

SEM studies of the type specimens of *Lamproderma biasperosporum*, *Macbrideola argentea* and *Trichia cascadiensis* (Eumycetozoa, myxomycetes)

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Singer H., Moreno G. & Illana C. (2008) SEM studies of the type specimens of *Lamproderma biasperosporum*, *Macbrideola argentea* and *Trichia cascadiensis* (Eumycetozoa, myxomycetes) – *Sydowia* 61 (1): 91–103.

The type specimens of *Lamproderma biasperosporum*, *Macbrideola argentea* and *Trichia cascadiensis* are studied by scanning electron microscopy. It is concluded that *L. biasperosporum* and *M. argentea* are very similar species that can be distinguished by the base of the stalk and the branching of the capillitium. The synonymy of *T. cascadiensis* and *T. alpina* is confirmed: supposed distinguishing characters between these taxa can be reduced to different stages of maturation of the capillitium; all other macro- and microscopic characters are identical. *Macbrideola argentea* is a new record for the American continent.

Keywords: chorology, nivicolous myxomycetes, scanning electron microscopy, taxonomy

Scanning electron microscope (SEM) has turned into an important tool in the taxonomy of the myxomycetes. Light microscopy is insufficient for studying the morphological details of the peridium, the capillitium and especially the spores. Magnifications between 4000- and 12000-fold are necessary to discover details of spore ornamentation. The scanning electron microscope allows us to distinguish between closely-related taxa, to demonstrate the variability of species at a morphological level, to support the validity of different taxa or to confirm already proposed synonymies. In the present work the morphologically very similar taxa *Lamproderma biasperosporum* Kowalski and *Macbrideola argentea* Nann.-Bremek. & Y. Yamam. are reconfirmed as distinct species. They share the same peculiar type of spore ornamentation but can be distinguished by their stalk and capillitial characters. *Trichia cascadiensis* H. C. Gilbert can be seen as a synonym of *T. alpina* (R. E. Fr.) Meyl. when the morphological variability of the capillitium is taken into consideration.

Materials and Methods

For light optic investigations, specimens were mounted on microscope slides in Hoyer's medium and studied with a Nikon

eclipse 80i microscope (Normarski interference contrast, oil immersion objective $\times 100$). Approximately 20 spores of each specimen were measured. Two sporocarps of each collection were studied, except for scarce and type specimens, where only one sporocarp was examined. Spores were measured including surface structures such as spines or warts.

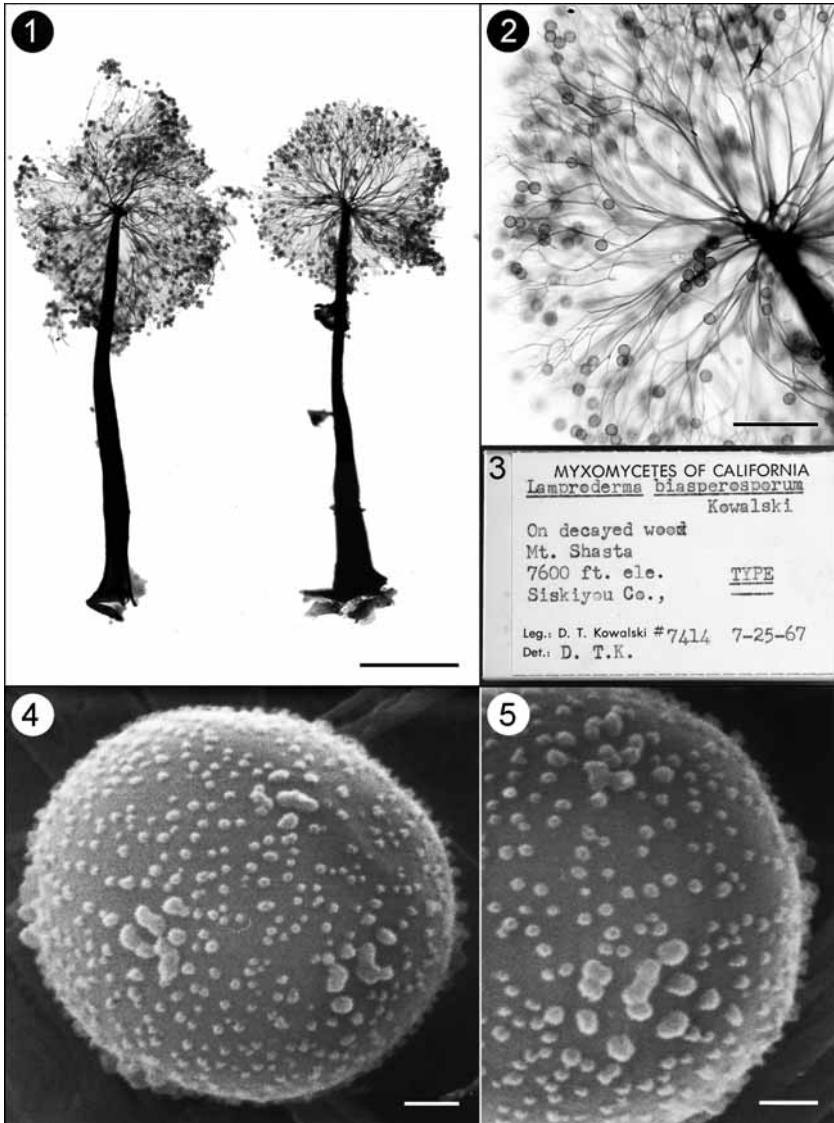
For scanning electron microscopy material was rehydrated in concentrated ammonium hydroxide (28–30%) for 30 minutes, dehydrated in aqueous ethanol (70 %) for 30 minutes, fixed for two hours in pure ethylene glycol dimethyl ether (= 1,2-dimethoxymethane) and finally immersed in pure acetone for at least two hours followed by critical point drying and sputter coating with gold-palladium. Micrographs were taken at 25 kV acceleration voltage using a Zeiss DSM-950 scanning electron microscope.

Taxonomy

Lamproderma biasperosporum Kowalski, Mycologia 60(4): 758. 1968.
– Figs. 1–5.

Latin diagnosis. – Sporangii dissipatis, stipitatis, globosis, atrobrunneis vel nigris, 0.25–0.5 mm diam; stipite nigra, in sporangium pro columella continuo, 0.1–1.5 mm in longitudinem; peridio membranaceo, persistenti vel evanescenti; hypothallo pusillo, rubido-brunneo, disciformi; columella nigra, attenuata, rare ramosa; capillitio reti flaccido formanti, filamentis primariis rubido-brunneis, circa 3 μm crassis, filamentis ultimis incoloratis, minus quam 1.0 μm crassis; sporis globosis, brunneis, minute spinulosis cum maculis verrucarum grandiorum, 7.5–8.5 μm diam; plasmodio ignoto.

The isotype is well conserved, stuck to the base of a cardboard box and consisting of three pieces of decorticate coniferous wood bearing numerous sporocarps. Sporocarps (Fig. 1) scattered, stalked, up to 1.5 mm in total height. Sporotheca globose, 0.3–0.5 mm diam. Peridium persistent, silver to greyish silver, shiny; irregular dehiscence, remaining in the base in the form of a small collar. Stalk dark reddish, up to 0.8 mm high, tapering slightly towards the apex. Columella as a continuation of the stalk, cylindrical to tapered slightly towards the branched apex, reaching half the height of the sporotheca. Hypothallus discoid, individual, reddish brown. Capillitium (Fig. 2) radial, originating from the apex of the columella, with ramifications from the outer half to the extremes, weak, lax, dark brown, becoming clearer and thinner towards the periphery, with abundant pointed, straight, hyaline free ends. Spores (Figs. 4–5) 8–9 μm , globose, dark brown in mass, light violaceous with darker areas by light microscope, warty, with warts of two types: small and dense ones and others larger and in small groups, giving the spores a typically dark appearance. By SEM the



Figs. 1-5. *Lamproderma biasperosporum* (i s o t y p u s). 1. Sporocarps (bar = 0.2 mm). 2. Detail of capillitium (bar = 60 μ m). 3. Box of type collection. 4-5. Spores (bar = 1 μ m).

presence of two types of warts is confirmed, small ones that are abundant and hardly visible by LM, and larger ones that appear in small groups and sometimes fuse to small crests.

Observations – Kowalski (1968) characterized *Lamproderma biasperosporum* by its scattered sporocarps (the species of *Lamproderma* usually have a more clustered growth pattern), by its small

sporothecae (less than 0.5 mm in diam.), its weak, lax, radial capillitium originating from the apex of a usually branched columella with many free hyaline ends, spores with clusters of larger, darker warts and its predominantly alpine distribution. Within the genus, *Lamproderma biasperosporum* is a species close to *L. arcyryonema* Rostaf. which also possesses a columella that branches at the apex and has spores of similar size and colour that sometimes can also bear clusters of larger, darker warts. *Lamproderma arcyryonema* however, can be distinguished by its clustered sporocarps, with larger sporothecae (more than 0.5 mm in diam.), dense and abundant capillitium, uniformly brown, composed of sinuous, branching and anastomosing threads that form a small-meshed net with few free ends and predominantly a non-alpine occurrence. Beyond the genus *Lamproderma*, the species most similar to *L. biasperosporum* is *Macbrideola argentea*. Differences between these two species are discussed below. *Lamproderma biasperosporum* has been combined and validated by Ing as *Collaria biasperospora* (Kowalski) Dhillon & Nann.-Bremek. ex Ing (Ing 1982), because the previous combination by Dhillon & Nannenga-Bremekamp (1977) was invalidly published for the reason that the basionym reference was omitted. Kowalski reported records of this species from USA (California, Kentucky and Oregon). Other records are published from moist chamber cultures like the single European record from France, Haute Savoie (Mitchell & Nannenga-Bremekamp, 1976), or the only African record from the Usambara Mountains in Tanzania, (Ukkola 1998). Also the specimens reported by Eliasson *et al.* (1988) from Arkansas and Snell *et al.* (2003) from Tennessee (USA) have been obtained in moist chamber cultures. The ecology of *Lamproderma biasperosporum* is very variable, according to publications cited above. It can be found both in nivicolous habitat, growing on decaying coniferous wood and at low altitudes on decaying grapevine or bark of ebony or maple.

Material examined. – USA, California, Siskiyou Co., Panther Meadow's Campground, Mt. Shasta, 2316 m, on decaying coniferous wood, 25 Jul 1967, *leg.* D. T. Kowalski (DTK 7414 in MICH 4822, isotypus).

Macbrideola argentea Nann.-Bremek. & Y. Yamam., Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 86(2): 228. 1983. – Figs. 6–19.

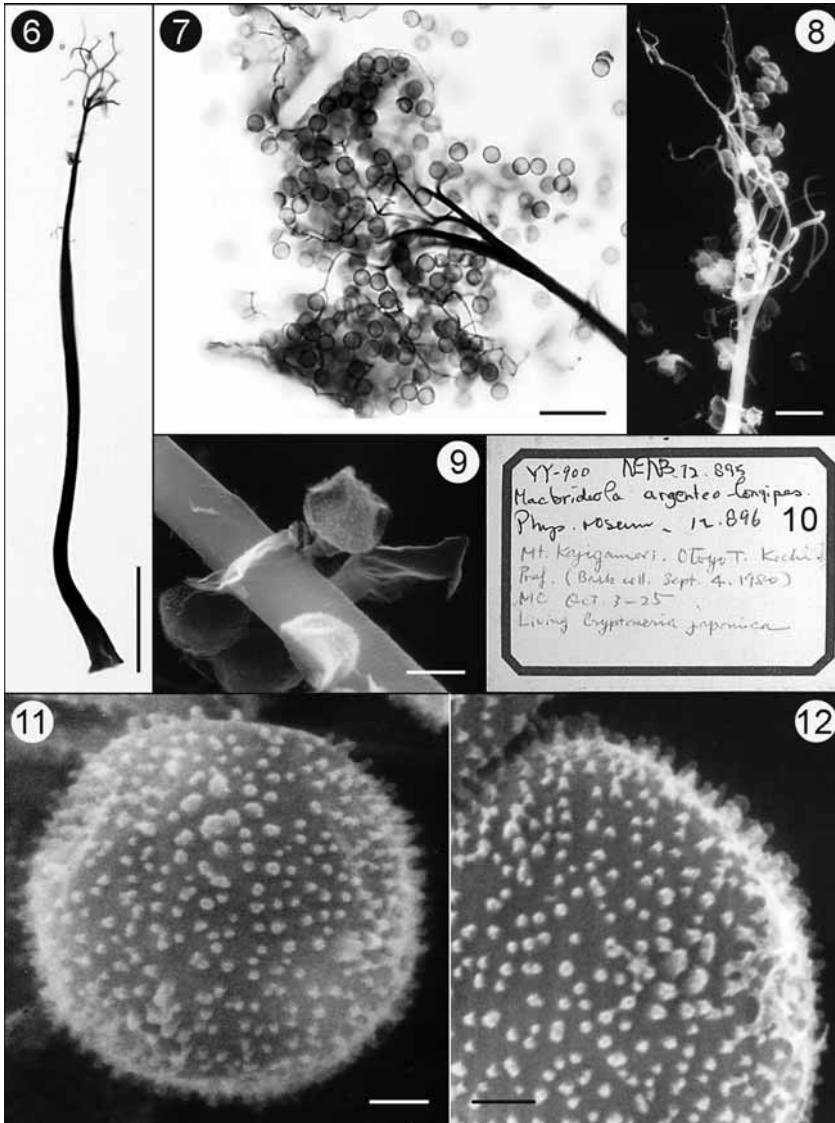
Latin diagnosis: Sporangia dispersa versus solitaria, longe stipitata, globosa, argenteo-nitentia, 0.5–1.0 mm alta, circa 0.1 mm diam. Stipes longus, gracilis, ad apicem obscurus, ad basim rubellus. Peridium persistens, membranaceum, incoloratum, laeve, post dehiscentiam collum circum basim sporangii relinquens. Columella 1/51/2 sporangii attingens, ad apicem in 2 vel 3 ramos principales capillitii ramificans. Capillitium ab apice columellae exoriens, gracile, brunneum, extremis liberis instructum. Sporae atro-brunneae per saturam, pallide canae luce transmissa, 7–8.5 µm diam., verrucis confertis, minutis et pallidis, et fasciculis verrucarum maiorum, instructae. Plasmodium ignotum.

The studied type material consists of a broad piece of bark stuck to the base of a plastic box with a few sporocarps. Sporocarps (Fig. 6) very scattered, stalked, up to 1 mm total height. Sporotheca globose, approximately 0.1 mm in diam. Peridium persistent, silvery iridescent, shiny; irregular dehiscence, leaving a collar (Fig. 9) around the stalk at the base of the sporotheca. Stalk blackish by hand-lens (10 x), dark brown in the upper part and reddish brown at the base under the LM, up to 0.9 mm high, tapering slightly towards the apex, hollow. Columella as a continuation of the stalk, cylindrical, reaching half the height of the sporotheca, branching in the apex. Hypothallus inconspicuous. Capillitium (Figs. 7–8) originating in the apex of the columella and branching dichotomously, scarce, dark brown, thinner at the periphery, with few free ends. Spores (Figs. 11–12) 7–9 (–10) μm , globose, dark brown in mass, light violaceous with small darker areas by LM, warted, with warts of two types: small densely distributed warts and larger warts forming small groups. By SEM the presence of the two types of warts is confirmed. Observations – *Macbrideola argentea* can be characterized by its scattered and largely stalked sporocarps, very small sporothecae (about 0.1 mm diam.) with a persistent, silvery iridescent peridium that remains after its dehiscence as a collar, columella branching in its apex where it turns into a lax capillitium that branches dichotomously and spores with small warts and groups of larger warts. This species is very similar to *Lamproderma biasperosporum*, as already indicated in the original description by Nannenga-Bremekamp & Yamamoto (1983). Like *Macbrideola argentea*, *L. biasperosporum* also has small sporocarps with a scattered growth habit, a columella that branches in its apex, a lax capillitium that has its origin in the apex of the columella, and spores of similar dimensions and spore ornamentation consisting of small warts and groups of larger warts (Table 1). Furthermore, they share the same type of habitat (grapevine and ebony). Consequently, the two species have been repeatedly confused, as it is the case for the American specimens (HWK 1802, HWK 1474, HWK 1788, HWK 2341; Figs. 13 – 19) erroneously determined as *L. biasperosporum*. *Macbrideola argentea* can be distinguished by a generally smaller sporotheca, about 0.1 mm diam., and especially by its hollow stalk being reddish in its base which is typical for the genus *Macbrideola*, and its capillitium whose main branches are dichotomously (and not radial) and with few free ends. *Macbrideola argentea* has only been known from Japan until now. This re-investigation of herbarium specimens lead to the first record of the species for the American continent (USA).

Material examined. – USA, Georgia, Clarke Co., near Athens, University of Georgia Botanical Garden, on bark surface of living grapevine, 25 Aug 1978, leg. H. W. Keller (HWK 2341 and HWK 2342 in GB). USA, Ohio, Greene Co., Xenia, on bark of *Diospyros virginiana* L., obtained from moist chamber culture,

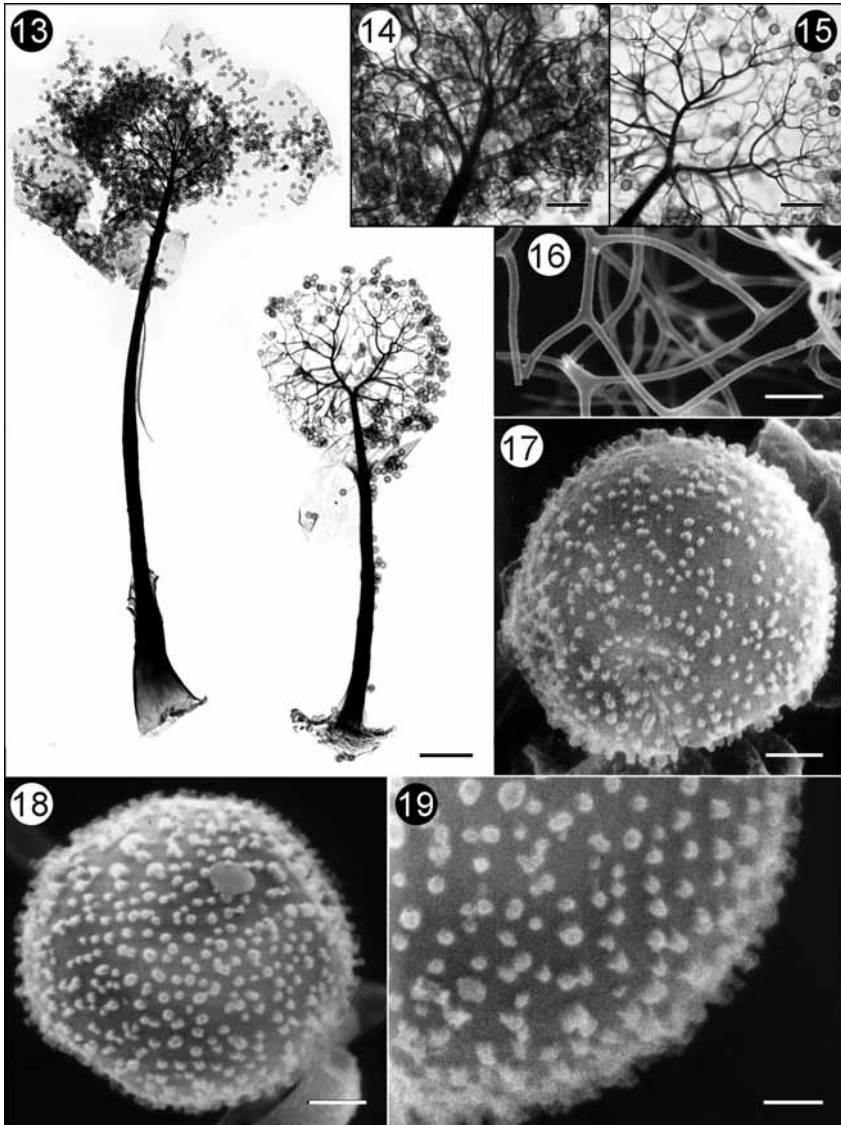
Tab. 1. – Comparison of the original descriptions of *Lamproderma biasperosporum* and *Macbrideola argentea*.

		<i>L. biasperosporum</i>	<i>M. argentea</i>
Sporocarps	Growth habit	predominantly widely scattered, occasionally slightly clustered	scattered to solitary
	Colour	variable, dark brownish to bluish black, strongly iridescent	shiny silvery
	Total height Diameter	n. d. 0.25–0.5 mm	0.5–1.0 mm circa 0.1 mm
Stalk	Height	attaining 1.5 mm, occasionally reaching only 0.1 mm	3/4–4/5 of total height
	Colour	shiny black in reflected light	black in reflected light, red-brown below and opaque above in transmitted light
	Hollowness	n. d.	hollow
Peridium	Persistence	persistent or evanescent, splitting irregularly, often remaining as a cup at the base of the sporotheca	persistent, when dehisced leaving a collar round the stalk at the base of the sporotheca
Columella	Height	reaching 1/2–2/3 of the sporotheca	reaching 1/5–1/2 of the sporotheca
	Apex	often branching, forming the primary branches of the capillitium	splitting into 2 or 3 branches and merged with the capillitium
Capillitium	Morphology	weak, lax net, primary branches straight, arising entirely from the apex, becoming progressively smaller from the base towards the extremities	rather lax, slender, dichotomously branched 3–5 times, with no or only a few anastomoses
	Colour	primary branches reddish brown, becoming progressively paler towards the extremities, free ends hyaline	brown
	Free ends	numerous small free ends	many, mostly about 10 µm long, slender attenuate free ends
Spores	Colour	dark purple brown in mass, violet brown by transmitted light	dark brown in mass, pale grey by transmitted light
	Diameter	7.5–8.5 µm	7–8 µm
	Ornamentation	minutely spinulose with clusters of larger, darker warts, 2–3 clusters per hemisphere	densely warted with minute, pale warts, and with groups of larger, darker ones



Figs. 6-12. *Macbrideola argentea* (isotypus). **6.** Sporocarp (bar = 0.2 mm). **7-8.** Columella and detail of capillitium (7: bar = 30 μ m; 8: bar = 20 μ m). **9.** Peridium collar around the stalk (bar = 5 μ m). **10.** Box of the type collection. **11.** Spore (bar = 1 μ m). **12.** Detail of the spore ornamentation (bar = 0.5 μ m).

wetted 29 Feb 1974, harvested 12 Apr 1974, leg. H. W. Keller (HWK 1802 in GB, as *Lamproderma biaspersporum*). USA, Ohio, Scioto Co., Shawnee State Forest, on bark surface of living grapevine, 27 Aug 1975, leg. H. W. Keller (HWK 1474 in GB, as *L. biaspersporum*). *Ibidem*, obtained from moist chamber culture, collected 27 Aug 1975, wetted 10 Oct 1975, harvested 12 Jan 1975, leg. H. W. Keller (HWK 1788



Figs. 13–19. *Macbrideola argentea* (13 left, 14, 17: HWK 1788; 13 right, 15: HWK 1474; 16, 18, 19: HWK 1802). **13.** Sporocarps (bar = 0.1 mm). **14–15.** Detail of columella and capillitium (bar = 30 μ m). **16.** Detail of capillitium by SEM (bar = 5 μ m). **17–18.** Spores (bar = 2 μ m). **19.** Detail of spore ornamentation (bar = 1 μ m).

and HWK 2341 in GB, as *L. biasperosporum*). JAPAN, Kochi Pref., Otoyoko-cho, Mt. Kajigamori, on living *Cryptomeria japonica* (L. f.) D. Don bark, obtained from moist chamber culture, wetted 4 Sep 1980, harvested 3–25 Oct 1980, leg. Y. Yamamoto (YY 900, NENB 12895, BR-MYCO 065113-26, isotypus). JAPAN, Kochi Pref., Geisei-mura, Wajiki, bark of *Chamaecyparis obtusa* (Siebold & Zucc.) Sie-

bold & Zucc. ex Endl., wetted 30 Jun 1986, harvested 19 Jun 1986, leg. Y. Yamamoto (YY 4183 in AH 31892, slides).

Trichia cascadenis H. C. Gilbert, in Peck & Gilbert, Am. J. Bot., Suppl. 19(2): 145. 1932. Figs. 20–24.

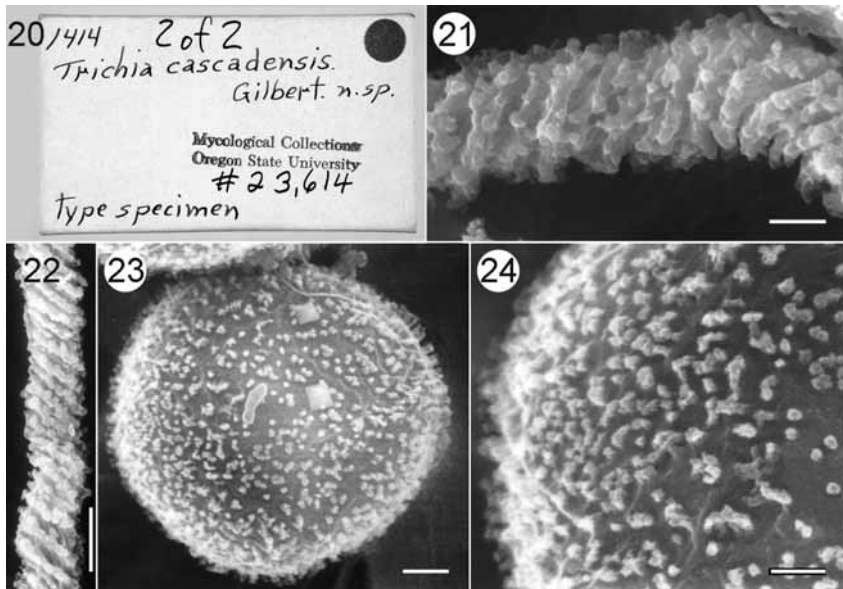
Diagnosis: Sporangia scattered or loosely clustered, sessile, globose or forming short plasmodiocarps, 1 mm in diameter and 1 to 4 mm long, dull black, with no hypothallus; peridium single, thick, granular, dull black on both surfaces, breaking irregularly to expose the yellow spores and capillitium; elaters numerous, long, 7 to 8 μm in diameter, the spiral bands thin, uneven, and closely wound, 3 to 5, covered with many fine warts which are most numerous along the edges of the bands, the tips of the elaters rounded, without enlargements, and entirely covered with the warted spiral bands, each elater with a conspicuous granular core; spores globose, yellow in mass, almost colorless under the lens, minutely and unevenly warted, 14 to 16 μm in diameter.

The type material is in a good condition and stuck on a card that is held in a cardboard box. Sporocarps scattered to gregarious, globose to elongated, 1–3 \times 0.8–1.2 mm. Hypothallus inconspicuous. Peridium simple, persistent, with irregular, lobed or stellate apical dehiscence, cartilaginous, coriaceous, brownish black by hand-lens (10 \times), dark brown with included amorphous matter by LM. Capillitium (Figs. 21–22) yellowish, elastic, composed of elaters 6–7 μm in diam., flexuous, tangled, bearing spiral bands with obvious warts in the edges; terminations rounded. Spores (Figs. 23–24) 14–16 (–17) μm , globose, golden yellow in mass, light yellowish by LM, warted, with small regularly distributed warts. By SEM, the spore ornamentation consists of small densely and regularly distributed baculae.

Observations – Hagelstein (1944) studied a specimen from Maine (USA) that coincided with the description of *Trichia cascadenis*, but which he considered only to be a variation of *T. alpina*. Pando & Lado (1997) investigated only one specimen of this species from the Iberian Peninsula and indicated that the capillitium of *Trichia alpina* is decorated with “4–6 faintly spinulose spiral bands”. The spinules drawn by these authors, however, correspond to the warts observed in our study as mentioned below. Gilbert, in Peck & Gilbert (1932), characterized *Trichia cascadenis* by its elaters being unlike those of any other *Trichia* and its simple peridium. The author considered *T. alpina* to be the closest species, but distinguished *T. cascadenis* by its larger elaters with distinctive bands, core, and tips. He also separated it by its simple peridium, very different from the double peridium of *T. alpina*. This latter character, however, is sometimes difficult to specify and depends on the degree of maturation of the sporocarp. The colour, morphology, dimensions of the sporothecae and spore ornamentation are similar in the two species. Thus, the main distinguishing microscopic character is the mor-

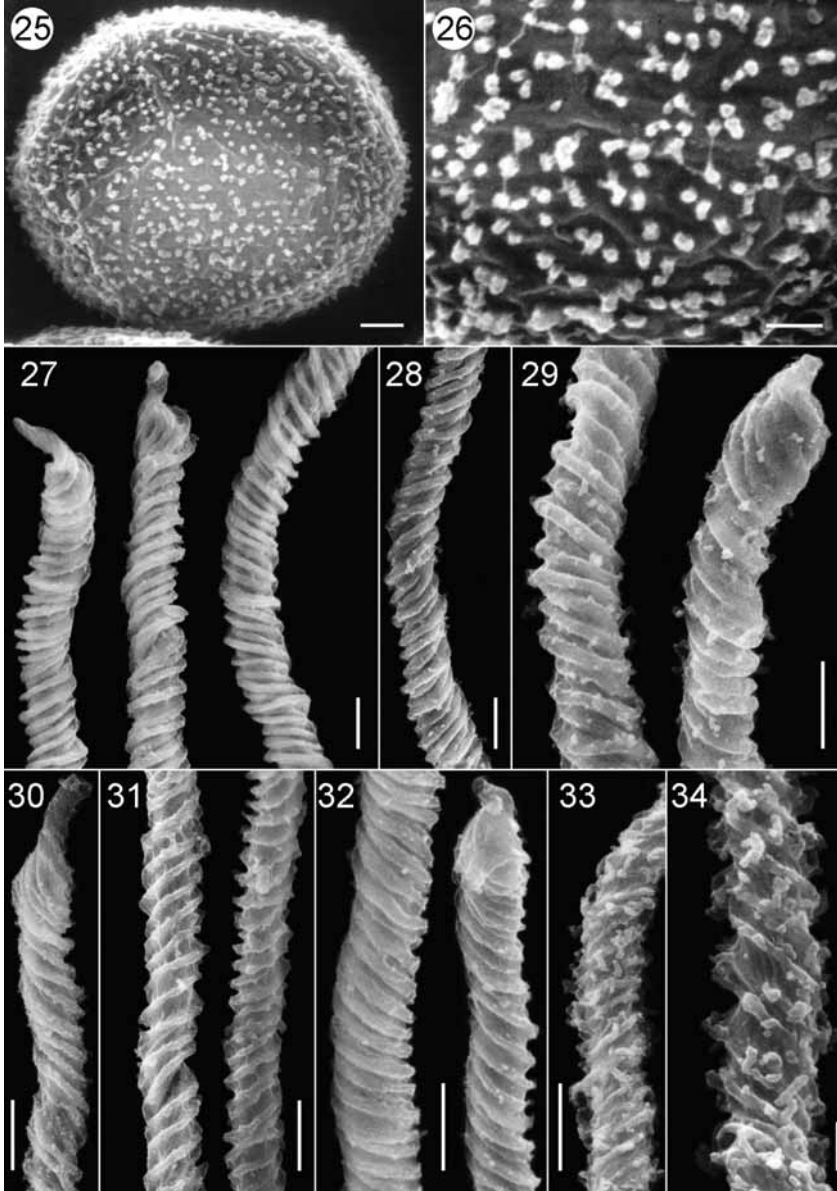
phology of the capillitium. According to own observations *Trichia alpina* (Figs. 25–34) is a nivicolous species usually fruiting on twigs and leaves at snow melt. Therefore, rapid changes of temperature can accelerate fructification or even induce formation of sclerotized sporocarps. In these cases the spores usually maintain their large dimensions and characteristic ornamentation composed of small and dense baculae (Figs. 25–26). The capillitium, however, can be subject to morphological changes that sometimes prevent a complete development. Filaments of variable length and ornamentation are formed (Figs. 27–34). These reactions to environmental influences may pretend the existence of more than one species. A synonymy of *T. cascadenis* with *T. alpina* was previously proposed by Martin & Alexopoulos (1969) after studying the type of *T. cascadenis* and two specimens determined by Meylan as *T. alpina*. These authors concluded that there were no significant differences between the two taxa. These conclusions could be confirmed by our SEM studies. It could be concluded that the variability of the ornamentation of the capillitium is a result of the maturation process of the sporocarps (Figs. 27–34). *Trichia alpina* is an abundant species in the Central System (Madrid and Segovia) of Spain.

Material examined. – *Trichia cascadenis* H. C. Gilbert: USA, Oregon, Cascade Mountains, in the foothills near Mount Jefferson, on old Thuya logs in dense timber, 5 Jul (Gilbert 1414 in OSC 23614, holotypus).



Figs. 20–24. *Trichia cascadenis* (holotypus). **20.** Box of type collection. **21–22.** Capillitium (21: bar = 2 μ m; 22: bar = 5 μ m). **23.** Spore (bar = 2 μ m). **24.** Detail of spore ornamentation (bar = 1 μ m).

Trichia alpina (R. E. Fr.) Meyl.: SPAIN, Huesca, Vallibierna valley, Puente de Coronas, 31TCH0520, on branch of *Pinus uncinata* Mill., 18 Jun 1991, leg. C. Lado & M. Dueñas (Lado 5250 in MA 33764). SPAIN, Madrid, Puerto de Cotos, 1900 m, on stalks of *Rubus ulmifolius* Schott, 10 May 1996, leg. A. Sánchez (AH 18418).



Figs. 25–34. *Trichia alpina* (25–27: AH 27193; 28–29: AH 27185; 30–31: AH 27278; 32: AH 27325; 33–34: AH 27324). **25.** Spore (bar = 2 μ m). **26.** Detail of spore ornamentation (bar = 1 μ m). **27–34.** Details of capillitium (27–33: bar = 5 μ m; 34: bar = 2 μ m).

SPAIN, Madrid, Puerto de Navacerrada, 2100 m, on stalks of *Senecio pyrenaicus* L., 8 Jun 1999, leg. A. Sánchez (AH 25975). *Ibidem*, 2100 m, on stalks of *Digitalis purpurea* L., 17 May 2000 (AH 25661). *Ibidem*, 2100 m, on stalks of *Senecio pyrenaicus*, 17 May 2000 (AH 25659). *Ibidem*, 2100 m, on stalks of *Senecio pyrenaicus*, 14 Jun 2000 (AH 26314). *Ibidem*, 2125 m, on stalks of *Senecio pyrenaicus*, 17 May 2000 (AH 25941). *Ibidem*, 2150 m, on stalks of *Digitalis purpurea* L., 17 May 2000 (AH 25660). *Ibidem*, 2150 m, on stalks of *Gentiana lutea* L., 16 Mar 2000 (AH 25658). *Ibidem*, 2150 m, on stalks of *Senecio pyrenaicus*, 17 May 2000 (AH 25902). *Ibidem*, 2150 m, on stalks of *Senecio pyrenaicus*, 20 Jul 2001 (AH 30456). *Ibidem*, 2175 m, on stalks of *Senecio pyrenaicus*, 20 Jul 2001 (AH 30455). SPAIN, Segovia, Puerto de Navacerrada, 2050 m, on fronds of *Cryptogramma crispera* (L.) R. Br. ex Hook, 11 Jun 2000, leg. A. Sánchez (AH 28709). *Ibidem*, 1950 m, on stalks of *Senecio pyrenaicus*, 14 May 1999 (AH 28700 and AH 30459). *Ibidem*, 1950 m, on stalks of *Senecio pyrenaicus*, 2 May 2001 (AH 28712). *Ibidem*, 1975 m, on stalks of *Gentiana lutea*, 24 May 1999 (AH 30460 and AH 30458). *Ibidem*, 2000 m, on stalks of *Gentiana lutea*, 17 Jun 1999 (AH 30457). *Ibidem*, 2050 m, on stalks of *Gentiana lutea*, 8 Jun 2000 (AH 25886). *Ibidem*, 2075 m, on stalks of *Gentiana lutea*, 8 Jun 1999 (AH 25892). *Ibidem*, 2075 m, on stalks of *Senecio pyrenaicus*, 7 Jun 1999 (AH 28703). *Ibidem*, 2100 m, on fronds of *Cryptogramma crispera*, 24 May 1997 (AH 18516). *Ibidem*, 2100 m, on stalks of *Gentiana lutea*, 8 Jun 1999 (AH 25971 and AH 25974). *Ibidem*, 2100 m, on stalks of *Senecio pyrenaicus*, 7 Jun 1999 (AH 28707). *Ibidem*, 2100 m, on stalks of *Senecio pyrenaicus*, 17 Jun 2000 (AH 25824). *Ibidem*, 2150 m, on stalks of *Senecio pyrenaicus*, 7 Jun 1999 (AH 25947). *Ibidem*, 2175 m, on stalks of *Gentiana lutea*, 30 May 2000 (AH 25923). *Ibidem*, 2200 m, on herbaceous remainders, 24 May 1997 (AH 18427).

Conclusions

Lamproderma biasperosporum is well characterized by its very scattered sporocarps, small sporothecae less than 0.5 mm diam., its weak, lax, radial capillitium with straight, hardly branched and sparsely anastomosed threads that originate from the apex of the columella and by its spores with clusters of larger, darker warts. It is a rare, predominantly alpine species known only from France, Tanzania and USA. *Macbrideola argentea* also forms scattered sporocarps, small sporothecae (about 0.1 mm) and warted spores with groups of larger warts. It can clearly be distinguished from *Lamproderma biasperosporum* by its capillitium that branches dichotomously along various primary branches and a hollow stalk with a reddish brown base. *Trichia cascadiensis* is considered to be a synonym of *T. alpina*. *Trichia alpina* is a common species known from Austria, Canada, France, Germany, Japan, Spain, Sweden, United Kingdom and USA.

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