New morphological and molecular data on *Gymnopilus purpureosquamulosus* and its phylogenetic relationships among similar species

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Guzmán-Dávalos L., Contu M., Ortega A., Vizzini A., Herrera M., Ovrebo C., Rodríguez A., Villalobos-Arámbula A. R., Palomera V., Vargas G. & Santerre A. (2008) New morphological and molecular data on *Gymnopilus purpureosquamulosus* and its phylogenetic relationships among similar species. – Sydowia 60 (1): 41–56.

Gymnopilus purpureosquamulosus Høil. is a rare fungus, previously known only from the type locality in Zimbabwe. In this paper, additional records from Italy, Nigeria, Panama, and Switzerland are presented. The main characteristics of this species are: pileus with purplish scales; membranous veil on the stipe; big, ellipsoid to oblong basidiospores, and subhymenium with inflated elements. Photographical illustrations, FESEM photographs of the basidiospores and drawings of the micromorphological features are provided. Notes on the type material of *G. peliolepis,* a related species, are presented. Based on ITS rDNA sequence data, the phylogenetic relationships of *G. purpureosquamulosus* with closely related species are discussed.

Keywords: Africa, Europe, Panama, Basidiomycota, Agaricomycetes.

During a field study on macrofungi growing on palms in northern Sardinia (Olbia and neighbouring areas), M. Contu and A. Vizzini collected a very interesting *Gymnopilus* from living plants of *Phoenix canariensis* Hort. ex Chabaud (Arecaceae). Its distinguishing characters are: (i) the purplish pileus colour, (ii) the membranous, although evanescent veil, and (iii) large basidiospores. A careful study allowed us to conclude that this material corresponds to a tropical species: *Gymnopilus purpureosquamulosus* Høil. described

from Zimbabwe (Høiland 1998). This species is interesting because *Gymnopilus* members forming basidiomata with purplish scales on their pilei are very rare in Europe; it may have been introduced from tropical or subtropical areas (Rees *et al*. 2004, Holec 2005). We studied other specimens from Nigeria, Panama and Switzerland that also turned out to represent *G. purpureosquamulosus*. To evaluate the phylogenetic relationships of this fungus with other members of the genus with similar basidiomata (purplish scales on the pileus) we analyzed the ITS rDNA sequences. It has been shown previously that ITS data were useful for phylogenetic evaluations on the infrageneric structure of *Gymnopilus* (Guzmán-Dávalos *et al*. 2003).

Materials and Methods

Morphology

The specimens studied are indicated under the morphological description of each species. The macro-morphological features were observed on fresh specimens by Contu and Vizzini (Italian material), and Ovrebo (Panamanian material). Micro-morphological analyses were made from hand-made sections of basidiomata or gill fragments mounted in 3 % KOH, Melzer's reagent, cotton blue or cresyl blue. Basidiospores were measured in 3 % KOH using a calibrated optical micrometer in a Zeiss K-7 optical microscope (oil immersion objective 100 ×). Measurements are given as follows: (minimum) interval (maximum), Q = length/width ratio (N = number of samples, n = sample size). The measurements include ornamentation but not the apiculus. The basidiospore ornamentations (warts) were measured through Axio Vision 4 software in a Zeiss Axioscop 40 microscope. The basidia length includes sterigmata. The morphological study of spores has been carried out by means of a Zeiss scanning electron microscope of the FESEM type (Field Electron Scanning Electron Microscope) model 1539 Geminis. The material studied is conserved in the herbaria: GDA (University of Granada, Granada, Spain), IBUG (Institute of Botany, University of Guadalajara, Zapopan, Mexico), K (Royal Botanic Gardens, Kew, England), O (Natural History Museum, University of Oslo, Oslo, Norway) and PMA (University of Panama, Panama).

Sampling for the DNA study

Twenty-five samples belonging to 16 species were included in this study representing an ample morphological variation of *Gymnopilus* basidiomata with pileal, purple scales (Tab. 1). Fourteen

Tab. 1. – *Gymnopilus* specimens used for molecular analyses in this study.

DNA number	Species	Collector, number and herbarium	Locality	Date of collection	GenBank No.
37	*G. aeruginosus* (Peck) Singer	P. Kroeger 1336 (DAOM-210322)	Canada	Oct 16, 1988	AY280974
205	*G. cyanopalmicola* Guzm.-Dáv.	F. Ramírez-Guillén 254a (XAL, **type**)	Mexico	Oct 19, 2003	EU401711
39	*G. dilepis* (Berk. & Broome) Singer	R. Treu (IMI-370900)	Malasia	1996	AY280980
89	*G. hispidellus* Murrill	Standley 53856 (F-1112036)	Honduras	Mar 20, 1928	AY280983
20	*G. hispidus* (Massee) Murrill	D.N. Pegler 3254 [K (M) 75211]	Dominica	Nov 8, 1977	AY280985
1	*G. lepidotus* Hesler	G. Guzmán 30374 (XAL)	Mexico	Sep 14, 1991	AY280989
22	*G. luteofolius* (Peck) Singer	L.R. Hesler & H. Ford (DAOM 80626)	USA	Oct 25, 1942	AY280992
152	*G. medius* Guzm.-Dáv.	J. García-Franco s.n. (XAL, **type**)	Mexico	Apr 19, 1990	AY280994
116	*G. ochraceus* Høil.	L. Ryvarden & K. Hțiland G23 (O-72838, **type**)	Zimbabwe	Jan 29, 1992	EU401709
48	*G. cf. punctifolius* (Peck) Singer	M. Evers & D. Sieger (L. Norvell 92-04.20-1)(WTU)	USA	Apr 20, 1992	AY280993
162	*G. purpuratus* (Cooke & Massee) Singer	H. Reis s.n. (L-0109669)	Germany	Aug 8, 1988	EU401710
18	*G. purpureosquamulosus* Høil.*	M.H. Zoberi 342 [K (M) 75214]	Nigeria	1968	AY280979
102	*G. purpureosquamulosus***	O. Röllin 89-16 (IBUG)	Switzerland	Jul 8, 1989	AY280998
115	*G. purpureosquamulosus*	L. Ryvarden & K. Hțiland G24 (O-72839, **type**)	Zimbabwe	Jan 29, 1992	EU401713
214	*G. purpureosquamulosus*	C.L. Ovrebo 3594 (IBUG)	Panama	Aug 10, 1997	EU401712
216	*G. purpureosquamulosus*	A. Vizzini AV G-1 (IBUG)	Italy	Jun 21, 2006	EU401714
218	*G. purpureosquamulosus*	M. Contu s.n. (IBUG)	Italy	Jun 29, 2006	EU401715
233	*G. purpureosquamulosus*	M. Contu s.n. (IBUG)	Italy	Oct 26, 2006	EU401716
234	*G. purpureosquamulosus*	M. Contu s.n. (IBUG)	Italy	Oct 25, 2006	EU401717
236	*G. purpureosquamulosus*	M. Contu s.n. (IBUG)	Italy	Oct 23, 2006	EU401718
237	*G. purpureosquamulosus*	M. Contu s.n. (IBUG)	Italy	Oct 12, 2006	EU401719
127	*G. subearlei* R. Valenz., Guzmán & J. Castillo	G. Guzmán 11648-A (ENCB, **type**)	Mexico	Jul 11, 1974	AY281013
172	*G.* cf. *subearlei*	L. Guzmán-Dávalos 7438 (IBUG)	Mexico	Aug 16, 1998	AY281014
5	*G. subpurpuratus* Guzm.-Dáv. & Guzmán	L. Guzmán-Dávalos 5303 (IBUG)	Mexico	Aug 2, 1991	AY281016
Outgroup					
52	G. sapineus (Fr.) Maire	I. Kytövuori 90-2488 (H)	Finland	Oct 9, 1990	AY281007

* As G. cf. *palmicola* Murrill in Guzmán-Dávalos *et al.* (2003). ** As *G. peliolepis* (Speg.) Singer in Guzmán-Dávalos *et al.* (2003).

samples were sequenced during a previous study (Guzmán-Dávalos *et al.* 2003). *Gymnopilus sapineus* was used as outgroup.

DNA extraction

DNA was extracted from small pieces (ca. 4 mg) of the pileus (including cutis, context and lamellae), using one of the following procedures: phenol method of Raeder and Broda (1985), CTAB method of Gardes and Bruns (1993), salt-extraction with 1 % PVP method of Aljabani and Martínez (1997) or employing an extraction kit (Nucleon PhytoPure, Amersham). The DNA extracts were diluted 1:2, 1:5 and 1:10 or were used undiluted in PCR reactions.

PCR amplification

Polymerase Chain Reaction (PCR) was performed to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene and the internal transcribed spacer 2 (ITS2) following the protocol indicated by Guzmán-Dávalos *et al.* (2003) with some minor modifications as follows: each 40 µL PCR reaction was prepared mixing 1 µL of undiluted DNA template, 33 µL of the mixture reaction [containing 4 µL of 10 × Taq DNA polymerase reaction buffer (–$MgCl_2$), 2 µL of 50 mM $MgCl_2$, 1 µL of 10 mM dNTPs, 2 µL of BSA (bovine serum albumine) at 2 µg/µL and 24 µL of MilliQ water] and 6 µL of Taq-primer mixture (containing 0.2 µL of Platinum Taq DNA polymerase (5 U/µL), 1 µL of each 10 µM primer and 3.8 µL of MilliQ water). All reagents were from Invitrogene® except BSA which was purchased from Sigma. Negative controls, without DNA template, were included to detect contamination in the reagents. The primer pairs ITS1F-ITS4, ITS1-ITS4, ITS1-ITS4S and ITS5-ITS4 were used to amplify the entire ITS. Likewise, ITS1F-ITS2, ITS1-ITS2 and ITS5-ITS5.8S were used to amplify the ITS1. Finally, ITS3-ITS4, ITS3-ITS4S and ITS5.8SR-ITS4S were needed to amplify the ITS2 (Vilgalys and Hester 1990, Kretzer *et al.* 1996).

PCR amplifications were performed in a MJ Research PTC 100 thermocycler. The DNA was denatured at 95 °C for 3 min. Twenty-five cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 45 s, and extension at 72 °C for 2 min were followed by 15 cycles of 95 °C for 1 min, 52 °C for 45 s and 72 °C for 2 min increasing 5 s each cycle with an extension step of 72 °C for 10 min and final incubation at 15 °C. Amplification products were separated by electrophoresis in 1.2 % TBE agarose gels (UltraPure grade, Invitrogen®), using a 100 bp DNA size marker and then stained in a solution of ethidium bromide (20 µL/500 mL, from a 10 mg/mL stock solution, Sigma).

Permanent record was obtained using a Kodak DC120 digital camera coupled with the EDAS 3.5 data analysis software.

Sequencing

Sequencing reactions were performed with BigDye™ Terminator v3.1 Cycle Sequencing (Applied Biosystems) in a 20 µL final volume following the manufacturer's protocols and using the same primers as in the PCR reactions. Sequencing reactions were purified with AutoSeq™ G-50 column (Amersham Biosciences), and finally 18 µL from formamide were added. Sequences were obtained by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems).

Sequencing edition was made with Chromas 1.45 (McCarthy 1996–1998). Assembly of sequence fragments and alignment of sequences were carried out with MacClade 4.0 PPC (Maddison and Maddison 2000). Alignments were checked by eye and manually corrected when necessary using MacClade 4.0. Eleven new sequences were generated and the rest were obtained from Guzmán-Dávalos *et al.* (2003). New sequences have been submitted to GenBank (accession numbers EU401709-EU401719).

Phylogenetic analyses

Twenty-five sequences from 16 taxa were used in the analyses (Tab. 1). Maximum-parsimony equally weighted analyses were performed with PAUP* 4.0b10 (Altivec) (Swofford 2000). Gaps were treated as missing characters. Heuristic searches were conducted under these conditions: starting trees obtained by stepwise addition, random addition sequence with 1000 replicates, tree-bisection-reconnection (TBR) as the branch swapping algorithm, branches collapsed if maximum branch length is zero, and MulTrees option in effect. Support for nodes was obtained from 1000 bootstrap replications. The conditions were the same as above, except that the number of random addition replicates was set to 10.

Taxonomy

Gymnopilus purpureosquamulosus Høil., Mycotaxon 69: 82, 1998. Figs. 1–21.

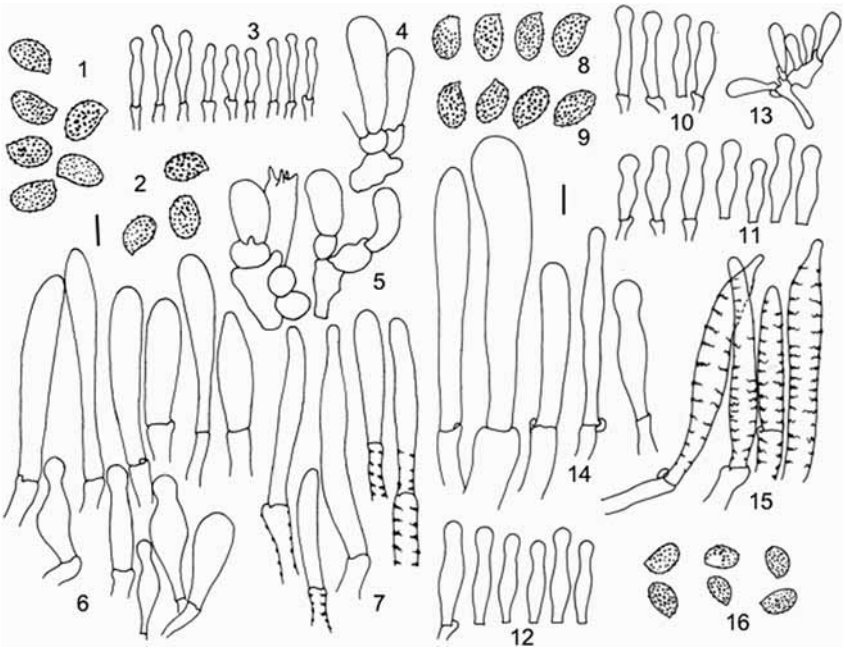
= *Gymnopilus aculeatus sensu* Zoberi in Kew Herbarium
= *G.* aff. *palmicola sensu* Guzmán-Dávalos *et al.* (2003)
= *G.* aff. *peliolepis sensu* Röllin (1998)
= *G. peliolepis sensu* Guzmán-Dávalos *et al.* (2003)

Macromorphological description from Italian and Panamanian specimens

Pileus (10–) 23–95 mm, obconic-campanulate to convex when young, expanding to broadly convex, plano-convex to plane, non-umbonate, slightly depressed at centre when older, surface dry to moist, raspberry-pink or raspberry-fulvous when young, then lilac-fuchsia, finally lemon-yellow, or dull yellow or golden-ochre overall at all stages, covered by dense, sharp and erect tiny fibrillose squamules, mainly present at disc, sometimes extending to 2/3 of the radius, squamules lilac-pink, red-fuchsia, reddish, rusty-red or pale brown when rain-soaked, purple when dry, in older basidiomata becoming appressed towards the margin and erect towards the disc; margin straight to revolute, wavy, translucent-striate up to 10 mm (most obvious in mature pilei), sometimes appendiculate with buff-yellow partial veil remnants. – Lamellae crowded to close, 4–8 mm wide, adnate, uncinate-adnate or shortly decurrent, ventricose, pale yellow when young, soon yellowish-orange, yellow-saffron, reddish-brown, finally dull ochre-orange or ferruginous then rusty when dry, not discolouring, edge entire, concolorous; lamellulae numerous. – Stipe 27–80 × 2–15 mm, central to eccentric, ± cylindrical to subclavate, flexuose, glabrous or fibrillose-striate, pruinose at the upper part, silky, faint traces of velar fibrils seen on some young basidiomata, light yellow to vinaceous-pink, not discolouring or rusty-brown when handled, occasionally turning very slightly bluish at the base, basal mycelium white; in young stages the hymenophore is covered by a yellow, membranous, partial veil; in mature basidiomata this veil forms a fugacious annulus, and apparently the stem is devoid of veil remnants. – Context of the pileus 2–4 mm thick, white to raspberry-fuchsia then pale sulphur-yellow or yellow in old pileus; context of the stipe solid, sulphur-yellow in the upper and lower part of stipe, light rusty-brown in the middle, often entirely yellow or light yellow, unchanging. – Odour and taste fungoid, sometimes with farinaceous odour. – Spore print rusty-brown. – KOH brown in fresh pileus and stipe in Panamanian specimen; KOH light green on fresh pileus, dark red on dry pileus and brownish on fresh stipe in Italian specimens.

Micromorphological description from all specimens

Basidiospores (6.4) 7.2–9.6 (10.4) × (4) 4.7–6 (6.4) µm, Q = 1.33–1.8 (1.9) [N = 11, n = 30], ellipsoid to oblong, amygdaliform, with obtuse to subacute apex, few with a truncate apex, wall thickish, verrucose, warts medium to large [0.3–1 (1.5) µm], anastomosing, without germ pore and plage, some with faint plage, without or with



Figs. 1–16. *Gymnopilus purpureosquamulosus* (1–15): **1–2.** Basidiospores. **3.** Cheilocystidia. **4–5.** Subhymenium cellular with some elongated elements. **6.** Caulocystidia **7.** Pileocystidia. **8–9.** Basidiospores. **10–12.** Cheilocystidia. **13.** Subhymenium ramose with some inflated elements. **14.** Caulocystidia. **15.** Pileocystidia. **16.** *Gymnopilus peliolepis:* basidiospores (1, 3–4, 6–7 = holotype; 2 & 5 = Zoberi 342; 8 & 10 = Contu 26 Nov 2006; 9, 12 & 15 = Vizzini AV G-1; 11 & 14 = Contu 12 Oct 2006; 13 = Contu 26 Oct 2006; 16 = holotype). Bar 1–2, 4–5, 8–9 & 16 = 5 µm, 3, 6–7 & 10–15 = 8 µm.

a slight suprahilar depression, yellowish-brown or orange-brown in KOH, dextrinoid, not metachromatic, ornamentation cyanophilic. – Basidia (20) 24.8–35 (39) × 6.4–8.8 (10) µm, clavate, with or without central constriction, tetraspored, with basal clamp connection, hyaline or with yellowish content, sterigmata 0.8–6.4 µm long. – Pleurocystidia not seen or extremely scarce. – Cheilocystidia 16–29 (40) × 3.2–8 (10) µm, lageniform, narrowly lageniform, utriform, widely utriform, cylindrical, fusiform, with a capitate or subcapitate apex (2.4–6.8 µm wide), with basal clamp connection, hyaline, yellowish, or with granulose yellow-brown content. – Hymenophoral trama subparallel. – Subhymenium cellular with some elongate elements, or, in some cases, ramose with some inflated or cellular elements. – Pileus trama radial, but with interwoven zones towards the pileipellis, hyphae 3.2–17.6 µm wide, with thin to thickish wall, hyaline or yellowish, occasionally with yellowish-brown content. – Pileipellis a cutis of prostrate hyphae, yellowish

or brown, with encrusted yellowish-brown pigment in bands; squamules formed by a trichoderm of (2.4) 3.2–13.6 (28) µm wide hyphae, elongate-fusiform, septate, with clamp connections, yellowish, with encrusted yellowish-brown pigment in bands, sometimes with pileocystidia-like terminal elements: 26.4–79 (92) × 5–11.2 µm, cylindrical or claviform, with acute, subcapitate, mucronate or obtuse apex, with clamp connections, yellowish or brown, sometimes with encrusted yellowish-brown pigment in bands. – Caulocystidia 22.4–73 (88) × 3.2–17 µm, apex 3.2–11.2 (16) µm wide, clavate, cylindrical, few utriform or lageniform, apex obtuse, subcapitate or capitate, with thin wall, with basal clamp connection, hyaline or yellowish, with or without granulose, hyaline or yellowish-brown content, in tufts at the stipe apex. – Tromboplerous hyphae with yellow content common in some basidiomata, absent in others. – Clamp-connections present.

Habitat. – Isolated, scattered, gregarious or caespitose, on decaying hard-wood in mixed riparian forest, on living trunks of *Phoenix canariensis* near the sea, on a log in tropical forest, or on a wooden wall next to a palm.

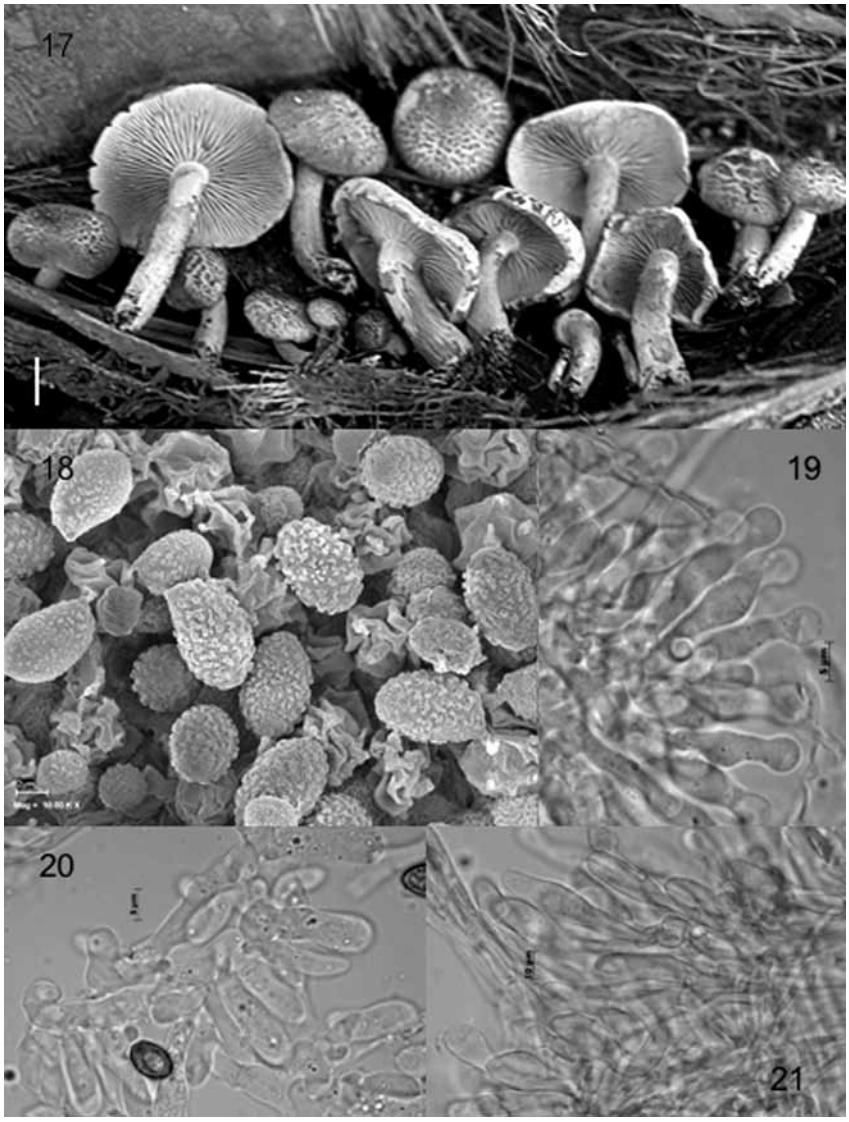
Distribution. – Previously known only from Zimbabwe; now recorded from Italy (Sardinia), Nigeria, Panama and Switzerland.

Material examined. – *Gymnopilus purpureosquamulosus* Høil.: ZIMBABWE, Victoria Falls, North Matabeleland, on decaying hardwood, 29 Jan 1992, *leg.* L. Ryvarden and K. Høiland GZ 4, *det.* K. Høiland (**holotype**, O-72839). ITALY, Prov. Sassari, Olbia, loc. Pittulongu, on a live trunk of *Phoenix canariensis* Hort. ex Chabaud near to the sea, 26 Sep 2006, 2 Oct 2006, 12 Oct 2006, 23 Oct 2006 (IBUG), 25 Oct 2006 (GDA 53540, IBUG), 26 Oct 2006, 28 Oct 2006, 3 Dec 2006, 24 Dec 2006, 16 Apr 2007 (IBUG), 3 May 2007 (GDA 53541), *leg.* M. Contu s. n., *det.* L. Guzmán-Dávalos; Prov. Sassari, Olbia, loc. Palau, on a living palm (*Phoenix canariensis*), 3 Jul 2006, *leg.* A. Vizzini AV G-1, *det.* L. Guzmán-Dávalos (IBUG). NIGERIA, Ife Biological Gardens, Ibadan, 1968, *leg. et det.* M.H. Zoberi 342 [K(M)75214] as *G. aculeatus* (Bres. & Roum.) Singer, *det.* as *G.* cf. *palmicola* Murrill in Guzmán-Dávalos *et al.* (2003), *det.* as *G. purpureosquamulosus* by L. Guzmán-Dávalos in this paper; PANAMA, Province of Panama, Gatun Lake, Barro Colorado Island, 10 Aug 1997, *leg.* C.L. Ovrebo 3594 (PMA, IBUG), *det.* L. Guzmán-Dávalos; SWITZERLAND, Geneva, 8 Jul 1989, *leg. et det,* O. Röllin 89-16 (IBUG) as *G.* cf. *peliolepis* (Speg.) Singer, *det.* as *G. peliolepis* in Guzmán-Dávalos *et al.* (2003), *det.* as *G. purpureosquamulosus* by L. Guzmán-Dávalos in this paper.

Notes on ***Gymnopilus peliolepis*** (Speg.) Singer, *Lilloa* **22**: 551, 1949 (1951).

= *Pholiota peliolepis* Speg., *Bol. Acad. Nac. Ciencias Córdoba* **23**: 394 (1918).

The type comprises two envelopes, each one with two specimens in regular condition but with moulds. Furthermore, there is an envelope with a spore print. A note in one of the envelopes reads: "Sombrero, escamas vinosas sobre el fondo blanco. Laminillas color



Figs. 17–21. *Gymnopilus purpureosquamulosus*: **17.** Basidiomata (Contu 2 Oct 2006). **18.** FESEM micrograph of basidiospores (Contu 25 Oct 2006, GDA 53540). **19.** Cheilocystidia (Contu 23 Oct 2006). **20.** Subhymenium ramose with inflated elements (Contu 26 Oct 2006). **21.** Caulocystidia (Contu 25 Oct 2006).

de paja (amarillo pálido), más tarde laminillas amarillo de manteca ... (anaranjado), ..." [Cap, vinaceous scales on white background. Short gills straw coloured (pale yellow), later, short gills butter yellow ... (orange), ...].

Observations from the type: Pileus approx. 18–30 mm as dried, 40 mm from the spore print, plane-convex, margin incurved to straight, surface pinkish-straw, with fibrillose squamules, reddish-brown to purple. – Lamellae ferruginous to dark ferruginous-brown. – Stipe stained reddish-black, apparently with a tomentose, whitish base; membranous ring thick, yellowish, leaves remains on the pileus margin. – Basidiospores from the spore print 6–8 × 4.4–5.2 µm, Q = 1.36–1.58 (N = 1, n = 20), ellipsoid, with obtuse apex, thick-walled, verrucose, warts medium, without germ pore, with plage, without suprahilar depression, yellowish-brown in KOH. – Basidiospores from the basidiomata 6.4–7.2 × 4–4.8 µm, Q = 1.33–1.8 (N = 1, n = 10), ellipsoid to oblong, with obtuse apex, thick-walled, verruculose, warts very small, almost asperulate, without germ pore, without plage or plage not distinguishable, without suprahilar depression, yellowish in KOH, dextrinoid, not metachromatic, ornamentation cyanophilic. – Basidia, pleurocystidia and cheilocystidia not observed. – Hymenophoral trama contaminated. Subhymenium not distinguishable. – Pileus trama interwoven, hyphae 1.6–13.6 µm wide, thin to somewhat thick-walled, hyaline or yellowish, septate, with clamp connections. – Pileipellis a cutis of prostrate hyphae, squamules a trichoderm of hyphae, 3.2–6.4 µm wide, hyaline to yellowish-brown. – Caulocystidia not observed. – Tromboplerous hyphae with yellow content. – Clamp connections at all septa.

Habitat. – Caespitose, on rotten branches and trunks in forests.

Distribution. – Type from Brazil; according to Singer (1951) and Hesler (1969) also in Argentina and Florida.

Material examined. – ***Gymnopilus peliolepis*** (Speg.) Singer: BRAZIL, Apiahy, Dec 1889, *leg.* J, Puiggari 87, *det.* Spegazzini (LPS-18270), type of *Pholiota peliolepis* Speg.

Results of sequence analysis

The alignment of the 25 ITS sequences consisted of 713 nucleotide positions after the introduction of gaps. Both ends of the sequences (1–53, 535–713) were excluded from the analysis being 481 characters included, from them 21 were parsimonious informative. Equally weighted parsimony analysis resulted in two trees, with a tree length of 53 steps. The consistency index (CI) excluding uninformative characters was 0.815, homoplasy index (HI) excluding

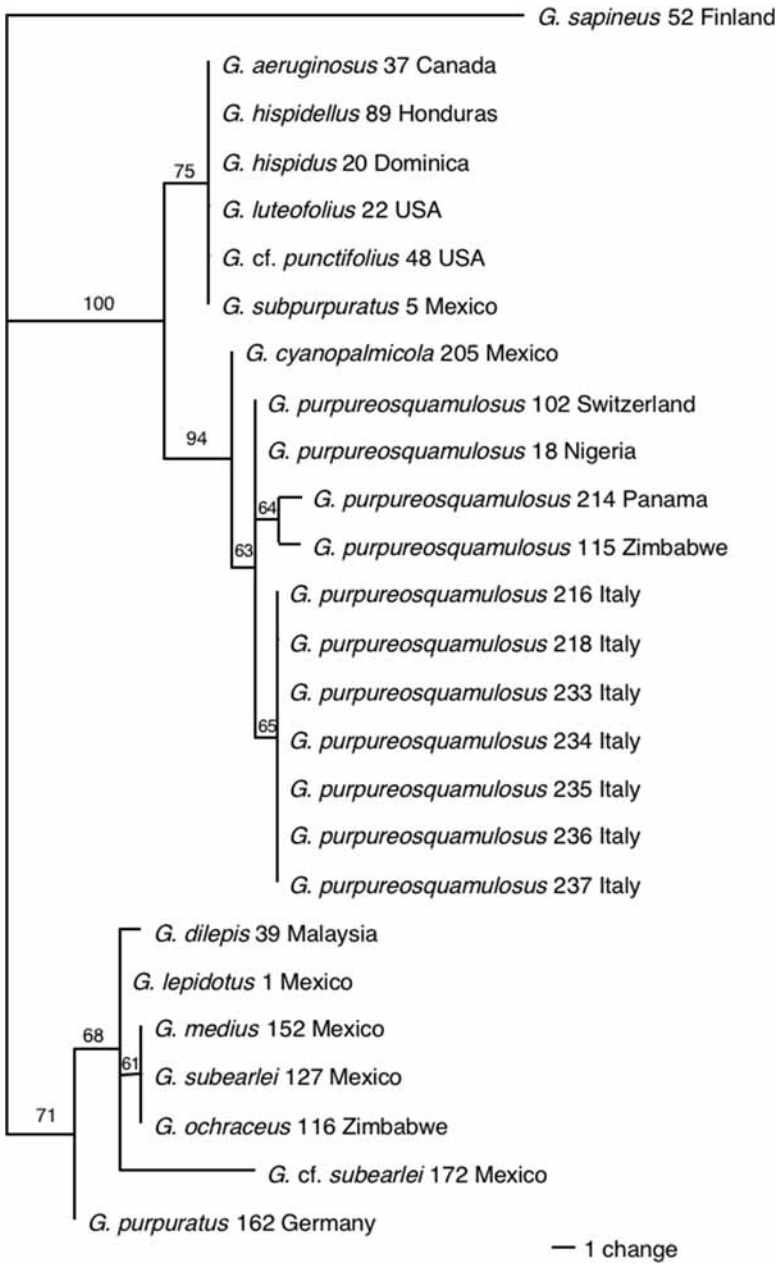


Fig. 22. Phylogram of one of two most-parsimonious trees with a length of 53 steps (CI excluding uninformative characters = 0.815, HI excluding uninformative characters = 0.185, RI = 0.961, RC = 0.871) of 24 ITS rDNA sequences of 15 species of *Gymnopilus* with purplish scaly pileus. *Gymnopilus sapineus* was used as outgroup. Bootstrap values > 50 % are given above each branch.

uninformative characters = 0.185, retention index (RI) = 0.961, and rescaled consistency index (RC) = 0.871. The phylogram in Fig. 22 has the same topology than the strict-consensus tree. Two large clades were obtained: (A) the *aeruginosus/luteofolius* clade with a bootstrap support of 100 %, and (B), the *lepidotus/subearlei* clade with a bootstrap of 71 %. Inside the first and larger clade, two groups with bootstrap support were found: (1) a clade with a bootstrap of 75 % which includes six species (*Gymnopilus aeruginosus*, *G. hispidellus*, *G. hispidus*, G. *luteofolius*, *G.* cf. *punctifolius* and *G. subpurpuratus*); (2) a clade with a bootstrap of 94 % with *G. cyanopalmicola* and *G. purpureosquamulosus*. The *lepidotus/subearlei* clade contains seven species: *G. dilepis*, *G. lepidotus*, *G. medius*, *G.ochraceus*, *G. purpuratus*, *G. subearlei* and *G.* cf. *subearlei*. The basal species of this clade is *G. purpuratus*.

Discussion

Høiland (1998) described *Gymnopilus purpureosquamulosus* based on a specimen collected in Zimbabwe, Africa. In this work we widen its distribution from a paleotropical to a tropical one, recording it again from Africa (Nigeria), for the first time from America (Panama) and from Europe (Italy and Switzerland), where it was probably introduced. The pileus of *G. purpureosquamulosus* was described with appressed squamules (Høiland 1998), but it also has erect to suberect squamules on the disk. The specimen from Nigeria was determined as *G. aculeatus* (Bres. & Roum.) Singer by Zoberi (indicated in the label at K). However, *G. aculeatus* has spores with small warts and a pileus trama with radially arranged hyphae (holotype, K-75166). Guzmán-Dávalos *et al*. (2003) considered the Nigerian specimen as *G.* cf. *palmicola* Murrill, but *G. palmicola* has larger spores (8–12 × 5.6–7.2 µm), with large to very large warts (Guzmán-Dávalos 2003).

The specimen from Switzerland was previously determined as *Gymnopilus* cf. *peliolepis* by Röllin (1998) and as *G. peliolepis* by Guzmán-Dávalos *et al*. (2003). Unfortunately, the type of *G. peliolepis* is in bad condition, so an adequate morphological description was not possible, nor were we able to obtain DNA for sequencing. The *G. peliolepis* type specimen presented two types of basidiospores: a) from the spore print, ellipsoid, with the typical ornamentation and colour of *Gymnopilus* basidiospores, and b) from the basidiomata, ellipsoid to oblong, lighter and with a very faint ornamentation, unusual in this genus. Singer (1951) found some material in Florida, USA, that he considered identical to the type; however, he described spores slightly bigger than those of the type (6.8–9 × 4.3–5.8 µm) and "distinctly rough and warty but warts sometimes con-

colorous with the episporium and not apparently different from the latter, often enveloped in a resinous chestnut brown incrustation, ellipsoid". On the other hand, Hesler (1969) described the spores as "6–8 (9) × 4–4.5 µm, ellipsoid, inequilateral, verruculose, ferruginous". So it is not possible to adequately interpret to which fungus the name *G. peliolepis* should be applied.

Rees *et al.* (2004) and Holec (2005) pointed out, that *Gymnopili* with purplish-scaly pilei are rare in Europe, and surely most of them were introduced from tropics and subtropics. They have a tendency to grow on warm or hot places (green houses or compost heaps of wood and bark remains), or in imported substrates [palm trees, Araceae (*Philodendron*), or ferns], or in Mediterranean regions of Italy and most likely of Spain. For instance, the type of *G. purpuratus* (Cooke & Massee) Singer was found on fern stems in a green house at Kew, England (Singer 1955). *Gymnopilus purpureosquamulosus* is distributed in the tropics and surely has been introduced to Europe. Very likely, European specimens with large basidiospores reported as *G. dilepis* or *G. purpuratus* might represent this species.

The material recently collected by Contu and Vizzini, corresponds to *Gymnopilus purpureosquamulosus* with minor differences: (i) KOH greenish in fresh basidiomata, (ii) the majority of the basidiospores oblong (Q = 1.6–1.8), with a faint plage and (iii) subhymenium ramose with some inflated or cellular elements. In the holotype and in specimens of *G. purpureosquamulosus* from Africa, Panama and Switzerland: (i) the KOH reaction is brown or unknown, (ii) most basidiospores are ellipsoid (Q = 1.4–1.6), without plage, and (iii) the subhymenium is cellular with some elongated elements. At the beginning we considered it as a new variety, but as the differences do not easily split the specimens in two groups, we prefer to keep all of them as *G. purpureosquamulosus*. A very closely related species is *G. cyanopalmicola* Guzm.-Dáv., which has oblong basidiospores without a plage and a cellular subhymenium with some elongate-inflated elements (Guzmán-Dávalos & Herrera 2006). More specimens of *G. cyanopalmicola* should be studied to clarify its position as an independent species or as a synonym under *G. purpureosquamulosus*. Bon & Roux (2002) described a Sicilian specimen as *G. luteofolius* (Peck) Singer, with spores 8–9.5 (11) × 5–6 µm, with plage, and with a green KOH reaction on the pileus that could represent *G. purpureosquamulosus*.

Many *Gymnopilus* members with purplish scales on the pileus stain greenish or bluish on the pileus or stipe surface, or, less frequently, in the context. But this feature might be overlooked when the basidiomata are not fresh enough or too humid, or because staining takes place a while after bruising. Høiland (1998) did not

mention any colour changes in the description of *Gymnopilus purpureosquamulosus*, and also not the reaction with 5 % KOH in fresh material (Høiland 1998); however, one of the two dry basidiomata from the type shows conspicuous olivaceous-blue/grey stainings. On the other hand, the reaction with KOH on the dry pileus of the type of *G. purpureosquamulosus* is orange or greyish-red, or greenish-black in previously stained zones. The Panamanian specimen was rain-soaked and no stainings were noticed by Ovrebo in fresh or dry basidiomata.

In the Italian specimens of *Gymnopilus purpureosquamulosus* the blueing of the flesh in the stem basis is occasional, since it has been observed only in three basidiomata (the collection of A. Vizzini AV G-1, and two collections of M. Contu: 26 Sep 2006 and 16 Apr 2007). This bruising reaction probably mirrors the presence of psilocybin compounds, as already demonstrated for *G. purpuratus* by Gartz (1989) and Gartz & Mueller (1990). Interestingly, *G. purpureosquamulosus* was collected in a similar place than the Mexican *G. cyanopalmicola:* on a palm (Arecaceae) in a restaurant (Guzmán-Dávalos & Herrera 2006). The Sardinian collections were made from a living trunk of *Phoenix canariensis* (Arecaceae), near the sea, also in a restaurant.

Discussion of molecular data

The variation of the ITS rDNA was not high enough to completely resolve the relationships among the studied species. They present very few changes in the nucleotide positions, and only 21 characters were parsimony informative. The same case has been described for *Antrodiella*, for which Johannesson *et al.* (2000) reported 34 informative characters for 12 taxa among 30 sequences with a sequence similarity ranging from 90.3 to 99 % excluding identical sequences. In this work, the sequence similarity ranged from 92.3 to 99.8 %. Rees *et al.* (2004) found in the *Gymnopilus* ITS sequences more variation, with 51 parsimony informative characters in 26 sequences and eight taxa. The difference between our results and those of Rees *et al.* (2004) is due to the inclusion of more samples without purplish-scaly pilei [*G. junonius* (Fr.) P.D. Orton group, *G allochrous nom. prov.* and *G. moabus* Grgur.] by Rees. Guzmán-Dávalos *et al.* (2003) analyzed 51 sequences from 32 taxa representing the whole variation of *Gymnopilus* and found 73 informative characters.

In the present study, we found the same two clades of *Gymnopilus* members with purplish pileal scales as described by Guzmán-Dávalos *et al.* (2003): the aeruginosus/luteofolius clade and lepidotus/subearlei clade. In the first clade there is a group formed by

G. aeruginosus, *G. luteofolius*, *G. subpurpuratus* and others with no differences in nucleotide positions among them, but clearly differing in morphology (Hesler 1969, Guzmán-Dávalos and Guzmán 1991). So, we have to assume that the high similarity of ITS sequences among them does not necessarily mean conspecifity. *Gymnopilus purpureosquamulosus* and *G. cyanopalmicola* are in the larger aeruginosus/luteofolius clade: they differ in only one nucleotide position (533).

Gymnopilus ochraceus was nested within the lepidotus/subearlei clade, another species described by Høiland from Zimbabwe (Høiland 1998), closely related to *G. medius* and *G. subearlei*. Basal to this clade, there is a specimen collected in Germany, determined as *G. purpuratus*, which is thought to have been introduced to Europe. Rees *et al.* (2004) speculated that this fungus is native to warmer climates, and might have been introduced from Chile in the late 19th century.

Finally, we conclude that *Gymnopilus* members with purplish-scaly pilei belong to a complex of many species, mainly with tropical distribution, which are grouped in two large clades. In this paper, we clarify the status of *G. cyanopalmicola, G. peliolepis* and *G. purpureosquamulosus* using morphological and molecular data. Similar studies should be conducted to elucidate the status of other species in the same complex.

Acknowledgments

We are grateful to DAOM, ENCB, F, H, IMI, K, L, O, WTU, and XAL curators for the loan of materials. Special thanks are given to Klaus Høiland for the loan of the type material of *Gymnopilus purpureosquamulosus*. Funds were obtained from CONACYT (project CONACYT-SEP-2003-C02-42957) and Universidad de Guadalajara (projects 62935, 72640, 62539, PIFI 3.1 & 3.3 UDG-CA-44). Miguel De Santiago is thanked for his valuable help in doing the ink drawings. Ovrebo thanks the Smithsonian Tropical Research Institute for permission to collect on Barro Colorado Island, and the Smithsonian Tropical Research Institute and Organization for Tropical Studies for funding.

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(Manuscript accepted 14 April 2008; Corresponding Editor: R. Pöder)