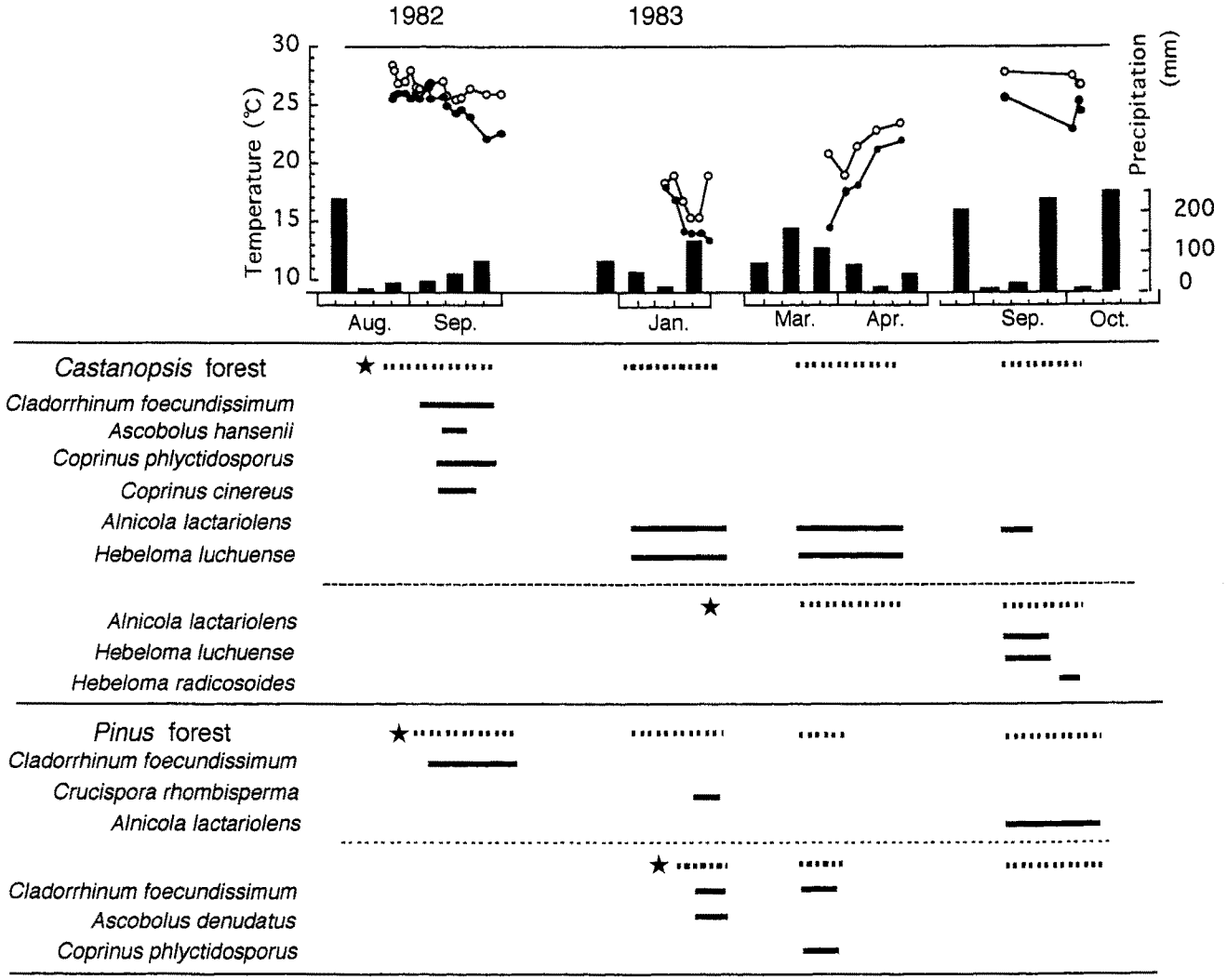


Fig. 1. Sporocarp phenology of ammonia fungi in *Castanopsis* and *Pinus* forests.
★ Urea treatment. ▪▪▪ Observation period. — Period of appearance of sporocarps on the ground. -○- Maximum soil temperature. -●- Minimum soil temperature. Temperatures were measured about 5 cm beneath the ground surface near the plots. Histogram shows the amount of precipitation. Precipitation data were obtained from Ishigaki Meteorological Station, located about 50 km from study area.

Among them, 8 species were collected from plots in the *Castanopsis* forest and 5 species from those in the *Pinus* plantation (Table 1). *Alnicola lactariolens* was first reported from a *Castanopsis* forest in Kyoto Prefecture as an ammonia fungus and tentatively identified as *Hebeloma* sp. (Fukiharu, 1991), before finally being described as a new species of *Alnicola* (Clémençon and Hongo, 1994).

The succession of the ammonia fungi can be divided into two stages, early stage and late stage (Sagara, 1992). The early stage comprises members of Deuteromycetes, Ascomycetes and saprophytic Basidiomycetes and the late stage comprises mainly ectomycorrhizal Basidiomycetes. In this study, two successional stages were also recognized (Fig. 1). Early-stage fungi, such as *Cladorrhinum foecundissimum*, *Ascobolus* spp. and *Coprinus* spp., began to appear about 3 weeks after urea treatment in both *Castanopsis* and *Pinus* forests in summer- and winter-treated plots and lasted for 1-3 months. Late-stage fungi, such as *Hebeloma* spp. and *Alnicola lactariolens*, occurred from September to October in the winter-treated plots, and even from January to April in the summer-treated plots. In the main islands of Japan, late-stage ammonia fungi appear only during the ordinary mushroom season and usually do not fruit in winter. For example, in *Castanopsis* forests of Kyoto Prefecture, late-stage ammonia fungi has been observed from late July to early August and from late September to November (Fukiharu, 1991), corresponding to the fruiting seasons of other fungi in non-urea-treated areas. In the areas around the experimental plots, mushroom sea-

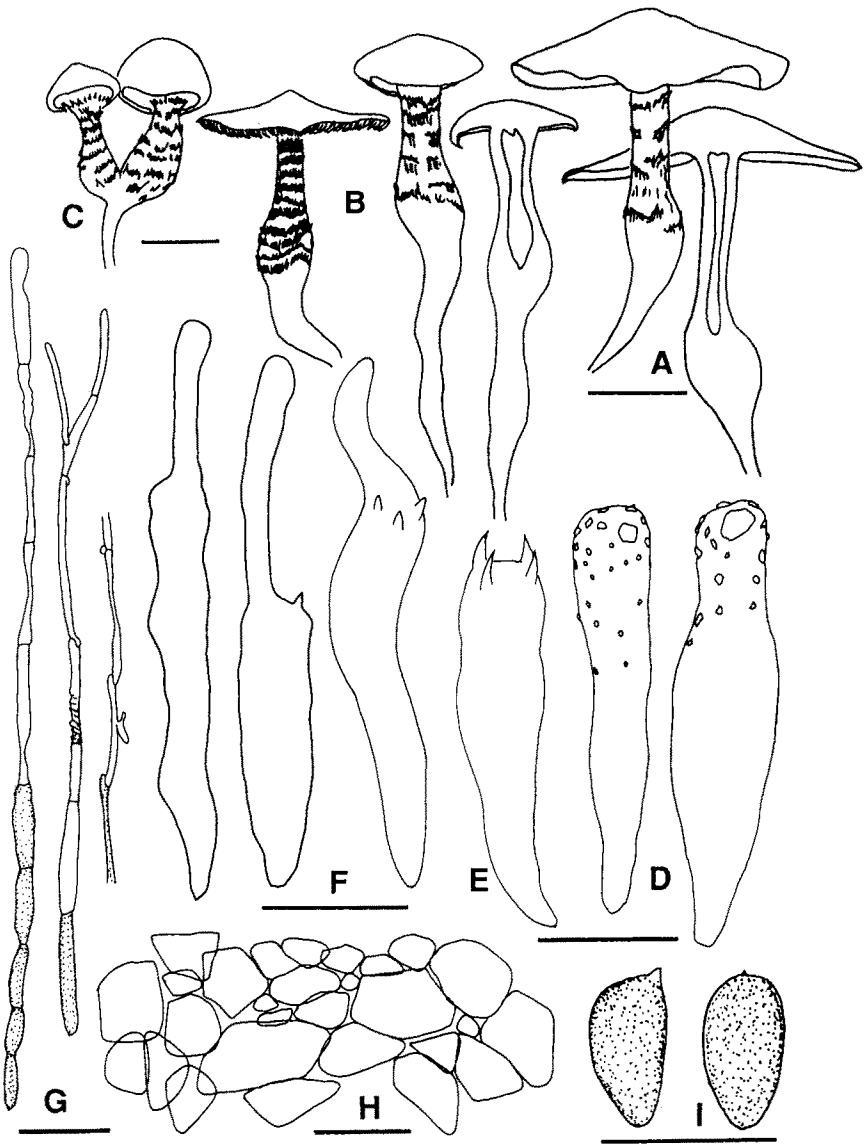
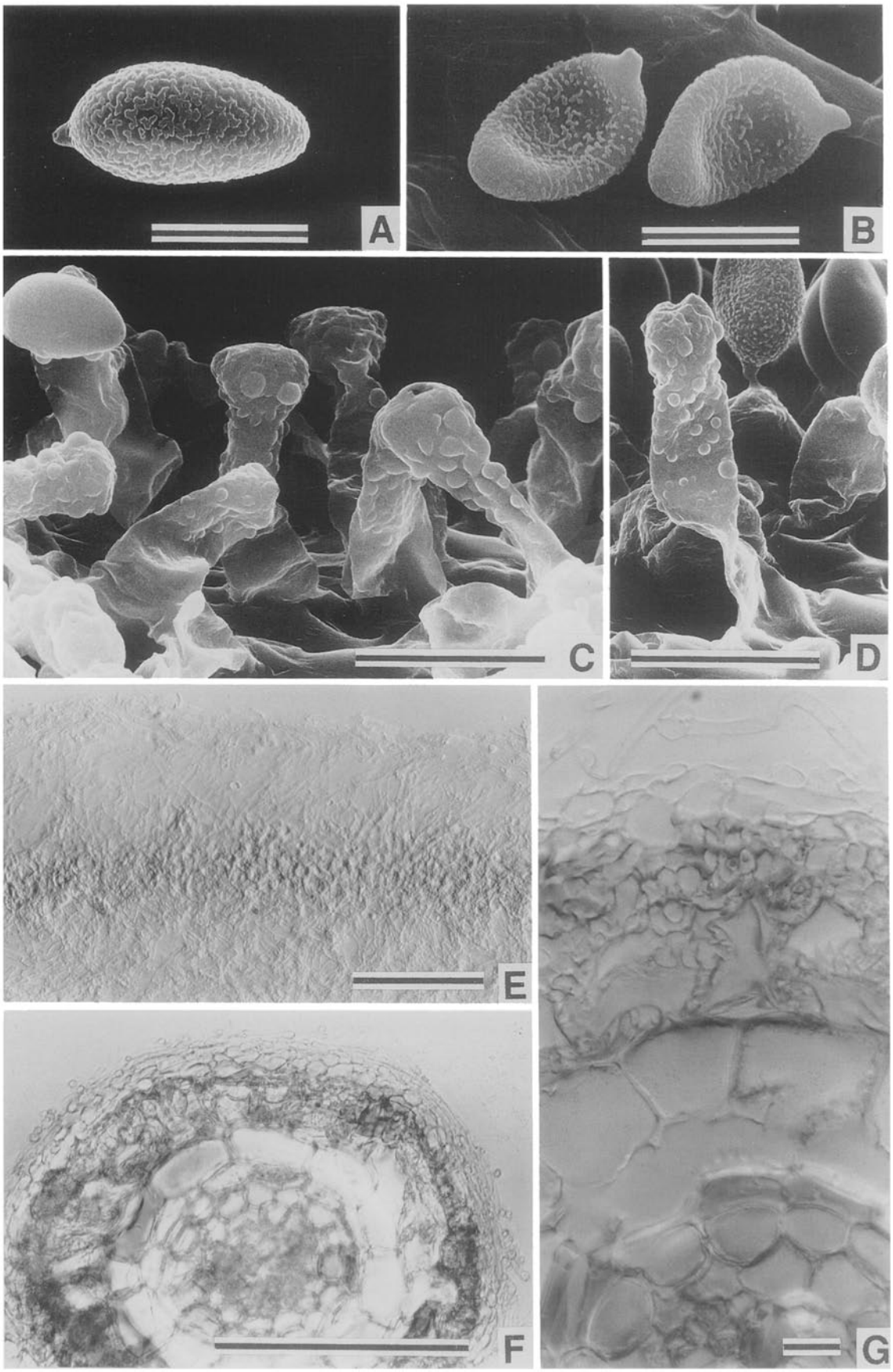


Fig. 2. *Hebeloma luchuense*.
A-C. Basidiocarps. D. Cheilocystidia. E. Basidium. F. Pleurocystidia. G. Hyphae of pileipellis. H. Cell of pileal hypoderm. I. Basidiospores, in face view (right), in profile view (left). Scale bars: A-C=10 mm; D-F, I=10 μm; G, H=20 μm. A-C, E-I from CBM-FB 3102; D from holotype.


A
B
C
D
E
F
G

son was very short but distinct and mushrooms such as members of *Amanita*, *Boletus* and *Russula* were observed from late August to early September. The continuous appearance of late-stage ammonia fungi other than in the general mushroom season in Iriomote Island might be related to the higher precipitation and temperature throughout the year than in the main Japanese islands. In this study, there was no clear floristic difference between summer- and winter-treated plots, perhaps in part because the field observation period was too short to cover all the members of ammonia fungi. Urea concentrations of 343 and 686 g/m^2 per experimental plot enhanced the floral richness and productivity of early- and late-stage ammonia fungi in both forests in comparison with lower urea concentration per plot (86 and 171 g/m^2). In the field, the flora and the productivity of ammonia fungi in *Pinus* forest was very poor compared to that in *Castanopsis* forest, and in *Pinus* forest late-stage fungi appeared in only one urea-treated plot. This difference might be attributed not to the difference of vegetation but to the conditions of the *Pinus* plantation forest. In the supplementary study of *Castanopsis* forests in 1992, *H. radicosoides*, *H. luchuense* and *A. lactariolens* were collected on 7 Sep. 1992.

New species of *Hebeloma*

Hebeloma luchuense Fukiharu & Hongo, sp. nov.

Figs. 2, 3

Pileo 15-40 mm lato, e convexo subumbonato, demum plano-convexo, margine primum incurvo, glabro, e viscido glutinoso, centro brunneo, margine pallide luteo; carne albida, miti, inodora; lamellis adnexis vel sinuatis, confertis, angustis, e pallide luteo cinnamomeis; stipite 30-60 mm longo, 3-7 mm crasso, basi bulbosa et deorsum radicante, sicco, pallide luteo, squamis brunneis revolutis ornato; velo fibrilloso, cortiniformi; basidiosporis in massa subargillaceis, sub microscopio 9-10×4.5-5 μm, e ovoideo amygdaliformibus, minute punctatis.

Holotypus: CBM-FB 5368, leg. T. Fukiharu, conservatus in herbario "the Natural History Museum and Institute, Chiba" (CBM).

Pileus 15-40 mm in diam, convex to subumbonate with an incurved margin at first, expanding to plano-convex at maturity; surface glabrous, smooth, viscid to glutinous, Brown (6E8-7E8) on the disc, Pale Yellow (4A3) on the marginal area. Context thick at the disc, Yellowish White (4A2) to white, odor and taste mild. Lamellae adnexed to sinuate, crowded, narrow (0.5-2 mm), Pale Yellow (4A3) to Clay (5D6), finally Cinnamon (6D6). Stipe 30-60 mm long, 3-7 mm thick, nearly equal or somewhat enlarged at the apex, bulbous (5-10.5 mm) at the base and rooting deeply, dry, Pale Yellow (4A3), beset with Brown (6D8, 6E8)±recurved scales, stuffed, sometimes becoming hollow with age. Partial veil fibrillose, cortinoid, evanescent, leaving remnants on upper portion of stipe.

Basidiospore 9-10×4.5-5 μm, ovoid in face view, obscurely inequilateral amygdaliform in profile view, faintly punctate-ornamented, Brownish Orange (5C5, 5C4) in deposit. Basidia clavate, 25-30×6-7 μm, hyaline, thin-walled, with 4 sterigmata. Cheilocystidia clavate, numerous, 30-40×10 μm, sometimes with tuberculate ornamentation on the surface. Pleurocystidia clavate, sometimes deformed basidia-like, 30-40×4-5 μm, protruding from hymenial layer. Pileipellis an ixotrichodermium ca. 100 μm thick (Fig. 3 E), consisting of chains of gelatinized, cylindric, ventricose, or elliptic cells, 10-40 μm long. Hypodermium cellular, consisting of globose cells, 15-30 μm in diam.

Habit and habitat: Solitary to gregarious, appearing 4-9 months after urea treatment in *Castanopsis* forest.

Distribution: Iriomote Island, Ryukyus, Okinawa Prefecture, Japan.

Materials examined: All materials were collected by T. Fukiharu in Iriomote Island, Ryukyus, Okinawa Prefecture, Japan. Dried basidiocarps: CBM-FB 5368 (holotype), CBM-FB 4985, CBM-FB 5367, CBM-FB 5369. Spore print: CBM-FB 3117, CBM-FB 3118. Basidiocarps in FAA: CBM-FB 3102. Ectomycorrhiza in FAA: CBM-FB 5384.

Observation: *Hebeloma luchuense* resembles *H. radicosum* in having the scaly stipe with a rooting base, but differs in lacking a membranous annulus and in its glabrous brown pileus. Just under the basidiocarps of this species, one type of ectomycorrhiza of *C. cuspidata* var. *sieboldii* was dominant, in which a fungal sheath and Hartig net had been formed (Figs. 3 F, G). This type of mycorrhiza was observed only inside urea-treated plots, where this fungus grew. Thus the mycorrhizae are considered to be formed by *H. luchuense*.

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Fig. 3. *Hebeloma luchuense* Fukiharu & Hongo.
A, B. Basidiospores. C, D. Cheilocystidia. E. Part of the pileus context. F, G. Transverse sections of ectomycorrhizae of *Castanopsis cuspidata* var. *sieboldii* from the soil colonized by *Hebeloma luchuense* showing fungal sheath (F) and Hartig net (G). Scale bars: A, B=5 μm. C, D, G=10 μm. E, F=100 μm. A, E from CBM-FB 3102 (stored in FAA). B from CBM-FB 3118 (spore print). C, D from holotype. F, G from CBM-FB 5384.

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