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# The myxomycete genus *Schenella*: morphological and DNA sequence evidence for synonymy with the gasteromycete genus *Pyrenogaster*

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***Abstract:*** The genus *Schenella* has proven difficult to classify since its description as a new genus in 1911. Macbride placed it with the Myxomycetes but it was unclear with which myxomycete, if any, it should be grouped. Recent identification of abundant samples of *Schenella* has aided a re-evaluation of its classification as a myxomycete. Morphological evidence based on light and scanning electron microscopy of recently collected specimens and on the type specimen of Macbride suggested that it might be synonymous with the gasteromycete *Pyrenogaster.* Analysis of DNA sequences from freshly isolated samples indicates that the genus *Schenella* is related closely to an anciently diverged, monophyletic group of fungi that includes several gasteromycete genera, among them *Geastrum, Sphaerobolus* and *Pseudocolus*. Comparisons of the morphology and DNA sequences of authentically identified specimens of *Pyrenogaster atrogleba* indicate that it is synonymous with *Schenella simplex*. The nomenclatural implications of this discovery are discussed.

***Key words:*** Eumycetozoa, gasteromycete, molecular systematics, Myxomycetes, plasmodial slime molds, *Pyrenogaster, Schenella*

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## INTRODUCTION

*Schenella simplex* was described and illustrated as a new genus and species by Thomas H. Macbride (1911). A single collection gathered in Aug 1903 on a decaying pine log in Yosemite Valley, California, was designated as the holotype. Macbride expressed doubt about this taxon in the first sentence of his brief paper: ''This following description and accompanying figures are submitted to mycologists partly for the sake of eliciting information.'' Macbride further noted that, ''If a slime-mould, the species is referable to the family Dianemaceae and is akin to those in which the capillitial threads pass from side to side of the fructification, attached at each end.'' This unique combination of characters—aethalioid fructifications, simple capillitial threads twisted together to form vertical columns attached to the outer peridium and at the base of the fructification along with tiny, smooth, spherical spores, 5–6 μm diam—were the stated hallmarks of this enigmatic taxon. The status of *Schenella simplex* as a myxomycete was so doubtful that Macbride omitted the taxon from his 1922 monograph (Macbride 1922), nor was it included in the Lister monograph (Lister 1925).

In the 1934 monograph by Macbride and Martin (1934) *Schenella simplex* again was included. Commentary after the species description again casts doubt on the status of this taxon: ''A very curious form, of somewhat doubtful affinities and placed in the Stemonitaceae on the basis of the dark violet brown spores and the dark capillitium. There is no suggestion of columellae in the pillar-like columns. To the naked eye it suggests an amaurochaete.'' Spores were described as ''rather coarsely tuberculate'' and not smooth.

Hagelstein (1944) made reference to *Schenella simplex* in the Amaurochaetaceae but again expressed doubt about its status: ''. . . might appropriately be placed in this family if conclusively proven to be a form of Mycetozoa. The original description and figures are not impressive, and clearly indicate the author was uncertain.'' *Schenella* was not included in his key to the genera.

Martin (1949) included a note about *Schenella* in the Stemonitaceae that, ''if it is a myxomycete, (it) would be placed in this family close to *Amaurochaete*.'' A dichotomous key to 10 genera in the family

fails to include *Schenella*. Martin (1961) described a second species of *Schenella, S. microspora,* after re-examination of the type of *S. simplex*. He concluded that *Schenella* was a valid genus in the Stemonitaceae, closely allied to *Amaurochaete*. Martin re-interpreted the morphology of this taxon as a pseudoaethalium, not an aethalium, with the sporangia arranged vertically much as in *Dictydiaethalium*. Each column (sporangium) appeared to have a distinct top with an individual peridium and a coiled, twisted mass of pseudocapillitial threads attached also at the base of the fructification. There was no trace of a columella. The capillitial system consisted of a bundle of threads within each sporangium. Martin (1961) emended the generic and species description, emphasizing the dark capillitium and dark spores as the salient morphological characters for assignment to the Stemonitaceae close to *Amaurochaete*. Spore diameter of *S. simplex* was given as 5–6 μm and for *S. microspora* 3.5–4(4.5) μm. Martin (1961) also noted that spores were small for a myxomycete but no smaller than other well known species. This statement is supported by examples such as *Stemonitis microsperma* B. Ing, and *S. smithii* Macbr., with the smallest spore diameters known in the Myxomycetes (Martin and Alexopoulos 1969).

Nannenga-Bremekamp (1967) used capillitial characteristics to transfer *Schenella* from the Stemonitaceae to its own monogeneric family, the Schenellaceae. This transfer was based exclusively on the literature because no specimens were examined.

The description of a second species in the genus, *Schenella microspora,* by Martin (1961) seems to have convinced other authors as to its identity as a myxomycete and recognition of a separate family, Schenellaceae (Martin and Alexopolous 1969, Nannenga-Bremekamp 1974, Farr 1976, Martin et al 1983, Hawksworth et al 1995).

Rammeloo (1985) examined spores of the type specimen of *S. simplex* using scanning electron microscopy, expressing doubt that the taxon was a myxomycete because the spores possessed a pore similar to that of some taxa in the fungal order Agaricales. One field collection from Mexico more recently was identified as *Schenella simplex* and compared with the type of *S. simplex*. Rodríguez-Palma and Estrada-Torres (1996) collected this specimen in the *Abies religiosa* forest in Malintzi National Park, Tlaxcala, México.

This paper reports comparisons of additional specimens from Cofre de Perote National Park, Veracruz, México, identified as *S. simplex,* previously known only from its type locality in California. Evidence from light microscopy and scanning electron microscopy indicates that the Mexican specimens are identical to the *S. simplex* type. Morphological data, as well as DNA sequence evidence from the Mexican specimens, also indicate that *S. simplex* is related to an anciently diverged group of gasteromycete fungi and should not be classified as a myxomycete. Further, morphological characterization indicates that *S. simplex* is synonymous with the gasteromycete fungus, *Pyrenogaster atrogleba* (Zeller) L.S. Domínguez & Castellano. In support of this, DNA sequences obtained from authentically identified *P. atrogleba* specimens are identical to DNA sequences from the Mexican specimens of *S. simplex*.

Preliminary work on the status of S*chenella* has been published as abstracts (Gaither and Keller 1997, Estrada-Torres et al 2002, Gaither and Keller 2002).

## MATERIALS AND METHODS

Recent field collections of *S. simplex* were examined from two Mexican states, Tlaxcala and Veracruz. Representative specimens are deposited in the TLXM herbarium with some duplicates at MA-Fungi. Specimens were washed with alcohol and distilled water to eliminate as many spores as possible. Freehand transverse sections were made of peridial fragments for microscopic examination and then rinsed and stained with a 1% aqueous solution of methylene blue following the technique described by Largent et al (1977). Permanent slides of these sections or of complete fruiting bodies were made in Hoyer's medium. Microscopic examination was made with a light microscope equipped with differential interference contrast imaging (Nomarski optics) or with bright field light microscopy. Fruiting bodies were mounted in either distilled water, clear lactophenol or Hoyer's medium. Selected specimens were photographed under 600× or 1000× under the oil immersion lens using either a Nikon E-600 microscope or an Olympus PM10 photomicrographic system. All SEM preparations used the critical point drying technique. SEM images were recorded on Polaroid 55 film using either of these SEMs: AMRAY 1200 at 35 kV, a JEOL JSM-35C, or a JEOL T-300 at 15 kV. Color standards followed the ISCC-NBS Color Name Charts Illustrated with Centroid Colors (Anon. 1976).

Spore germination was observed using a suspension of spores in sterile distilled water placed in a depression microscope slide. Hanging drop spore preparations using sealed cover glasses over a depression microscope slide were observed periodically up to 1 wk. Spore cultures were prepared by spreading spores uniformly using a sterile glass rod with 0.1 ml aliquots of a spore suspension on the surface of these culture media: 2% water agar, potato-dextrose agar and a modification of Melin & Norkrans medium for ectomycorrhizal fungi (Marx 1969).

*Specimens examined.—Schenella simplex* T.H. Macbr., Type specimen. USA. CALIFORNIA: Yosemite Valley, on a decaying pine log, Aug 1903, *T.H. Macbride* (HOLOTYPE, BPI839197). MÉXICO. TLAXCALA: Huamantla municipality, Malintzi National Park, cañada central, 19°14′29″N, 97°59′38″W, 3300 m, 13 Apr 1990, on leaves of *Alnus* sp. in

*Abies-Pinus* forest, A. Estrada-Torres & M. Rodríguez-Palma, TLXM sub RP-1775. VERACRUZ: Perote municipality, Cofre de Perote National Park, 19°31′11″N, 97°09′28″W, 3390 m, 9 Mar 1998, on trunks, needles and wood of *Abies religiosa,* and bryophytes, A. Estrada-Torres, C. Lado, M. Rodríguez & A. Varela, TLXM sub ET 4029, 4030, 4031, 4032, 4033, 4034, 4035, 4036; ibídem, 1 Mar 2001, on bryophytes, A. Estrada-Torres & M. Ramírez-Ortega, TLXM sub ET 7511, 7512. *Pyrenogaster atrogleba* (Zeller) L.S. Domínguez & Castellano: ITALY: Ravenna, under *Pinus pinea,* 18 Dec 1993, leg. A. Randi, MA-Fungi 32070. MÉXICO. MORELOS: Lagunas de Zempoala National Park, under *Abies,* 12 Oct 1965, ENCB sub G. Guzman 4946 (as *Radiigera atrogleba* Zeller). TLAXCALA: Huamantla municipality, Malintzi National Park, cañada central, 19°14′29″N, 97°59′38″W, 3300 m, under *Abies religiosa,* 9 Mar 1990, TLXM sub A. Kong Luz 1338 (as *Radiigera atrogleba*). USA. WASHINGTON: Pend Orielle County, USA. Pend Orielle County Park, along U.S. 2, under *Pseudotsuga menziesii,* and other conifers, 6 Oct 2000, M. Castellano, collector, collection number Trappe 25561 (OSC80968).

*Molecular techniques.—DNA isolation.* Total cellular DNA was released from the Mexican *S. simplex* specimens by suspending a small portion of the material in BEST (0.1% bovine serum albumin, 60 mM EDTA, 300 mM sucrose, 20 mM Tris-HCL, pH 7.4) and disrupting membranes by the addition of sodium dodecyl sulfate (SDS) to a concentration of 2%. The lysate was centrifuged at 8000 × *g* 10 min at 4 C. After centrifugation the supernatant was extracted with an equal volume of phenol and then with an equal volume of chloroform. Total nucleic acids were precipitated by the addition of 0.2 volumes of 3M sodium acetate, pH 7.8 and 3 volumes of 95% ethanol. Nucleic acids were pelleted by centrifugation at 12 000 × *g* 10 min at 4 C. After decanting the supernatant ethanol, the pellet was dried in vacuo and resuspended in TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA).

*Oligonucleotide primers.* Primers used for the polymerase chain reaction (PCR), and DNA sequence analysis were synthesized commercially (Sigma-Genosys).

*Mitochondrial SSU primers*

Mt SSU Bam HI 5′ CGGGATCCAGCAGCCGCGGTAA 3′
Mt SSU PstI 5′ AACTGCAGTCGAATTAAACCACAT 3′
Fungal 5′ mtSSU 5′ CTGGTGCCAGAAGACTCGGT 3′
Fungal 3′ mtSSU 5′ GTACTCACAAGGCGGAATGG 3′

*Nuclear SSU primers*

Nuc SSU BamHI 5′ CGGGATCCAGCACCCGCGGTAA 3′
Nuc SSU EcoRI 5′ GGAATTCGTCAAATTAAGCCGCAGG 3′

*Polymerase chain reaction.* Total nucleic acids were resuspended in TE (10mM Tris-HCl, pH 8; 1 mM EDTA), and RNA was removed by digestion with DNAase-free RNAase A (Sigma Chemical Co.) at 37 C for 30 min. The DNA was used as template for PCR (Saiki et al 1988) with the set of either SSU mitochondrial primers or the SSU nuclear primers to amplify the core region of the small subunit ribosomal RNA from the mitochondria or the nucleus respectively. The amplification was performed in 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.5 mM $MgCl_2$; 200 μM deoxynucleotide triphosphates; 2.5 units Taq DNA polymerase (Fisher Biotech). The thermal cycle regimen consisted of 94 C for 1 min, 55 C for 2 min and 72 C for 2 min for 35 cycles. During the final cycle an extension of 10 min at 72 C was performed and the reaction products were slowly cooled to 4 C for complete annealing.

*Recovery of PCR amplification products.* PCR amplification products were separated from the PCR primers by electrophoresis in 1% agarose-TAE (40 mM Tris-acetate, pH 7.9, 1 mM EDTA) gels. The amplification products were excised from the gels and removed from the agarose by binding to glassmilk (GENECLEAN II kit, BIO101).

*Sequence analysis.* DNA amplification products recovered from agarose were sequenced directly. Both strands of the amplification products were sequenced individually using each of the PCR primers. Sequences of the amplification products were determined using the cycle sequencing, dye-labeled terminator procedure (ABI PRISM, Applied Biosystems). Thirteen pmol of primer in 4 μL of water was extended on a template of 80 ng of PCR amplification product in 4 μL of water using ampliTaq DNA Polymerase FS in the presence of Big Dye terminator mix. Thermocycling conditions were 90 C for 30 s, 50 C for 15 s and 60 C for 3 min. Sequence data were analyzed using the MicroGenie sequence analysis package (Queen and Korn 1984).

*Phylogenetic analysis.* DNA sequences were aligned initially using the multiple alignment algorithm of MicroGenie sequence software (Queen and Korn 1984) and then manually adjusted. Regions of length variation were omitted in the alignment. Maximum parsimony trees were produced from alignments using the DNAPARS algorithm from the PHYLIP package, version 3.57c (Felsenstein 1995). Distance matrices were produced from pairwise comparisons of aligned sequences or by using the maximum likelihood (ML) option of the DNADIST algorithm of PHYLIP on multiple alignments. Trees were produced from these matrices using the FITCH algorithm of PHYLIP. Bootstrap values were determined using the SEQBOOT algorithm of PHYLIP.

*Nucleotide sequence accession numbers.* The mtDNA and nuclear DNA core SSU rRNA sequences from *S. simplex* have been submitted to GenBank under accession numbers AY573059 and AY573060 respectively.

## RESULTS

*Description of specimens identified as* Schenella simplex *from México* (FIGS. 1–16).—Fruiting bodies pulvinate, sessile, slightly adhering to the substratum, spreading over 1–5 cm, grayish (265. med. Gy), formed by cylindrical cavities of 5–6 × 0.5 mm, concrescent, closely compacted in a palisade layer, divided by peridial plates (FIGS. 14, 15). Hypothallus lacking, not observed on the substratum. Peridium thick, evanescent or persistent at the apex when remaining as a cap, grayish, opaque, brown by transmitted light, formed by irregular cells, branched and intermixed with hy-

phae; peridial plates limiting the cylindrical cavities formed by a compact system of colorless hyphae interwoven with brown and granular hyphae (conductive hyphae) when seen with light microscopy, 2.4–4 μm diam; peridium persistent and coriaceous at their base, areolate, olive-brown (95. m. Ol Br-96. d. Ol Br) by transmitted light. A stratification of four different layers of cells can be observed (FIG. 1), the external 10–15 μm thick, formed by crystalline deposits (birefringent by polarized light) and with some emergent fibulate hyphae, a second compacted stratum of 10–15 μm thick, formed by irregular cells (FIGS. 3, 4), a third stratum 30–35 μm thick, formed by irregular or subglobose cells, 8–24 × 4–9.6 μm, interwoven, and finally a compact stratum, 5–10 μm thick, formed by hyphae with irregular and branched cells (FIGS. 3, 4); jointly the layers form a pseudoparenchymatous tissue easy to observe by transverse cuts with LM (FIG. 1) which freely absorb methylene blue. The cellular nature of the peridium can be observed by SEM (FIG. 6). Capillitium composed of elastic, brownish gray (63. l. brGy-64. br Gray), bright threads in groups of helical filaments (FIG. 8) that occupy almost all of the cavity of the peridiole, and joined to the extremities, elastic at maturity and procumbent; threads single (FIG. 9) or branched at the ends, smooth, bright, grayish yellow (91. d. gy. Y) to olive brown (95. m. Ol Br) by transmitted light, 2.4–4.8 μm diam, thick-walled (up to 1.5 μm; FIG. 5), occasionally septate (FIG. 16), arising from irregular and branched cells of the inner layer of the peridium (FIG. 5). Spores free, dark brown (62. d. gy. Br-65. br Black) in mass, dark yellowish brown (78. d. y Br-75. deep y Br) to dark olive brown (96. d. Ol Br) by transmitted light, subglobose to slightly elliptical, 5.6–6.4 × 4.4–5.6 μm, thick walled, 1.0–1.5 μm, paler at the end with a pore or a scar, the pore circular and depressed, the ornamentation consists of dense, irregular warts, confluent into a subreticulum, fused and smaller toward the pore or scar forming radial crests as seen with SEM (FIGS. 10–13). Based on these characteristics, these specimens were tentatively identified as *S. simplex*.

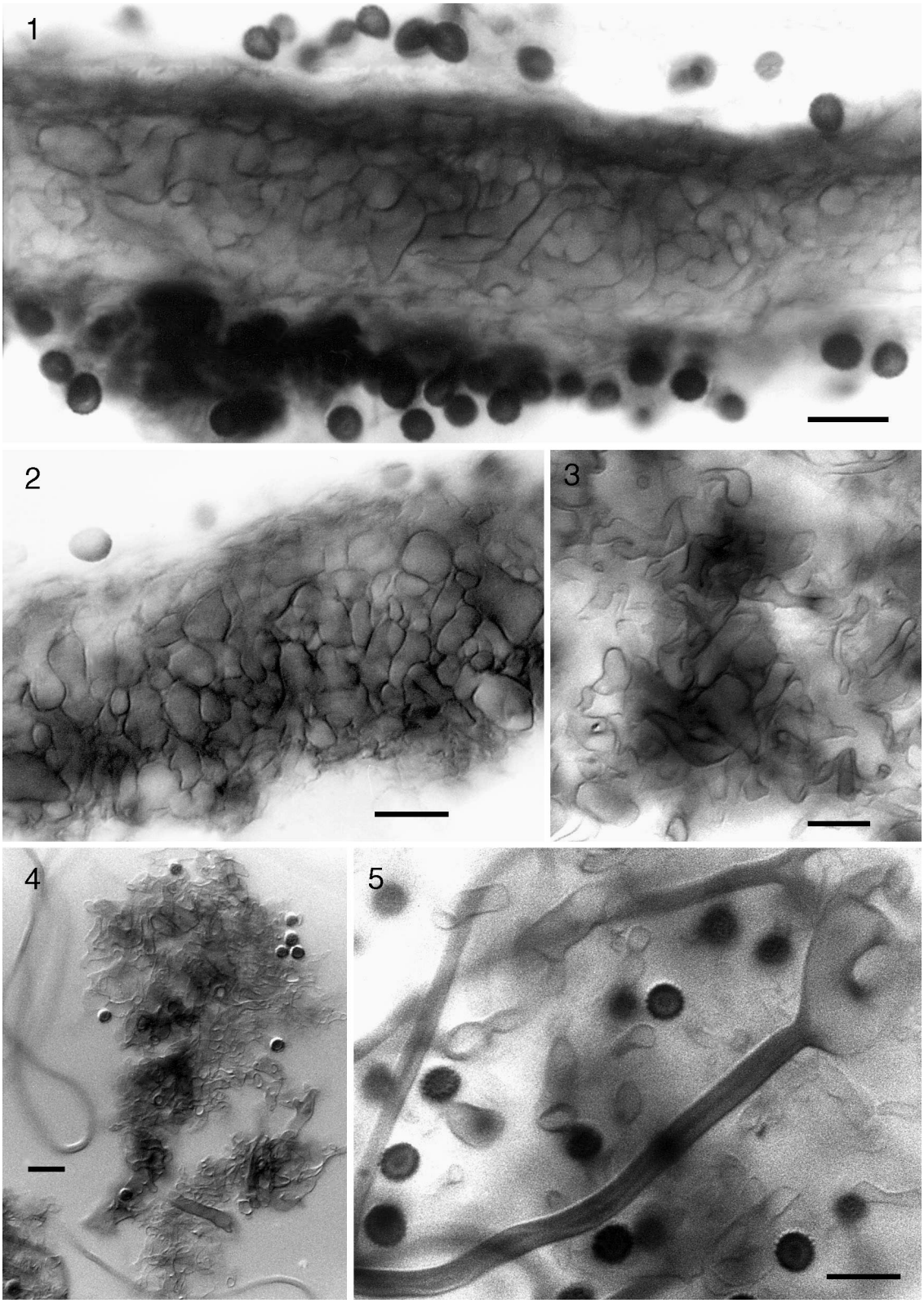
*Germination and culture*.—Spore germination or production of myxamoebae or hyphal filaments were not observed in water or in selected media.

*Commentary*.—The microscopic characters are reminiscent in color, form and habit of some species of myxomycetes (*Amaurochaete*), but the spore ornamentation and the scar, which is discernible by LM (Rammeloo 1985) and clearly visible by SEM, have not been seen in any myxomycete.

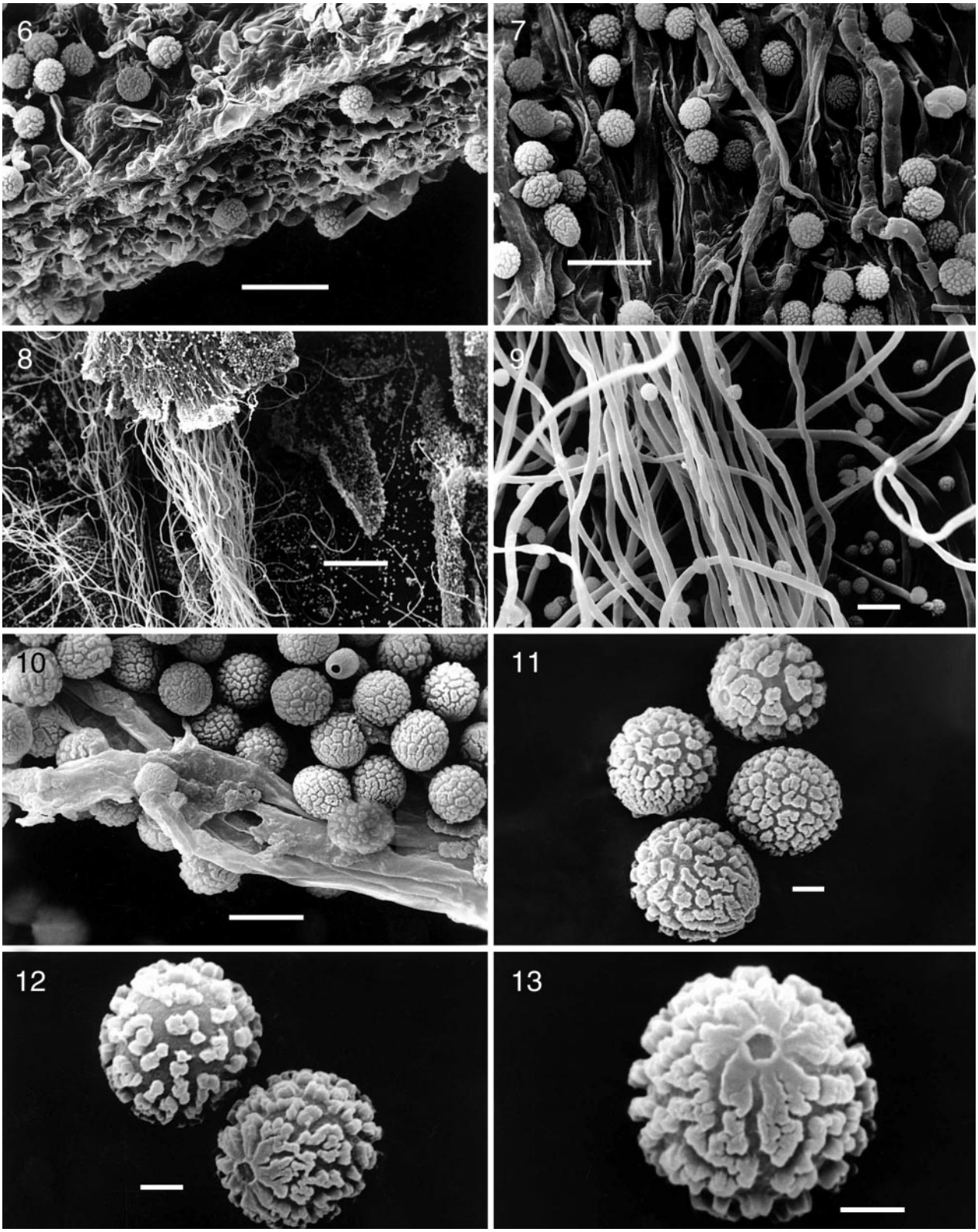
*Analysis of* Schenella simplex *type specimen*.—To confirm that the Mexican specimens were *S. simplex*, parallel analyses of the type specimen for *Schenella simplex* were done with light and scanning electron microscopy.

*Commentary on the* Schenella simplex *holotype*.—The type is represented by ample material with the following morphological features. Re-examination of the holotype of *S. simplex* with LM and SEM showed the vertical chambers (peridioles) packed with spores and coiled, twisted capillitial threads. Peridial fragments are scattered among the capillitial threads but no longer form an outer crust (intact glebal layers) as indicated in the original species description (FIG. 17). It is understandable that the holotype, now close to 100 y old, has undergone additional fragmentation. The combination of the morphology of the capillitial threads and spores separates this taxon from all other myxomycete species. The tubular threads and spore mass appear black in mass. The capillitial threads are unbranched, thick walled, lack ornamentation and have occasional septations (FIG. 18). These features alone would eliminate *S. simplex* as a myxomycete. Spore morphology when compared to typical myxomycete spores appears quite different. The small, thick-walled spores with elliptical shapes when observed in side view are different from all other myxomycete spores. Observations with SEM clearly demonstrate the morphological features of a basidiospore. The apiculate basal area represents the former attachment to the basidium. Generally in SEM preparations the distal polar view is directed upward and the apiculate view downward, but a few spores with the basal scar usually can be found. Unlike typical myxomycete spores, *Schenella* spores do not collapse when dehydrated and the size, 3.5 μm, extends beyond the smallest known range for myxomycete spores. The basal scar is recessed in the center with a smooth, raised margin, distinctly different from the rest of the surface ornamentation (FIG. 19). Capillitial threads and spore morphology are identical to the Mexican specimens identified as *S. simplex*.

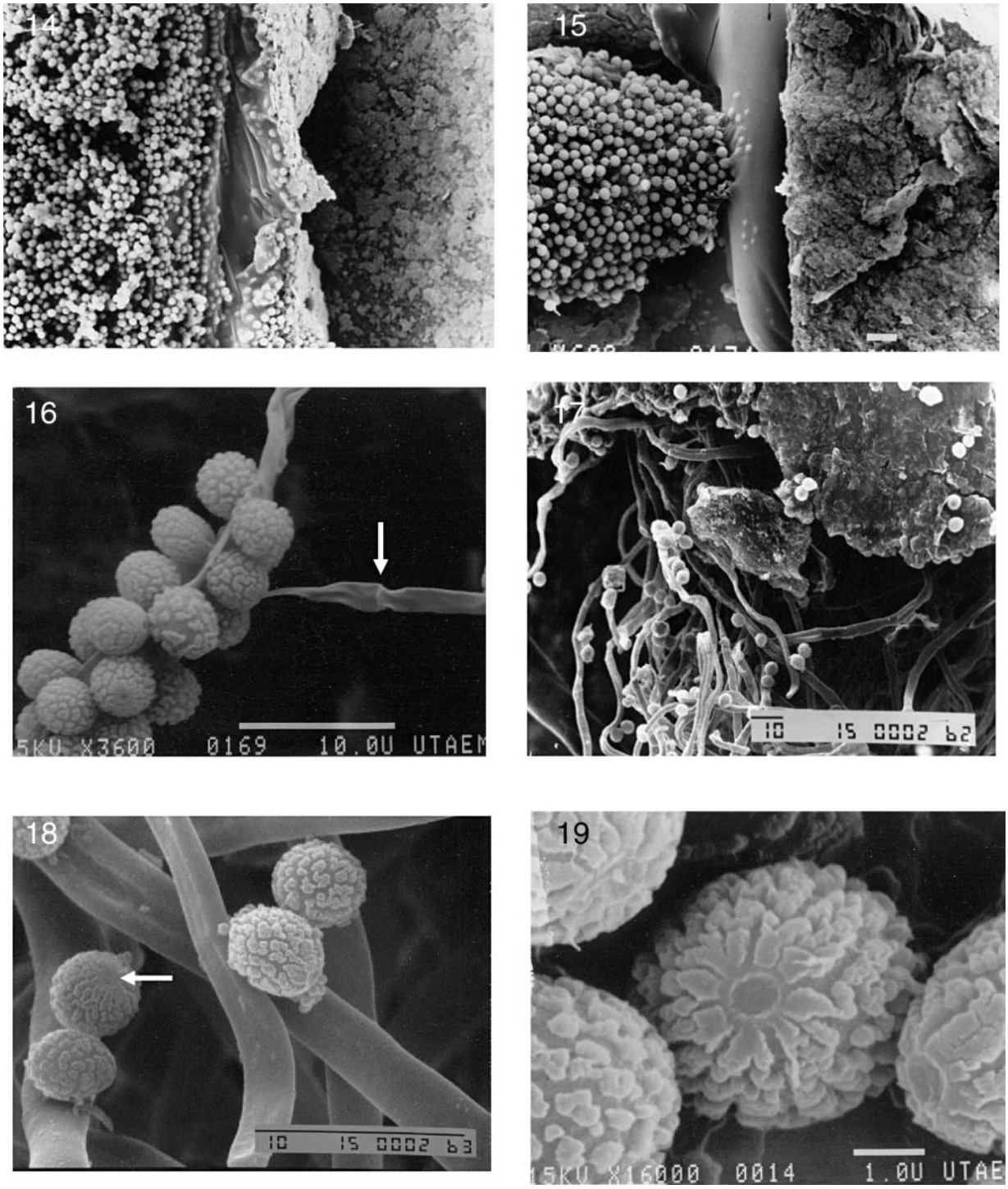
The most obvious morphological characters of the genus *Schenella* were studied. In all cases the presence of a cellular peridium (pseudoparenchyma) (FIGS. 1, 2), a hyphal network with clamp connections in the external layers of the peridium and spores with a scar or pore (FIGS. 12, 13) similar to that illustrated by Rammeloo (1985) were confirmed. This combination of characters belongs only to some groups of fungi in the strictest sense of the word and prompts us to remove *Schenella* from the myxomycetes. The presence of fibula would fit this genus in




FIGS. 1–5. *Schenella simplex* Mexican specimen (ET-4036). 1. (top panel) Pseudoparenchymatous tissue with a stratification of different layers of cells. 2. (middle left) Middle stratum of the peridium with globose cells. 3–4. (middle right, lower left) Irregular cells of the peridium. 5. (lower right) Capillitial thread branched at the end. Scale bars = 10.0 μm.




FIGS. 6–13. *Schenella simplex* Mexican specimens. 6–9. Specimen ET-4031. 6. (upper left) Peridium. 7. (upper right) Spores interwoven with hyphae. 8. (second row left) Peridiole with capillitial threads. 9. (second row right) Capillitial threads and spores. 10–11. (third row) Spores of the specimen ET-4036. 12–13. (bottom row) Spores of the specimen ET-4031. 13. (bottom right) Spore with basal scar. Scale bars: 6–9 = 10 μm, 10 = 5 μm, 11–13 = 1 μm.




FIGS. 14–19. *Schenella simplex* Mexican specimen (ET-4034) and holotype (BPI 839197). 14–16 Mexican specimen (ET-4034). 14. (upper left) Vertical wall of cylindrical chamber (peridiole) packed with spores and capillitial threads. 15. (upper right) Spore mass retaining shape of vertical chamber. Note spores embedded in gelatinous material. 16. (middle left) Spores adhering to capillitial thread. Note collapsed thread and possible septum (arrow). 17–19 Holotype (BPI 839197). 17. (middle right) Peridial fragment with underlying entangled mass of long unbranched capillitial threads intermingled with spores. 18. (lower left) Smooth capillitial threads. Spore with basal scars (arrow). 19. (lower right) Spore with basal scar. Compare spore with Figs. 12, 13, and Fig. 19 in Domínguez de Toledo and Castellano (1996). Scale bars: 15–18 = 10.0 μm, 19 = 1.0 μm.

the Basidiomycetes, and the existence of a capillitium would put it in the Gasteromycetes *sensu lato.*

*Sequence analysis of DNA extracted from a Mexican* Schenella simplex *sample.*—To test the conclusion, based on morphological features of the Mexican and type specimen, that *Schenella* is a gasteromycete fungus, DNA was isolated from a Mexican specimen (ET-7512) and used as the template in PCR experiments. Primer pairs were used that specifically amplified core regions of myxomycete small subunit ribosomal RNA (SSU rRNA) genes from nuclear chromosomal DNA and mitochondrial (mt) DNA (Miller pers comm). While no amplification product was obtained for the mitochondrial primer pair, a 639 base pair amplification product consistent with a fungal nuclear template, but inconsistent with a mitochondrial or bacterial template (Miller pers comm), was obtained with the nuclear primer pair. Sequence analysis of the amplification product obtained with the nuclear primer pair revealed a sequence similar to those found in an anciently diverged, monophyletic group of fungi that includes *Geastrum, Sphaerobolus* and *Pseudocolous* (Hibbett et al 1997) but is quite different from sequences of myxomycetes. The sequence was most similar to the nuclear SSU rDNA sequence of *Geastrum saccatum* with 96.4% sequence identity. A primer pair designed to amplify fungal mitochondrial small subunit ribosomal RNA sequences with PCR was used to amplify sequences from *Schenella* nucleic acids. An amplification product of the size predicted (383 base pairs) for fungal mitochondrial rDNA sequences was obtained. Sequence analysis of the mitochondrial amplification product revealed sequences similar to the mitochondrial DNA of the same monophyletic fungal group. Again the closest match was with *Geastrum saccatum* with 88.3% sequence identity. Phylogenetic analysis of both nuclear and mitochondrial DNA sequences amplified from *Schenella* nucleic acids indicates that *Schenella* should be grouped with these fungi and that it is most closely related to *Geastrum* (FIG. 20).

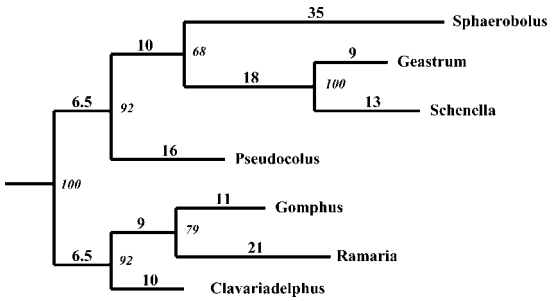



FIG. 20. Phylogeny of the monophyletic fungal group including *S. simplex.* The mtDNA sequences of the six anciently diverged, monophyletic fungi grouped by Hibbett et al (1997) with a bootstrap frequency of 100% (*Sphaerobolus stellatus, Geastrum saccatum, Pseudocolus fusiformis, Gomphus floccosus, Ramaria stricta,* and *Clavariadelphus pistillaris)* were aligned with the analogous mtDNA sequence from *S. simplex.* Tree topology was determined using maximum parsimony analysis (DNAPARS) of the mitochondrial SSU rDNA core sequence alignments. Numbers on the branches indicate the branch length in terms of the minimum number of base substitutions between nodes on the single most parsimonious tree. Numbers by the nodes (in italics) indicate the bootstrap frequencies.

*Morphological evidence that* Schenella simplex *is synonymous with* Pyrenogaster atrogleba.—The fruiting body, with subcylindrical compartments arranged as palisades and filled with capillitium and spores originally perceived as sporocarps or sporangia, are the most distinctive features of this taxon. This type of morphology is known only from one hypogeous genus of Gasteromycetes, *Pyrenogaster* and is termed a peridiole.

*Pyrenogaster* was described in France by Malençon and Riousset (1977) as *P. pityophilus* and was considered to be a monospecific genus until Domínguez de Toledo and Castellano (1996) transferred *Radiigera atrogleba* Zeller to the genus under the name of *P. atrogleba* (Zeller) L.S. Domínguez & Castellano. The presence of a peridiole and the shape and size of the spores are the salient characters for this change.

Of the two known species of the genus, *S. simplex* specimens agree with the detailed description of *P. atrogleba* by Domínguez de Toledo and Castellano (1996). This was confirmed by examination of *P. atrogleba* material kept at TLXM (Holmgren and Holmgren 1995). The characters that we observed in our material of *S. simplex* are the same as those described for *P. atrogleba*: the diameter (0.5 mm) and the arrangement of the peridiole with evanescent walls, and without evidence of adhesive material, the kind of capillitium, and the shape, size, color and type of ornamentation of the spores. The subcylindrical peridiola are arranged as palisades, with persistent caps of peridium at the apex, capillitium arranged in a helicoidal manner (FIG. 8), and subglobose to slightly elliptical spores (FIGS. 10, 11), with a circular pore and ornamented with irregular warts (FIGS. 12, 13). The illustrations by Domínguez de Toledo and Castellano (1996) (FIGS. 18, 19) of the spores by SEM also agree with those obtained in this study (FIGS. 11–13, 18, 19). Both of these authors and Malençon and Riousset (1977) described basidiomata with thick peridia for each species of *Pyrenogaster.* For *P. atrogleba,* Domínguez de Toledo and Castellano (1996) described a peridium made up of two layers, one mycelial layer 175–500 μm thick and another of stratified pseudoparenchyma approximately 45–100 μm

thick. Of these layers, the field specimens had only the innermost stratum of the second layer (see FIG. 1) illustrated by Domínguez de Toledo and Castellano (1996, Fig. 1J) due to deterioration of the material. The characteristics of the strata observed are highlighted in the description. We have compared an exsiccatum of *P. atrogleba* from the same locality of the first record of *S. simplex* (Rodríguez-Palma and Estrada-Torres 1996), another from Morelos, México and another from Italy, all coincide with *S. simplex* in the characters mentioned above.

One character not pointed out by Domínguez de Toledo and Castellano (1996) is the presence of the irregular branched tips of the capillitial filaments (FIG. 5) that mingle with the cells of the internal layer of the peridium and are distinguishable from them by their thick walls. These already had been described, however, by Malençon and Riousset (1977) and illustrated for the other species of the genus. These also were present in the field collections of *S. simplex*.

The interpretation of *S. simplex* as a myxomycete could be due to the advanced maturity of the fungus and its fragmentation, which reveals structures of similar appearance to myxomycetes such as capillitium, dark spores and peridium. Malençon and Riousset (1977) noted the difficulty in identifying any very mature stages of the fungus. The appearance of this hypogeous fungus on the surface of logs, dead wood and bryophyte layers covering the ground is an enigma, perhaps explainable by the activities of some animal.

*DNA sequence evidence that* Schenella simplex *is synonymous with* Pyrenogaster atrogleba.—To confirm the supposition that *Schenella simplex* is synonymous with *Pyrenogaster atrogleba,* DNA was isolated from authentically identified *P. atrogleba* specimens (Trappe 25561, OSC 80968) from M. Castellano and used as the template in PCR reactions with the nuclear mtSSU primers and, in a separate reaction, with the fungal mtSSU primers. Amplification products of a size identical to those produced from the Mexican *Schenella simplex* samples (639 and 383 base pairs from nuclear and mitochondrial primer pairs respectively) were obtained. These amplification products were isolated and sequenced. These sequences were compared with those obtained from the Mexican *Schenella simplex* specimen and found to be identical.

## DISCUSSION

*Taxonomy.*—Morphological and molecular evidence leads us to conclude that *S. simplex* and *P. atrogleba* are identical and should be considered synonymous. From a nomenclatural point of view, the genus *Schenella* is transferred from the Myxomycetes to the Gasteromycetes and given its priority (art. 11.3 International Code of Botanical Nomenclature) over *Pyrenogaster,* these synonyms and combinations are proposed:

*Schenella* T. Macbr., Mycologia 3(1):39. 1911
= *Pyrenogaster* Malençon & Riousset, Bull. Soc. Mycol. France 93: 289. 1977, **syn. nov.**

Generitypus: *Schenella simplex* T. Macbr., Mycologia 3(1):39. 1911.

Generitypus specimen: BPI 839197. USA. California, Yosemite Valley, Aug 1903, on a decaying pine log, T.H. Macbride.

*Schenella simplex* T. Macbr., Mycologia 3(1):39. 1911
= *Radiigera atrogleba* Zeller, Mycologia 36(6):634. 1944, **syn. nov.,** ≡ *Pyrenogaster atrogleba* (Zeller) L.S. Domínguez & Castellano, Mycologia 88(5):866. 1996.

Typus.- BPI 839197. USA. California, Yosemite Valley, Aug 1903, on a decaying pine log, T.H. Macbride, holotypus.

*Schenella pityophilus* (Malençon & Riousset) Estrada & Lado, **comb. nov.**
≡ *Pyrenogaster pityophilus* Malençon & Riousset, Bull. Soc. Mycol. France 93:290. 1977 [basion.].

Typus.—MPU (in coll. G.J.L. Malençon). FRANCE: near Aigues-Mortes, Mar-May 1976, under *Pinus pinea* needles, L. Riousset, holotypus (Isotypus at OSC).

*Schenella romana* (Quadracia) Estrada & Lado, **comb. nov.**
≡ *Radiigera romana* Quadracia, Mycotaxon 58:336 (1996) [basion.]; ≡ *Pyrenogaster romana* (Quadracia) Calonge, Bol. Soc. Micol. Madrid 22:108. 1997)

Typus—ROHB (1325 LQ). ITALY: Roma, Orto Botanico, 22 Jan 1922, under *Cupressus sempervirens,* L. Quadracia, holotypus.

The identity of *S. microspora* (G.W. Martin, Mycologia 53[1]: 29. 1961, BPI 839199, U.S.A.: CALIFORNIA, Big Basin State Park, San Mateo County, 26 August 1957, on fallen trunk of *Sequoia sempervirens* [Lamb.] Endl., G.W. Martin 6547) still has to be clarified. *Schenella microspora* was studied using LM and SEM, but these results will be published elsewhere. There are still some questions if this taxon is synonymous with *P. atrogleba* (Gaither and Keller 2002, Gaither and Keller 2003).

*Spore germination.*—Further evidence of the fungal nature of *Schenella* might have been provided by germination of the spores from the Mexican specimens. Spores did not germinate in either media for ectomycorrhizal fungi (potato-dextrose agar or modified Melin & Norkrans medium) or on 2% water agar. Most ectomycorrhizal fungal species such as *Pyrenogaster* have a low percentage of germination on artificial laboratory media (Molina & Palmer 1982), although the use of active charcoal to remove inhibitors or the presence of some stimulators (living organisms or chemical substances) can greatly improve the success of these procedures (Fries 1977, Ali and Jackson 1989, Iwase 1991). It also is relevant that spores of truffle-like fungi, such as *Pyrenogaster,* seem to be activated by passage through the intestine of mycophagous animals (Fogel and Trappe 1978). These two observations might explain why *Schenella* spores were not germinated using the techniques described. Germination of *Schenella* spores using alternate techniques or media is the goal of future experiments.

*Ecology.*—The basidiomata studied were found on diverse substrata such as trunks, needles and fallen branches of conifers or bryophyte layers growing on the ground. These habitats are typical of many species of myxomycetes. This hypogeous taxon apparently is dug up by an animal and transported to above ground sites so that its appearance on these substrata is accidental.

As with other hypogeous Gasteromycetes, *S. simplex* must be dug up and carried away to be eaten by small rodents, a fact that favors its dispersal as has been demonstrated for other species of hypogeous ectomycorrhizal fungi (Maser et al 1978, Malajczuk et al 1987, Trappe 1988). The abundance of fruiting bodies (in 400 $m^2$ about 20 were observed) and the way some were found, on trunks more than 1 m above ground or on mounds covered with bryophytes, supports this hypothesis. Furthermore rodent feces occasionally were observed alongside fruiting body remains.

None of the field collections contained complete basidiomata and in the majority of cases they were composed of spore masses and capillitium. This can be reconciled with the differential use of the fruiting body by animals (Cork and Kenagy 1989). Rodents feed on the fleshy parts of the fungus such as the columella and external layers of the peridium. As the columella and probably other fleshy parts of the unripe fungus are consumed the fruiting body is inverted leaving the outer peridium against the substrate and the base of the peridiole in the upper part, exposing the capillitium. Because the capillitium is elastic, it gives the procumbent appearance of some myxomycetes such as *Comatricha longa* Peck or species of *Amaurochaete.*

The fact that animals dig up and consume the whole fruiting structure appears to be one of the reasons why, in spite of intense searching in one locality, it was found again only after 3 y. It also is interesting to note that all collections of *S. simplex* (including those identified as *Radiigera atrogleba*) from Tlaxcala and Veracruz were collected during the end of the dry season (March and April) (Tejeda-Martínez et al 1989), when most of the mushroom species had not yet fruited in this area, indicating a peculiar phenology of the species. Furthermore in 1998 the collection of specimens in Cofre de Perote, one of the most productive localities, coincided with the dry period which that year was aggravated by the effects of El Niño.

The distribution of *S. simplex* is wider than was once thought since it was reported as *Radiigera atrogleba* from southwestern Canada (British Columbia), from USA (California, Colorado, Idaho, Montana, Oregon, Utah and Washington), from México (Baja California Norte and Morelos), from Sweden and Italy (Zeller 1944, Guzmán 1971, Kers 1976, Ayala and Guzmán 1984, Domínguez de Toledo and Castellano 1996, Calonge 1997) and as *S. simplex* from USA (California) and México (Tlaxcala and Veracruz) (Macbride 1911, Rodríguez-Palma and Estrada-Torres 1996).

Considering the ectomycorrhizal character of most hypogeous species, this species appears to be exclusively associated with conifers of such diverse genera as *Abies, Picea, Pinus, Pseudotsuga, Thuja* and *Tsuga,* although Kers (1976) also cited it under *Fraxinus excelsior.*

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