

# **Article**



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# Two new species of *Pluteus* (Agaricales, Pluteaceae) from China

JIANG XU<sup>1,2</sup>, TAI HUI LI<sup>2\*</sup>, ALFREDO JUSTO<sup>3</sup> & ZAI WEI GE<sup>4</sup>

- 1 School of Bioscience & Bioengineering, South China University of Technology, Guangzhou 510006, China,
- 2 State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China,
- 3 Instituto de Biología. Departamento de Botánica. Universidad Nacional Autónoma de México, México Distrito Federal 04510, México 4 Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China
- \* Corresponding author: mycolab@263.net

#### **Abstract**

Two new species of *Pluteus* section *Pluteus*, *P. griseodiscus* and *P. purpureofuscus*, are described from China. *Pluteus griseodiscus* is characterized by the pale basidiomata, non-pigmented lamellar edges, thick-walled cheilocystidia and the presence of caulocystidia. *Pluteus purpureofuscus* is characterized by a purple-brown pileus, presence of clamp-connections, and growth on coniferous wood. The phylogenetic position of both species is also discussed based on nrITS data.

**Key words:** Basidiomycota, biodiversity, phylogeny, taxonomy

#### Introduction

*Pluteus* Fr. (Pluteaceae, Agaricales) is a large and widely distributed genus typically classified in the Pluteacaea Kotl. & Pouzar (Singer 1986), with approximately 300 known species worldwide (Kirk *et al.* 2008). It is characterized by free lamellae, pinkish spore print, absence of annulus and volva, and inverse hymenophoral trama. Most of taxa in the genus are lignicolous (Orton 1986).

Morphologically (Singer 1986), the genus *Pluteus* was subdivided in three sections, viz., section *Pluteus* with metuloid cystidia and pileipellis as a cutis, section *Hispidoderma* Fayod without metuloid cystidia, but with pileipellis composed of elongated elements organized as a cutis, an hymeniderm or a trichoderm, and section *Celluloderma* Fayod without metuloid cystidia, but with a pileipellis composed of clavate or spheropedunculate elements organized as an hymeniderm, with transitions to an epithelium. This morphological subdivision has been supported by molecular data with some minor rearrangements (Justo *et al.* 2011)

Until now, about 38 species of *Pluteus* have been reported in China (Teng 1963, 1996; Tai 1979; Bi 1988; Bi *et al.* 1993; Yang *et al.* 2010, 2011; Yang 2011). During a recent survey on the genus *Pluteus* in China, several noteworthy collections were found which did not correspond to any previously described taxa in the genus. Further observation revealed that they possessed metuloid hymenial cystidia and a pileipellis organized as a cutis, which conformed the definition of the genus *Pluteus* section *Pluteus* (Singer 1986, Justo *et al.* 2011). Molecular analysis also indicated they were close to the taxa of genus *Pluteus* section *Pluteus*. Therefore, they were described as two new species belonging to genus *Pluteus* section *Pluteus*.

#### Materials and methods

Taxon sampling and morphological studies:—Macro-morphological descriptions were based on the field notes and photos of the fresh basidiomata collected from southern China. Color descriptions are according to Kornerup & Wanscher (1978). The types and the other examined collections are deposited at the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM) for *P. griseodiscus* and at the Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) for *P. purpureofuscus* (Hao *et al.* 2014).

Micro-morphological data were obtained from sections made from the dried specimens mounted in 5% KOH and/ or Congo Red before making line drawings. In the descriptions of the basidiospores, the abbreviation [n/m/p] means that n basidiospores were measured from m basidiomata of p collections. At least 30 basidiospores were measured for each collection. The length of basidia measurements excludes the sterigmata. Dimensions for basidiospores are given as (a-)b-c(-d). The range b-c contains a minimum of 90% of the measured values. Extreme values (a and d) are given in parentheses, and avl/avw represents the average length/width of all measured basidiospores. Q is used to mean "length/width ratio" of the basidiospores in side view; and avQ refers to the average Q of all observed basidiospores  $\pm$  sample standard deviation.

*Molecular methods and phylogenetic analyses:*—Genomic DNA was isolated from fresh materials (for *P. griseodiscus*) or from fragments preserved on silica-gel (for *P. purpureofuscus*) using the CTAB procedure of Doyle & Doyle (1987). The nuclear ribosomal internal transcribed spacer (nrITS) region was amplified using the universal primers ITS4/ITS5 (White *et al.* 1990). Amplified PCR products were purified, sequenced, and edited by Invitrogen Biotechnology (Invitrogen Life Technologies Co., Ltd, Shanghai). Sequences generated in this study have been subjected to BLASTn (http://www.ncbi.nlm.nih.gov) analysis and deposited in GenBank.

An nrITS dataset was constructed with all currently sequenced species of *Pluteus* section *Pluteus* (Justo *et al.* 2011; Justo *et al.* 2014) and the newly generated sequences. The final dataset includes 78 sequences from *Pluteus* section *Pluteus*, with *Pluteus* aff. *romellii* and *Pluteus aureovenatus* Menolli & Capelari (Menolli *et al.* 2010) of *Pluteus* sect. *Celluloderma* as outgroup taxa. GenBank numbers and geographic origins for previously generated sequences are given in Fig. 1. GenBank numbers for the new taxa are given with the respective collection information. Sequences were aligned using MAFFT version 7 (Katoh & Toh 2008) and the strategy FFT-NS-i was selected. The alignment was inspected and manually corrected using AliView (Larsson 2014). A Maximum likelihood analysis was run in the RAxML servers, (Stamatakis *et al.* 2008), under a GTR model with one hundred rapid bootstrap (BS) replicates.

#### Results

#### Molecular phylogeny

The final dataset consists of 76 ingroup sequences and a total of 707 characters (gaps included). The alignment file has been deposited in TreeBASE (S17591). The phylogenetic positions of *Pluteus griseodiscus* and *P. purpureofuscus* are highlighted in Fig. 1. Clade names used in the discussion follow Justo *et al.* (2014).

Pluteus griseodiscus appears as a member of the atromarginatus clade (100% BS) and forms a long branch with respect to the rest 3 taxa in the clade, i.e. P. atromarginatus (Konrad) Kühner (1935: 51), P. atrofibrillosus Vellinga & Justo (Justo et al. 2014: 72) and Pluteus sp. MES523 from Chile.

Pluteus purpureofuscus is placed as a sister taxon of the Indian collection (KJ009752) reported by Pradeep et al. (2002) as P. subcervinus (Berkeley & Broome) Saccardo (1887: 666) with a 100% BS (Fig. 1). The percentage similarity between the nrITS sequences of P. purpureofuscus and P. subcervinus (KJ009752) is 95% (27 base pair differences). These two species forms an independent clade sister to the all species of the salicinus clade with 84% BS.

#### **Taxonomy**

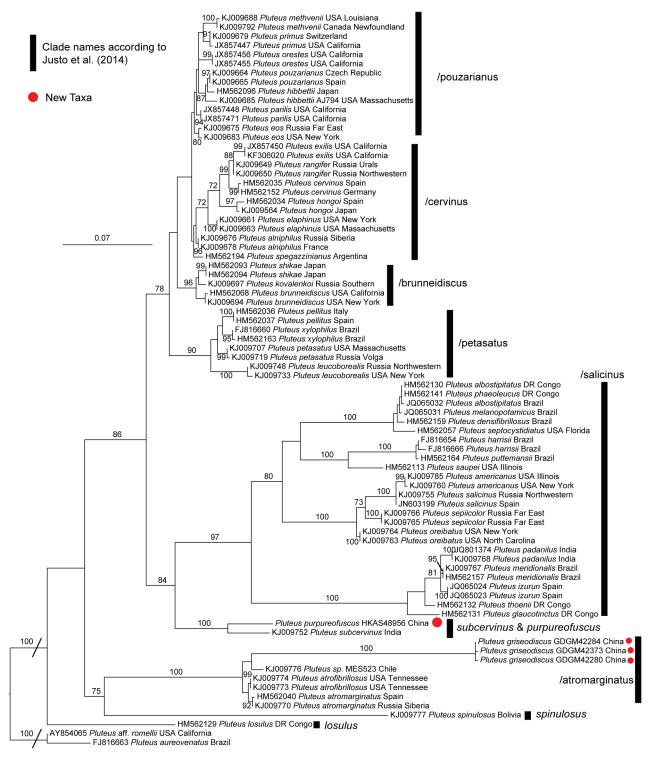
Pluteus griseodiscus Jiang Xu & T.H. Li, sp. nov. Fig. 2 a-c, Fig. 3

MycoBank: MB 812366; Fungal Name: FN570175.

**Diagnosis:**—Differing from *Pluteus atromarginatus* in having paler pileus, concolorous lamellar edges, thick-walled cheilocystidia, and abundant caulocystidia.

Type:—CHINA, Guangdong Province, Guangzhou City, Tianlu Lake Forest Park, elev. 268 m, at 113°25.058'E, 23°13.383'N, 29 May 2013, Jiang Xu, M. Zhang & S.H. Zhou, GDGM42280 (Holotype; nrITS KR350490).

Etymology:—the epithet *griseodiscus* is from the Latin words "*griseus*" (gray) and "*discus*" (umbo), making reference to the grayish umbo of the pileus.



**FIGURE 1.** Best tree from the ML analysis of the nrITS dataset of selected *Pluteus* sect. *Pluteus* species. Support values are given on or below the branches.

*Pileus* 65–76 mm in diam. campanulate when young, becoming plano-convex to applanate with a low and broad umbo at the center; surface glabrous or with some innately fibrils at center or around, dry or viscid when moist, whitish to brownish gray (6C2), with some pinkish tints when mature; margin exceeding lamellae and short translucently striate. *Lamellae* free, moderately crowded, thin, ventricose, up to 13 mm wide, white to pinkish; about (9–10) complete lamellae per cm at pileus margin, with 2–3 lamellulae between two lamellae; edge entire, concolourous. *Stipe* 80–90 ×5–6 mm, central, equal or slightly enlarged towards base, occasionally curved, white or paler in mature basidiomata, solid, longitudinally striate and covered by whitish fibrils, with white mycelium at base. *Context* white, unchanging. *Odor* not distinctive. *Taste* not recorded.

Basidiospores [90/3/3] 6.0–8.0(–10.0) × (4.0–)5.0–6.0(–7.0) μm, avl × avw=7.5 × 5.5 μm, Q=1.17–1.80, avQ=1.42, broadly ellipsoid to elongate, smooth, thick-walled. Basidia 26–37 × 6–10 μm, clavate, 4-spored, thin-walled. Pleurocystidia abundant, 65–115 × 12–27 μm, metuloid, (narrowly) fusiform or lageniform, with 2–4 hooks at apex, thick-walled (2–5 μm thick at neck), colorless. Intermediate cystidia rare, with a sharp transition between pleurocystidia and cheilocystidia. Lamellar edge sterile. Cheilocysidia abundant, 47–72 × 9–12 μm, fusiform or bifurcate, strongly tapering towards apex, thick-walled, colorless or with brown intracellular pigment. Hymenophoral trama made up of non-septate hyphae in 9–12 μm diam., convergent, thin-walled, hyaline. Pileipellis a cutis of cylindrical hyphae; terminal elements 75–100 × 8.5–15 μm, strongly tapering towards apex, with brown intracellular pigment, thin-walled. Stipitipellis a cutis of hyphae 6–10 μm wide, hyaline or with brown intracellular pigment, thin-walled. Caulocystidia abundant, present over the whole stipe surface, 40–90 × 7–14 μm, cylindrical to sub-fusiform with acute apex, thin walled, hyaline. Clamp connections common, especially in pileipellis hyphae.



**FIGURE 2.** a–c. Basidiomata of *P. griseodiscus* (b, c. GDGM42280, d. GDGM42377). d. Basidiomata of *P. purpureofuscus* (HKAS48956–holotype, photo by Z.W. Ge.). Scale bars: a, b, c, d = 1 cm.

**Habit, habitat and distribution:**—Solitary. A total of four basidiomata was collected on the rotten wood of broad-leaved trees (e.g. *Castanopsis* sp., *Quercus* sp.) between mid-May and mid-July in a forest park at elve. 250–300 m. Asia: Known only from southern China.

**Additional specimens examined:**—China, Guangdong Province: Guangzhou city, Tianlu Lake Forest Park, in broad-leaved forests, at 113°25.060'E, 23°13.385'N, elev. 270 m, 6 July 2013, Jiang Xu (GDGM42284; nrITS KR350491); *ibid.*, 3 July 2014, Jiang Xu (GDGM42373; nrITS KR350492); *ibid.*, 3 July 2014, Jiang Xu (GDGM42377)

**Discussion:**—The combination of whitish to pale greyish pileus, habitat on angiosperm wood, thick-walled cheilocystidia and abundant caulocystidia are diagnostic for *P. griseodiscus*.

Although *P. griseodiscus* is placed with high support (100% BS, Fig. 1) in the *atromarginatus* clade, its paler pileus and the habitat on broadleaf wood are quite distinctive. All known taxa in this clade have darker colors of the basidiomata, pigmented lamella edges, thin-walled cheilocystidia, and habitat on coniferous wood (Justo *et al.* 2014).

Other taxa in *Pluteus* section *Pluteus* that have white or paler basidiocarps are also morphologically distinguished

easily from the new species: *P. hongoi* Singer (1989: 95), as well as its synonyms *P. albineus* Bonnard (2001: 131) and *P. nothopellitus* Justo & M.L. Castro (2007b: 222), differs in the lack of clamp-connections and mainly in the thin-walled cheilocystidia (Justo *et al.* 2014); *P. orestes* Vellinga & Justo (Justo *et al.* 2014: 41) has relatively larger basidiospores (9.0–9.9 × 6.0–7.3 μm), narrow cylindrical thin-walled cheilocystidia, and habitat exclusively on coniferous wood in mountainous areas in Western North America; *P. pellitus* (Pers.: Fr.) P. Kumm. (1871: 98), known only from Western Europe, has smaller basidiospores (5.8–6.5 × 4.3–4.6 μm), thin-walled cheilocystidia and no caulocystidia (Justo *et al.* 2014); while *P. petasatus* (Fr.) Gillet (1876: 395) and *P. leucoborealis* Justo, E.F. Malysheva, Bulyonkova & Minnis (Justo *et al.* 2014: 58), though morphologically similar, lack clamp-connections and caulocystidia, and have thin-walled cheilocystidia (Justo *et al.* 2014).

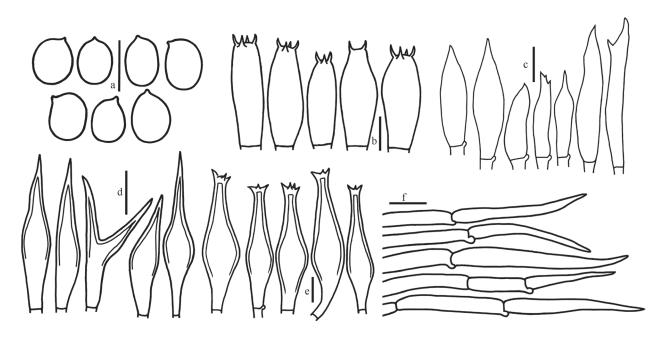


FIGURE 3. Pluteus griseodiscus (GDGM42280-holotype). a. Basidiospores. b. Basidia. c. Caulocystidia. d. Cheilocystidia. e. Pleurocystidia. f. Pileipellis elements. Scale bars: a, b,  $d = 10 \mu m$ ; c, e,  $f = 20 \mu m$ .

The presence of thick-walled cheilocystidia is though unusual in section *Pluteus*, a few species with this invite comparison with the new species: *P. amphicystis* Singer (1959: 213), known from Bolivia (Singer 1959), Martinique (Pegler 1983), India (Pradeep *et al.* 2002) and Mexico (Rodríguez & Guzmán-Dávalos 1997), can be separated by its yellow pileus, pleurocystidia without apical hooks, and lack of clamp-connections. Another species known from the Americas is *P. spinulosus* Murrill (1917: 138), but it differs from *P. griseodiscus* in the brown pileus, the pleurocystidia mostly without apical hooks and common in lateral ornamentation, and in the absence of caulocystidia; in addition, although *P. spinulosus* was described having exclusively thick-walled cheilocystidia (Pegler 1983), the observation on the type collection at NY (A. Justo, pers. obs.) has revealed that the lamellar edge is mostly composed of a continuous strip of thin-walled cheilocystidia, and along that strip some metuloid cystidia do occur. A modern collection of *P. spinulosus* from Bolivia appears in the phylogeny as sister to the *atromarginatus* clade (Fig. 1). The Asian species *Pluteus horridilamellus* S. Ito & S. Imai (1940: 46) has thick-walled and fusiform-lageniform pleurocystidia, cheilocystidia without apical or lateral hooks, even thick-walled pileocystidia and caulocystidia, and pileipellis without clamp-connections, which are different from those of *P. griseodiscus* (Kobayashi 2002).

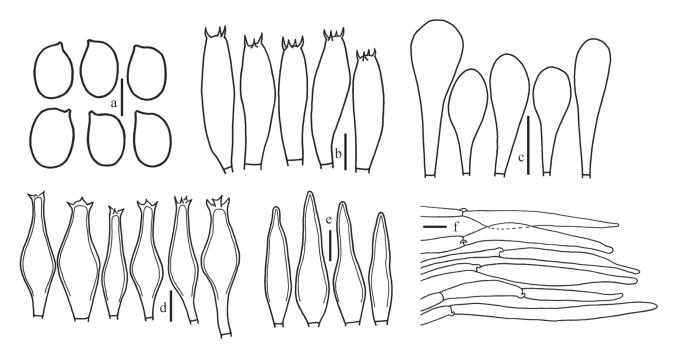
*Pluteus purpureofuscus* Jiang Xu, T.H. Li & Z.W. Ge, *sp. nov.* Fig. 2 d, Fig. 4 MycoBank: MB 812365; Fungal Name: FN570174.

**Diagnosis:**—Differing from *Pluteus subcervinus* in having purple pileus, relatively bigger basidiospores and habitat on coniferous wood **Type:**—CHINA, Sichuan Province, Mianning County, Lamagetou Nature Reserve, elev. 3110 m, at 28°56′07″N, 102°12′42″E, 12 July 2005, Z. W. Ge 460, HKAS48956 (Holotype; nrITS: KR350489).

**Etymology:**—the epithet *purpureofuscus* is from the Latin words of "*purpureus*" (purple) and "*fuscus*" (dark colored), making reference to the dark purple color of the pileus.

*Pileus* 40–60 mm in diam., subconical to convex, fleshy, purple to dull purple (13C4–13D4), slightly paler towards the margin (15C6), not hygrophanous, innately or conspicuously radially fibrillose; margin thin, non-striate. *Lamellae* free, white when young, becoming pinkish, slightly ventricose, crowded, with lamellulae of 1–2 lengths; lamellar edge slightly eroded, dark-brown for whole length or only in some areas. *Stipe* 50–70 × 4–7 mm, cylindrical to subcylindrical, central, equal with a slightly enlarged base, curved, pale purplish brown (15C4–15D4), longitudinally striate, slightly pubescent or fibrillose, solid. *Context* in pileus and stipe white, unchanging when injured. *Taste* and *odor* not recorded.

Basidiospores (40/3/1) 6.0–8.0(–9.0) × 5.0–6.0(–6.5) μm, avl × avw = 7.4 × 5.5 μm, Q = 1.20–1.60, avQ = 1.34, broadly ellipsoid to ellipsoid, smooth, slightly thick-walled. Basidia 21–40 × 7–10 μm, 4-spored, clavate or cylindrical, colorless, thin-walled, sterigmata 3–4 μm long. Pleurocystidia 55–80 × 12–30 μm, abundant, metuloid, fusoid-ventricose or lageniform with 2–4 apical or subapical horns, occasionally with fewer or without horns; horns sometimes forked; wall thickened in upper half (2.0–2.8 μm thick at neck), smooth, colorless or with pale brown intracellular pigment. Intermediate cystidia rare, predominantly fusiform and without apical hooks, sometimes similar to the pleurocystidia but smaller and/or with thinner walls. Lamellar edge sterile. Cheilocystidia abundant, 30–58 × 10–20 μm, subclavate to clavate, thin-walled, with or without brown intracellular pigment. Hymenophoral trama convergent; hyphae 3–12 μm in diam., hyaline, smooth, thin-walled. Pileipellis a cutis, with cylindrical hyphae 5–18 μm wide, filled with grayish red to reddish brown (8C4–8D4) intracellular pigment in 5% KOH; terminal elements 98–230 μm long, sub-cylindrical, usually tapering towards an obtuse apex. Stipitipellis a cutis; hyphae 4–10 μm wide, cylindrical, colorless or with brown content, thin-walled. Caulocystidia absent. Clamp connections present in hyphae of pileipellis and stipitipellis.



**FIGURE 4.** *Pluteus purpureofuscus* (HKAS48956–holotype). a. Basidiospores. b. Basidia. c. Cheilocystidia. d. Pleurocystidia. e. Intermediate cystidia. f. Pileipellis elements. Scale bars:  $a = 5 \mu m$ ;  $b = 10 \mu m$ ; c, d, e,  $f = 20 \mu m$ .

**Habit, habitat and distribution:**—Solitary to scattered. On rotten logs of *Picea* sp. in meadow with shrubs and remnant trees apparently once forested. Asia: Known only from Hengduan Mountains of southwest China.

**Discussion:**—The cutis pileipellis, the metuloidal hymenial cystidia, together with the molecular data (Fig. 1), place *Pluteus purpureofuscus* in section *Pluteus*. It is characterized by the combination of the (i) medium sized basidiomes with convex to plano-convex dark purpure pileus; (ii) curved and pale purplish brown stipe; (iii) pileipellis elements contained with grayish red to reddish brown intracellular pigment; (iv) subglobose to broadly ellipsoid spores sized  $6.0-8.0(-9.0) \times 5.0-6.0(-6.5)$  µm; (v) cheilocystidia clavate and filled with brown intracellular pigment; (vi) obvious clamp connections; (vii) and habitat on coniferous wood.

On the molecular study, *P. subcervinus* (KJ009752) appears to be the closest relative taxa of *P. purpureofuscus* (Fig. 1). However, *P. subcervinus* is distinct by its brown pileus, smaller basidiospores (5.4–8.5  $\times$  4.4–5.5  $\mu$ m, avl  $\times$ 

avw =  $6.9 \times 5 \mu m$ ) (Justo & Castro 2007a), and habitat on angiosperm wood. *Pluteus subcervinus* is only known with certainty from Sri Lanka and India (Pegler 1977, 1986, Pradeep *et al.* 2002). An additional record from China remains to be confirmed with molecular and/or morphological data (Bi *et al.* 1993).

All known members of the *salicinus* clade, which appear close in the phylogeny (Fig. 1), lack the purple-brown coloration and habitat mostly on angiosperm wood. The two species of the *salicinus* clade found on coniferous wood include *P. sepiicolor* E.F. Malysheva (Justo *et al.* 2014: 64) and *P. oreibatus* Justo (Justo *et al.* 2014: 66). However, both species differs from the present taxon by the brown pileus and the white lamellar edges. Aomong the compared species with coniferous habit, mainly in the *pouzarianus* and *atromarginatus* clades (Fig. 1), none of them has any purple coloration and habitat at high elevation above 3000 m as *P. purpureofuscus*; besides, their basidiospore, cheilocystidia and geographic distribution are also different (Justo *et al.* 2014).

Among the other known species, two species of *Pluteus* section *Hispidoderma*, viz. *Pluteus hispidilacteus* Horak (2008: 22) and *P. decoloratus* Horak (2008: 24), also can develop pink colors on the pileus with age but they are clearly different from *P. purpureofuscus* in the non-metuloid and absent to very rare pleurocystidia. Besides, they are only known from New Zealand (Horak 2008). *Pluteus lilacinus* (Sacc.) Singer (1961: 337), with lilac or purplish brown to cocoa brown pileus, is somewhat morphologically similar and also belongs to *Pluteus* section *Pluteus*, but it is distinctive by its glabrous pileus, smaller basidiospore  $5-7.5 \times 3.5-5.7 \mu m$ , more elongate and narrower cheilocystidia  $40-75 \times 5.5-8.8 \ (-12) \mu m$ , the mainly *Magnus*-type pleurocystidia  $42-70 \times 9.5-16 \mu m$  with sharply acute apex, and absence of clamp connections (Singer 1961).

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