The Genus Strobiloscypha: A New Species And An Unresolved Phylogenetic Placement

Mycol. Monten. XVI (2013): 7-22

THE GENUS STROBILOSCYPHA: A NEW SPECIES AND AN UNRESOLVED PHYLOGENETIC PLACEMENT

Branislav Perić[1], Katherine F. LoBuglio[2], Donald H. Pfister[2]

[1]University of Montenegro. Faculty of Biotechnology.
81000 Podgorica, Mihaila Lalića 1, Montenegro.
branislav@mycolmonten.org
[2,]Department of Organismic and Evolutionary Biology,
Harvard University, Cambridge, Massachusetts 02138.
klobuglio@oeb.harvard.edu
dpfister@oeb.harvard.edu

Abstract

A new species of the genus *Strobiloscypha*, *S. cupressina*, is described from collections made in Montenegro. This species, like the other species in the genus, *S. keliae*, occurs on rotting leaves and cones of *Cupressaceae*. The genus had been assigned to the *Sarcosomataceae* but molecular phylogenetic analysis shows that it falls outside that family with no resolved placement elsewhere. Morphologically it is perhaps most close to the *Chorioactidaceae*.

Keywords : *Chorioactidaceae, Strobiloscypha, S. cupressina,* Taxonomy, Montenegro.

Introduction

The genus *Strobiloscypha* was described from material collected in Oregon, USA. A single species was included, *S. keliae*, and the genus has remained monotypic. Two collections of *S. keliae* were included both from the Peavy Arboretum near Corvallis; these were on damp decaying cones of *Chamaecyparis lawsoniana* and *Sequoiadendron gigantea*. So far as is known this species has not been collected again. Collections referable to the genus now have been discovered in the country of Montenegro. Apothecia were collected on decaying cones and branches of *Cupressus sempervirens*. These collections represent an undescribed species and allow us to further discuss the phylogenetic placement of the genus and the particular ecology and habitat of these rarely collected taxa. The genus was placed in the *Sarcosomataceae* because of its ascus structure and coloration of the ascoma. Previous phylogenetic studies have placed *S. keliae* outside the core group of the *Sarcosomataceae* in an unresolved position but in relationship to the *Sarcosomataceae*, *Chorioactidaceae*, *Sarcoscyphaceae* and the *Pyronemataceae* (Harrington et al., 1999; Perry et al., 2007 and Pfister et al., 2008).

Materials and methods

Materials examined

The majority of collections were examined in the fresh, living state in tap water (see Baral, 1992), using a Leica DMLS microscope. The sections and other materials for microscopic observations were made in distilled water, lactic cotton blue, Congo red, and 3% KOH .The iodine reaction was tested with Melzer's Reagent, without potassium hydroxide (~3–5% KOH) pre-treatment. Air-dried material was examined in H_2O, or in 5% KOH to which Congo Red (CR, ~1% in H_2O) was later added. All measurements and all the drawings were obtained from preparations made in distilled water, measurements from fresh material are indicated by the symbol * measurements from dry material by the symbol †. The statistical values were based on measurement of 30 ascospores from multiple collections. The space occupied by ascospores in living asci is defined by the term *pars sporifera*. Macrophotos were obtained using a analog camera Nikon FM3 and digital camera Canon 7D while; microphotos were obtained using a Leica DC 300. All drawings were done by hand. Type material is deposited in the Farlow Herbarium (FH), Harvard University. Isotype is deposited in the Herbarium of Biotechnical Faculty of the University of Montenegro in Podgorica.

Pure cultures of were established from fragments of ascocarp (apothecium) on modified Melin-Norkrans (MMN) medium (Marx, 1969) amended with 50 ppm of Streptomycin (Galenika, Serbia) and ampicilin (Panfarma, Serbia) and 5ppm Benomyl (Zorka, Serbia). The culture was transferred, maintained and grown on MMN medium.

DNA extraction and sequence analyses

The Qiagen DNeasy Plant Mini Kit (Qiagen, Germany; cat. no. 69104) was used to extract genomic DNA from the Montenegro specimen (Dgf/C7D-19-12-12) of *Strobiloscypha* collected by Branislav Perić. A 1/10 and 1/100 dilution of the DNA was used for PCR amplification of the SSU and LSU rDNA (small subunit and large subunit ribosomal DNA) regions, and the RPB2 gene (DNA-dependent RNA polymerase II) between conserved motif 6 and 11 (Liu et al. 1999). The SSU was amplified using the primers NS1, NS2, NS4, NS8 (White et al., 1990) and SL1, SL122, and SL344 (Landvik et al., 1996) primers. Amplification of the LSU rDNA region utilized the primers LROR and LR5 (Moncalvo et al., 2000). The 6-11 region of the RPB2 gene utilized the primers RPB2-P6Fa, RPB2-P7Ra, RPB2-Pb7F, and RPB2-P11aR as designed by Hansen et al. (2005). PCR parameters were as previously described (Hansen et al., 2005). All PCR reactions were done in a Peltier Thermal cycler PTC–200 (MJ Research, Watertown, MA). The SSU and LSU PCR reactions used EconoTaq DNA Polymerase (Lucigen, Middleton, WI) whereas the RPB2 6-11 region was amplified using Platinum Taq DNA polymerase (Invitrogen, Life technologies, Carlsbad, California, USA).

PCR purification and sequencing techniques were as described in Hansen et al. (2005). Sequencher 4.6 (GeneCodes, Ann Arbor, Michigan) was used to edit the DNA sequences obtained. The DNA sequences determined in this study for the Montenegro isolate (GenBank Number KJ812103, KJ812104, and KJ812105) were aligned manually

with DNA sequences from the Treebase alignment S1946 (Pfister et al., 2008). Using Se-Al v 2.0a8 (Rambaut, 1996).

DNA sequence alignments were analyzed using Maximum Parsimony, PAUP 4.0b10 (MP; Swofford, 2002) and Maximum-Likelihood with RAxML–HPC2 on Abe through the Cipres Science Gateway (ML; Miller et al., 2009). Branch support for MP and ML analyses was determined by 1000 bootstrap replicates. The outgroup species was *Neolecta vitellina*.

Fig. 1. *Strobiloscypha cupressina*: apothecia on *Cupressus* cones (PG, Masline, 16.12.2012). Right on the other cones *Resupinatus alboniger* (Pat.) Singer, (photo: B. Perić)

RESULTS

Taxonomy

Strobiloscypha cupressina Perić & Pfister sp. nov.

MycoBank MB 809574

Diagnosis :

This species is similar to S. keliae in the morphology of the sterile tissues of the ascomata, the pigmentation of the hymenium and hairs; both species have brown pigments that encrust the cells of the outer excipulum. The species differ in that S. cupressina has ascospores that are smaller than those of S. keliae, are more acute at the poles, have smooth walls and contain discrete lipid bodies. Both species occur on fallen twigs and cones of members of the Cupressaceae.

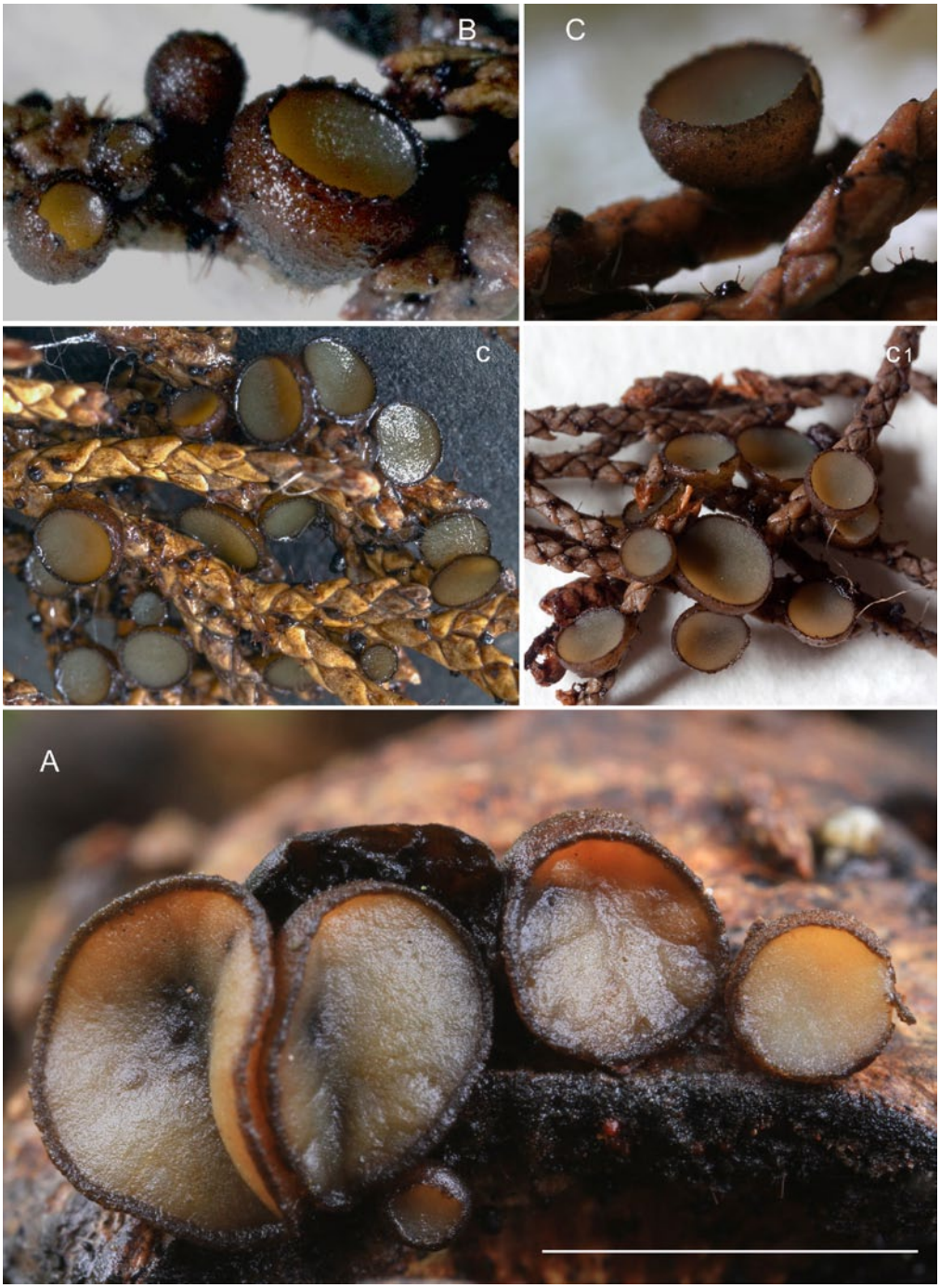

Fig. 2. *Strobiloscypha cupressina* (photo B. Perić)*:*
A – apothecia on *Cupressus* twig (PG, Masline, 16.12.2012); **B** – apothecia on *Cupressus* twig (PG, Gorica, 18.12.2012); **C, c-c$_1$** – apothecia on *Cupressus* twigs (PG, Gorica, 19.12.2012). Scale bar = 5 mm

Habitat: on the cones and tips of rotting one-year-old branches of *Cupressus sempervirens* L.

Holotipus: is deposited in the Farlow Herbarium (FH), Harvard University (FH 00377333). Isotipus is deposited in the Herbarium of Biotechnical Faculty of the University of Montenegro in Podgorica.

Etymology: from *Cupressus* on which occurs.

Description

Apothecia 1-5 mm in diam., sessile, hemispherical, with a circular opening at in the distal portion, expanding during development, thus creating a ± deepened disk, like a shallow bowl, margins raised. Hymenium smooth, finely granulated, shiny and moist, gray whitish with olive or light brownish reflex, depending on the humidity and age. Hymenium of dried apothecia golden yellow, ectal excipulum brown to purple brown. Margin circular, rarely slightly undulate, (when growing close together) raised, at first finely toothed, than granulated, of dark brown color. The outer surface finely granulated, shiny, brownish-red, finely decorated with brown granules like those on the edge.

Asci *233–315 × 11–17.6 µm, †230–260 × 12–13 µm, operculate, octosporous, hyaline, cylindrical, apices straight, obtuse rounded, rarely slightly curved, mainly before and after discharged, with operculum laterally positioned, J-, cell wall 1–1,5 µm, *pars sporifera* *95.5–106 µm (spores uniseriate), †145–190 µm, base narrowed, aporhyncous type (without croziers), ascoplasm of live mature asci transparent, in some places finely granulated, becomes red brown in Melzer's reagent.

Mature ascospores *15,2–20 × 9,6–12 µm, X = 18 × 11,4, Q =1,6; † 14,7– 18,7 × 8,7–11,2 µm, X=16,8 × 10, Q =1,7; broadly ellipsoid or subfusiform with rounded or subacute at poles, on occasion one pole sharper than the other, which can be rounded and blunt, hyaline or light yellowish, thin-walled 0,5–0,8 µm, cyanophilic, containing 1–3 larger oil drops (Lbs) *2,5–4 µm in diameter, variously positioned from the center toward the poles, the area around them filled with tiny droplets, with a tendency to fuse over time, in the dead ascospores †4,5–6 µm in diam., some ascospores collapse in lactic cotton blue, occasionally with one longitudinal or transverse depression 7–10 µm long. Germination was observed in nutrient medium (PDA, MMN) after 8 days. Germ tubes 1,5–4,5 µm in diam., occur usually at the poles and develops into bifurcating hyphae 3–4,5 µm in diam. Ascospores in asci and free ascospores germinate. Conidia obtained from the culture cylindrical, hyaline to pale yellowish, measuring 6–7,2 × 1,8–2,5 µm.

Paraphyses *2,5–4 µm, straight, slightly enlarged at the apices 5–7,5 µm, bifurcate base, some with bifurcate apices too, anastomozed, especially in the basal part, densely septate, containing fine-granulated hyaline protoplasm which is cyanophilic, but not reacting in the iodine preparations, terminal cells *56–110 × 4,5–7 µm, lower cells *37–75 × 3–4 µm.

Subhymenium 40-70 µm, hardly distinct from the medullary excipulum, of *textura intricata* composed of elongated, hyphoid, hyaline and septate cells, some of them bifurcate, *5–37 × 3–5,2 µm. Ascogenous hyphae often with bud extensions or branched.

Medullary excipulum 315-726 µm thick, with perpendicular orientation to the hymenium, of *textura intricata*, individual cells *13,7–93 × (3–) 4,3–10,3 µm, hyaline, hyphoid, straight or curved, elongate, septate, slightly constricting towards septae, often

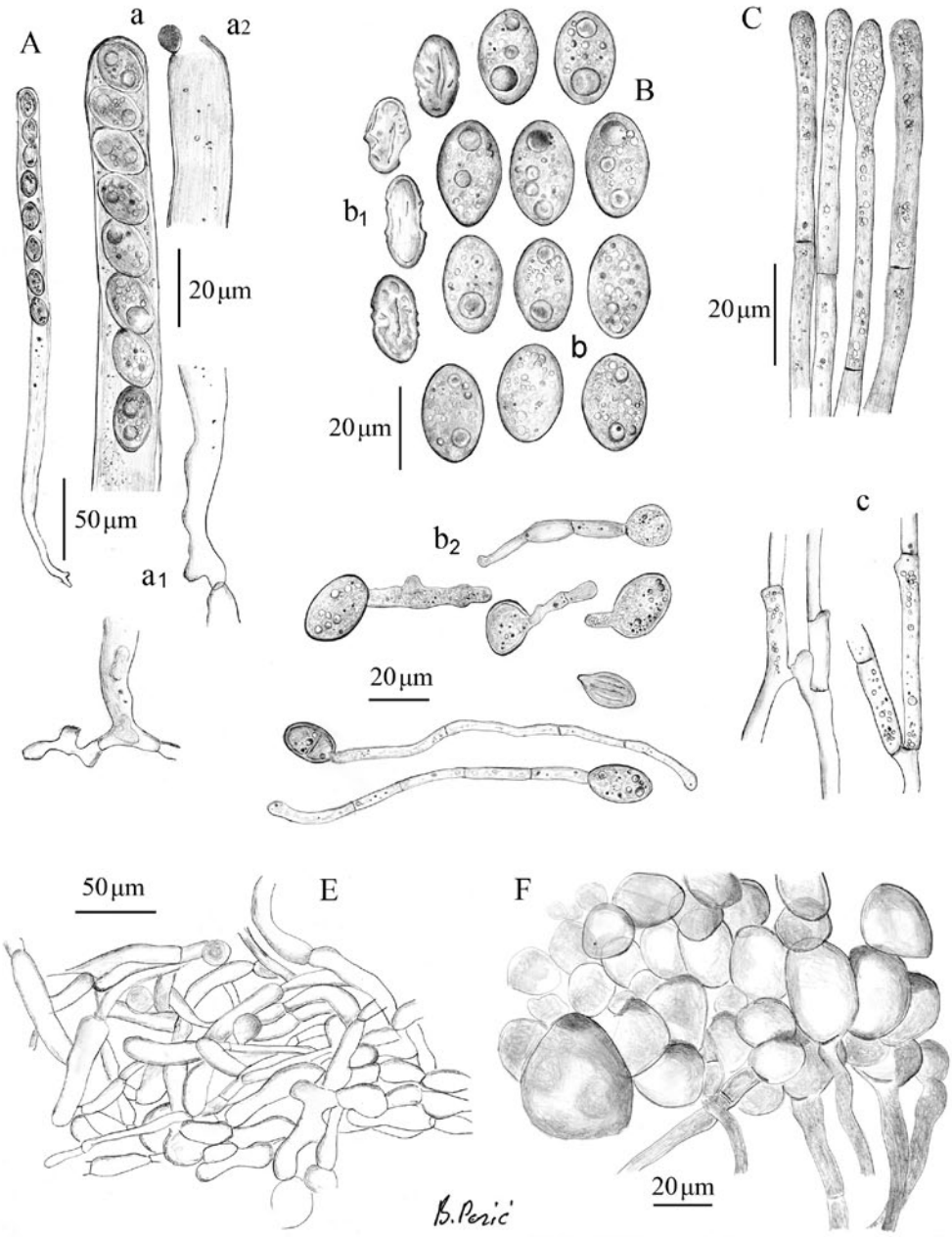

Fig. 3. *Strobiloscypha cupressina*:
A – ascus, **a** – ascus apex and *pars sporifera,* $\mathbf{a_1}$ – ascus base, $\mathbf{a_2}$ – operculum;
B – †ascospores from the paper, **b** – * ascospores, $\mathbf{b_1}$ – † collpased ascospores, $\mathbf{b_2}$ – germinated ascospores; **C** – paraphyses in water, **c** – bifurcate and anastomosate paraphyses;
E – medullary excipulum; **F** – ectal excipulum

branched, those near ectal excipulum more tortuous and forked, cell wall 0,5–1 µm.

Ectal excipulum 48–109 µm wide, with a parallel orientation to the hymenium, of *textura globulosa* to *globulosa-angularis*, composed of two layers: along medullary excipulum of subspherical, spherical, ovate or irregularly elongated cells, hyaline, thin-walled (0,5–1 µm), *12–40 µm in diam., while toward the outside mainly spherical cells, with thicker cell walls (1–1,8 µm), *8–18 µm in diam., hyaline, intercellular spaces with brown deposits of irregular shape.

Marginal ectal excipulum of *textura globulosa*, with 10–25° orientation to the hymenium, consists of predominantly spherical cells but also of oval and irregularly ovoid cells, *6–46 µm in diam., of light brown, coated with dense, extracellular layers of brown pigment; the cells to the outside lose spheroidal becoming increasingly irregular, elongate, oval, then spreading to *textura angularis*, the outer cells of the margin, cylindrical, of *textura porrecta*, *14,5–52 × 5–10 µm, cell wall 1–1,5 µm thick, with a thick deposits of extracellular brown pigment, 1–3,5 µm in diam., from 1–3,5 µm.

Hairs arise from spherical cells of ectal excipulum, light yellowish-brownish, 50–600 µm long or more, 5,5–12 µm wide, denser and longer in the base of apothecia; toward the margin much shorter and lighter, sometimes hyaline also; septated, some slightly twisted, some branchat at the base, the apices rounded and blunt; cell walls 1–2 µm.

Anamorph *Verticicladium*-like.

Habitat

On the cones and tips of rotting one-year-old branches of *Cupressus sempervirens*.

Note

The appearance of this small discomycete is usually related to long moist periods with heavy rain. Interestingly, the snow that fell in Podgorica in November 2012, and stayed ten days, did not disrupt, but even facilitated, the development of this small fungus. At the same time, in the same habitats, we found *Pseudopithyella minuscula* (Boud. & Torrend) Seaver, *Pithya vulgaris* Fuckel and *Kotlabaea benkertii* Perić. All of these are associates of *Cupressaceae* and were in a layer of *Pinus halepensis* needles mixed with tiny twigs of *C. sempervirens*.

Collections examined

Montenegro, Podgorica, Forest Park Gorica, altitude 97 m, 42°27'01"N, 19°16'13"E on the tips of rotten branches of *C. sempervirens* in the mixed association of *P. halepensis* and *C. sempervirens.* Leg. B. Perić, 19.12.2012. Exsicc. Dgf/C7D-19-12-12. Holotipus is deposited in the Farlow Herbarium (FH), Harvard University (FH 00377333). Isotipus is deposited in the Herbarium of Biotechnical Faculty of the University of Montenegro in Podgorica.

Podgorica, Forest park on the hill Ljubović, altitude 94 m, on the peaks of *Cupressus sempervirens* branches in artificial forest of *Pinus halepensis* and *C. sempervirens*. Leg. B. Perić 12.0.1998. Exsicc. Ef-2(36a).

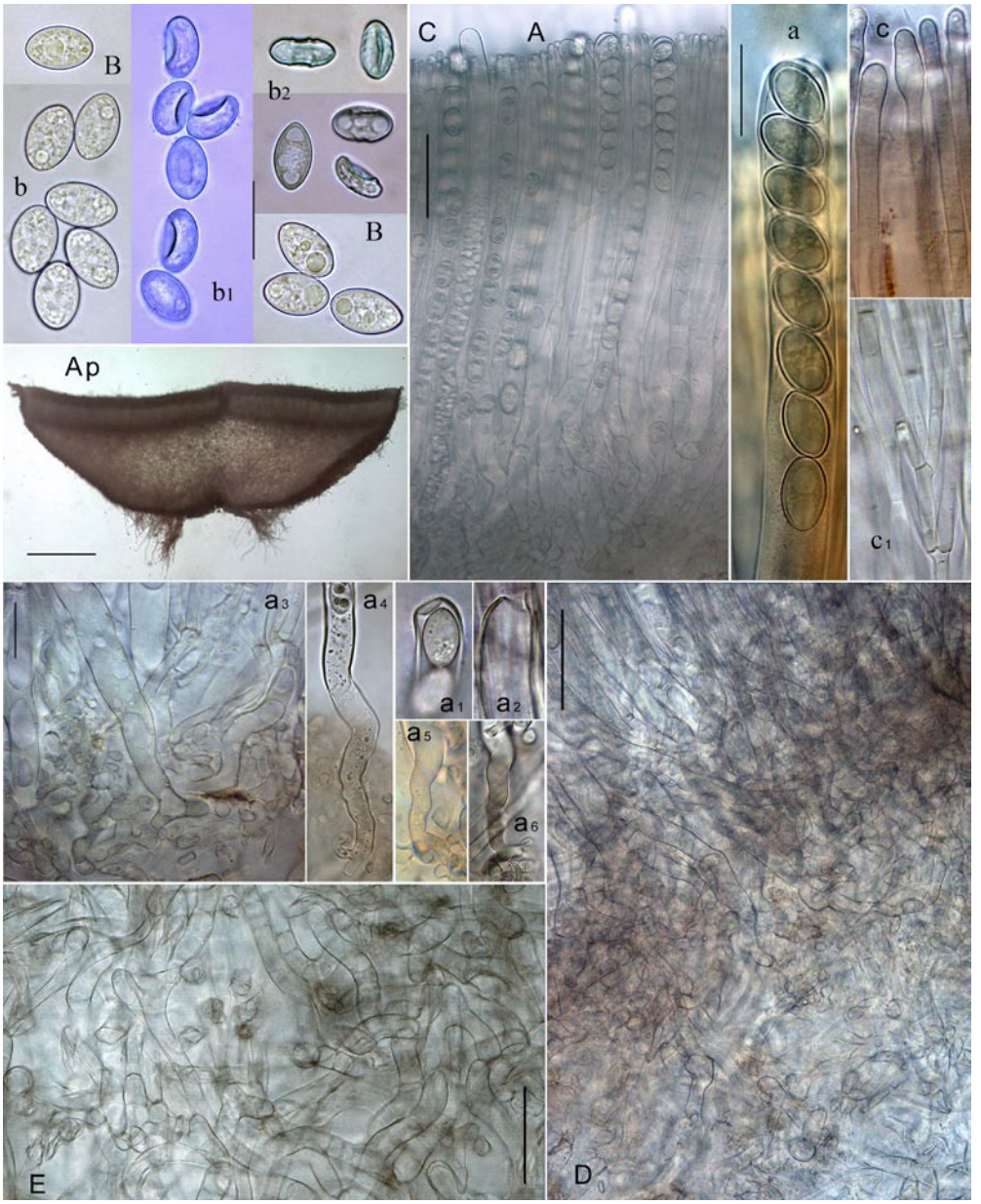

Fig. 4. *Strobiloscypha cupressina* (photo B. Perić):
Ap – apothecium in median section, at the base in the middle is where apothecium was bound to the substrate; **A** – asci and paraphyses in water, **a** – ascus apex J- and *pars sporifera,* in Melzer's Reagent, $\mathbf{a}_1$ – ascus apex before discharged, $\mathbf{a}_2$ – ascus apex after dehiscence operculum, $\mathbf{a}_3$ – ascus base with ascogenous hyphae in water, $\mathbf{a}_4$ – ascus base without croziers in water, $\mathbf{a}_5$ – ascus base in Melzer's Reagent, $\mathbf{a}_6$ – ascus base in water; **B** – †ascospores from spore deposit in water, **b** – *discharged ascospores in water, $\mathbf{b}_1$–ascospores in lactic cotton blue, $\mathbf{b}_2$ – †collapsed ascospores from the paper in lactic cotton blue; **C** – paraphyses in water, **c** – paraphyses apices in water, magnified, $\mathbf{c}_1$ – bifurcate and septate paraphyses in water; **D** – subhymenium in water; **E** - medullary excipulum. Scale bars: **Ap** 300 µm; **A, D, E** =50 µm; **a, $\mathbf{a}_3$, B, b-$\mathbf{b}_2$** = 20 µm.

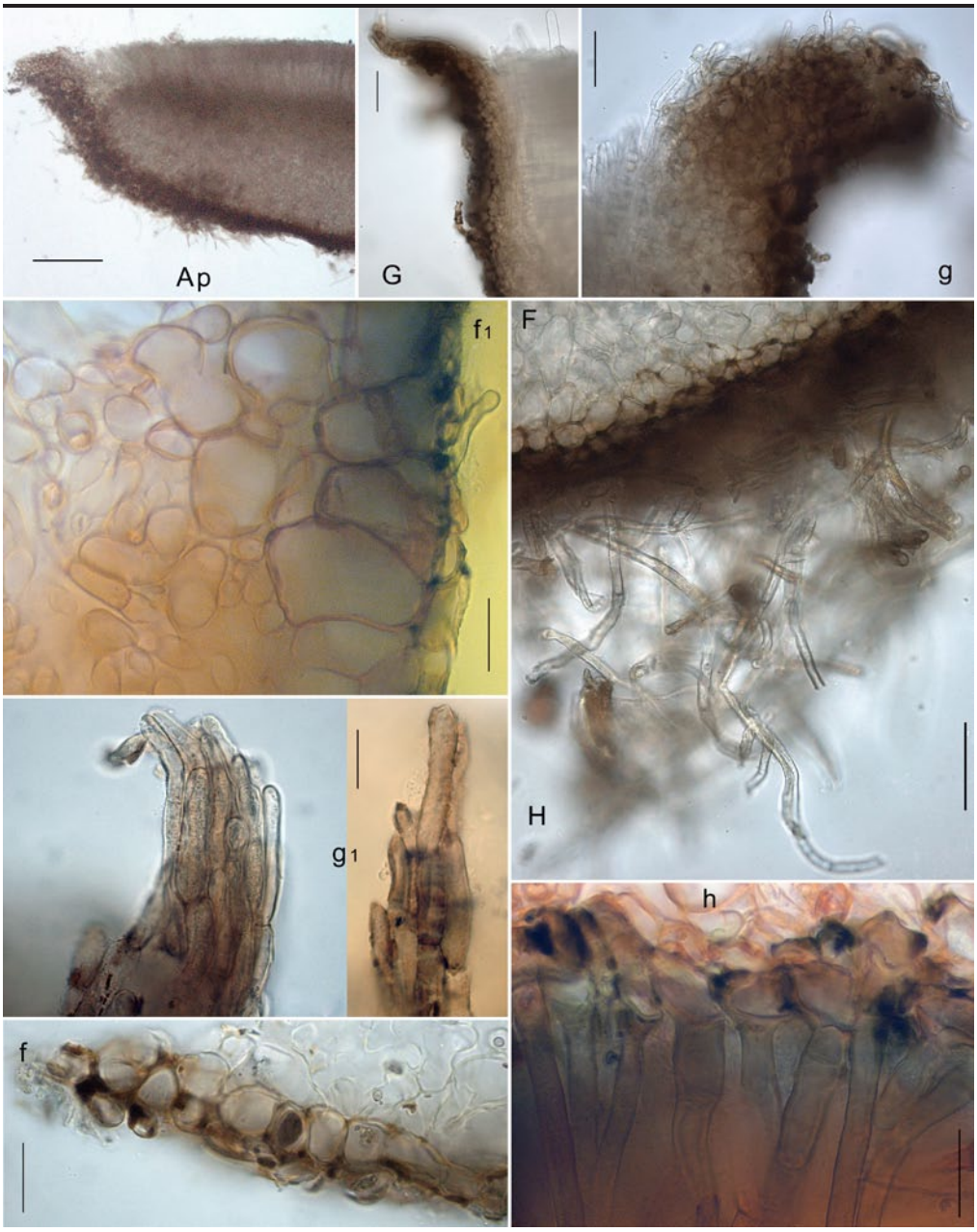

Fig. 5. *Strobiloscypha cupressina* (photo B. Perić):
Ap – part of apothecium in median section; **F** – ectal excipulum cells in water,
f – magnified, in 3% KOH, $\mathbf{f_1}$ – marginal ectal excipulum cells in Congo red;
G – cells of apothecium in water, **g** – magnified, $\mathbf{g_1}$ – terminal cells of margin of apothecium;
H – hairs of apothecium base, **h** – hairs of apothecium base arising from ectal excipulum.
Scale bars: **Ap** = 300 μm; **H, G, g** = 50 μm; **f, $\mathbf{f_1}$, $\mathbf{g_1}$, h** = 20 μm

Fig. 6. *Strobiloscypha cupressina* (photo B. Perić): pure culture

- Podgorica, Forest Park Gorica, on cones of *C. sempervirens* in artificial forest of *P. halepensis* and *C. sempervirens,* Leg. B. Perić & Olgica Perić, 12.11.2000. Exsicc. Hf=28(27-31), Hf=29(21-23, 25-28).

- ***Idem***, on the tips of *C. sempervirens* branches. Leg. B. Perić, 07.12.2003. Exsicc. Kf-22 (14-23).

- Podgorica, Masline, in Perić`s family garden, altitude 50 m, on the cones of *C. sempervirens.* Leg. B. Perić, 16.12.2012. Exsicc. Dgf/C7D-18-12-12.

- Podgorica, Forest Park Gorica, altitude 120 m, on the tips of rotten branches of *Cupressus sempervirens* in mixed association of *P. halepensis* and *C. sempervirens.* Leg. B. Perić & O. Perić, 18.12.2012. Exsicc. Dgf/C7D-18-12-12.

DISCUSSION

With analysis of these recent collections in living state and through DNA sequence analysis we were able to demonstrate that there is a second species in the rare or elusive genus. *Strobiloscypha keliae* and *S. cupressina* share characters that place them in the same genus. Their apothecia are similar in form, size and color; especially the hymenial color both in fresh and dry specimens. *Strobiloscypha keliae* develops on the cones of *Chamaecyparis* and *Sequoiadendron*, while *S. cupressina* often develops on tips of rotting branches of *Cupressus sempervirens* and rarely on cones. The hosts for both species are members of the *Cupressaceae*. Ascospores of both species are elliptical but those of *S. keliae* are larger, are

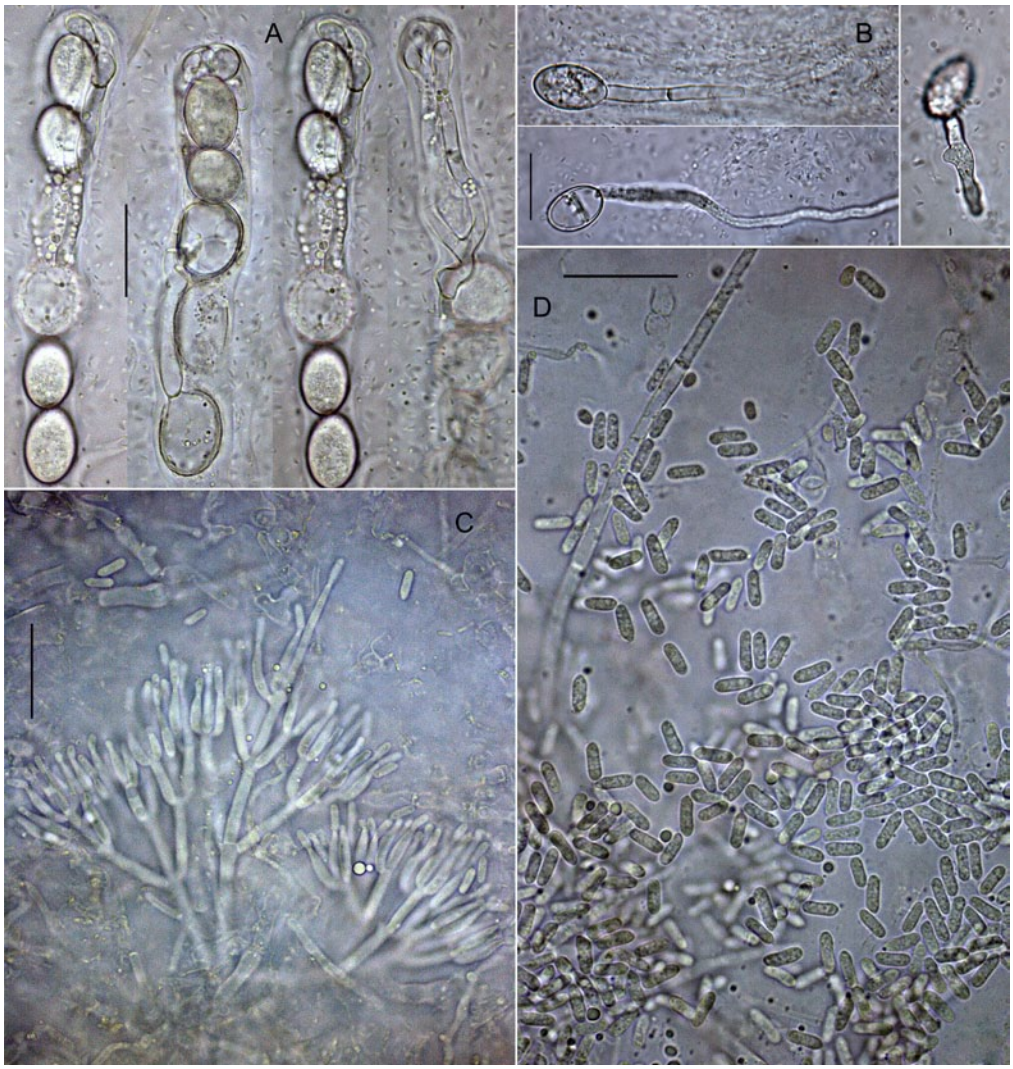

Fig. 7. *Strobiloscypha cupressina* (photo B. Perić):
A – ascospores germination within asci in water; **B** – discharged germinated ascospores in water; C – conidiophore of *Verticicladium*-like anamorph in water; **D** – conidia in lactic cotton blue. Scale bars = 20 μm

finely ornamented and lack larger oil guttules. Ascospores of *S. cupressina* are usually less blunt at the poles, often appearing to be nearly subfusiform, with smooth surface and contain larger oil droplets, which in dead ascospores are united and enlarged. Ascospore contents are described by Weber and Denison (1995) as "refractive granular contents but no distinct large guttules at maturity." Asci of these two species are similar in the upper and the basal part, but in *S. cupressina* they are somewhat smaller. Ascus bases are aporhyncous and therefore without croziers, with well separated ends arise from ascogenous hyphae. The two species are also similar in the structure of the subhymenium, medullary and ectal layer. In both species the outer two layers are not distinctly differentiated. In the ectal layer

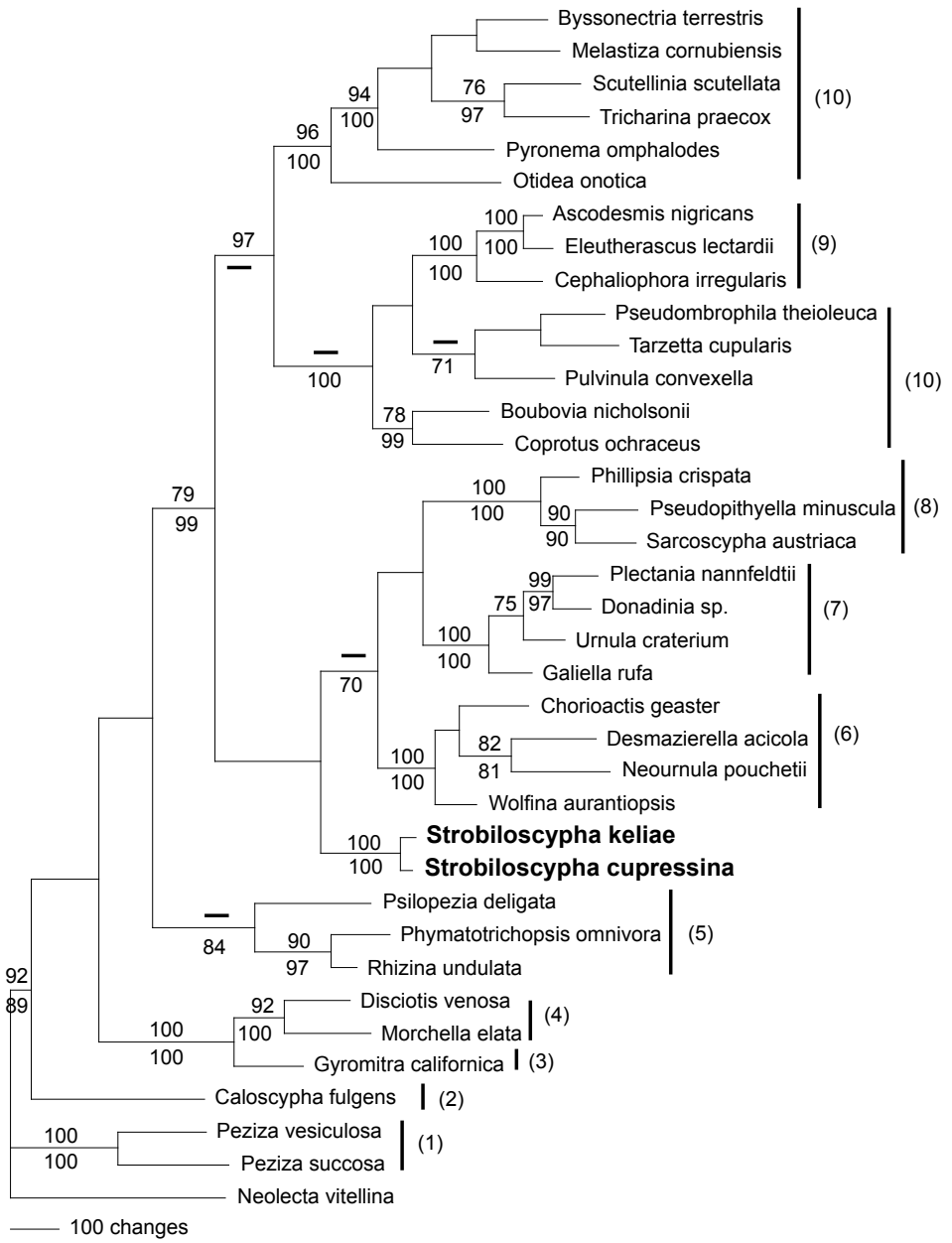

Fig. 8. Phylogenetic placement of *Strobiloscypha cupressina* among the Pezizales: (1) *Pezizaceae*; (2) *Caloscyphaceae*; (3) *Discinaceae* (4) *Morchellaceae*; (5) *Rhizinaceae*; (6) *Chorioactidaceae*; (7) *Sarcosomataceae*; (8) *Sarcoscyphaceae*; (9) *Ascodesmidaceae*; (10) *Pyronemataceae*. Phylogeny presented is the most parsimonious tree from combined SSU rDNA, LSU rDNA, and RPB2 sequence data with *Neolecta vitellina* as the outgroup species. Maximum Parsimony and Maximum Likelihood bootstrap values (above 70%) are above and below the branches respectively. Dashes represent bootstrap values below 70%.

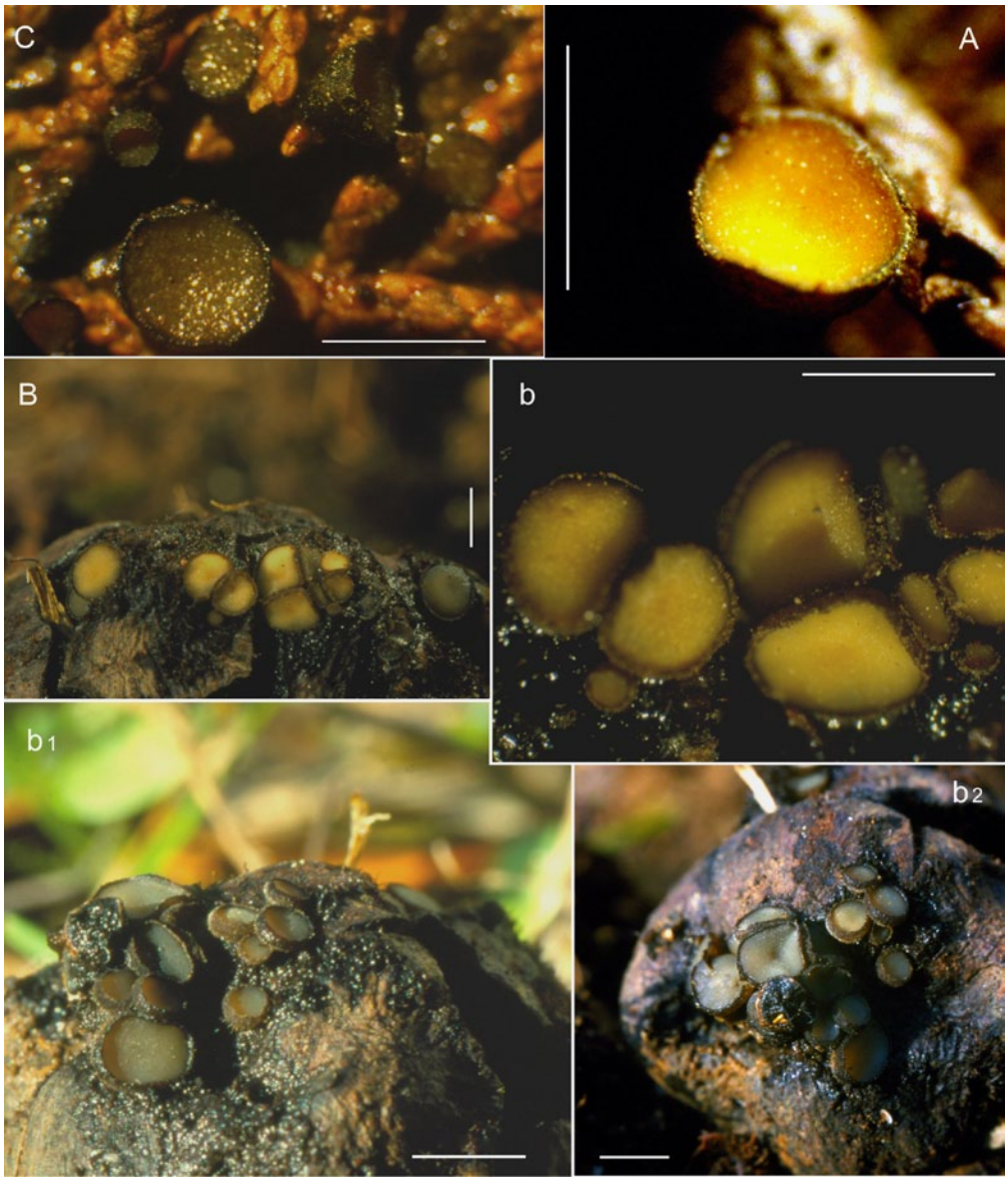

Fig. 9. *Strobiloscypha cupressina*:
A – apothecium on *Cupressus* twigs (PG, Ljubović 1998), **B, b-b$_2$** – apothecia on *Cupressus* cones (PG, Gorica 2000), **C** – apothecia on *Cupressus* twigs (PG, Gorica, 2003). Magnified on **A, C** and **b.** Scale bar = 2,5 mm

the intercellular spaces of both species have brown pigmented deposits that are clearly visible. These deposites that are seen the best with 3% KOH (2.5%. KOH by Weber & Denison, 1995).

Fig. 10. *Strobiloscypha cupressina*:
A – live specimens on *Cupressus* twigs (PG, Gorica 19-XII-2012);
B – dried specimen of the same sample, **b-b$_2$** – magnified

The host plants, *Cupressaceae*, of both species are members of the subfamily *Cupressoideae*. These represent living representatives of the Laurasian continent. The taxa in the *Cupressoideae* have experienced extinction and range reduction, particularly since the last ice age (Mao et al., 2012) These two species of *Strobiloscypha* perhaps also reflect a reduction in range similar to that experienced by the host family. Conifers in the northern hemisphere show a higher rate of speciation and extinction that may reflect shifts in climatic cycles over the Neogene (Leslie et al., 2012). Whatever the case these disjuntions are noteworthy both in regard to the hosts themselves but also in regard to the fungi that occur on them.

The broadly disjunct distributions of these species is noteworthy but not unusual. In the *Chorioactidaceae Neournula pouchetii* is reported from Western North America and North Africa, France, Spain and Italy. *Chorioactis geaster* is known from Texas, USA

and Japan (Pfister et al. 2008). Such distributions might be dismissed as artifacts of collecting practices or they may also be the result of human transport of plants and spores as is surely the case of *Neournula pouchetii* in France and of the reports of *Desmazierella acicola* in North America (Pfister et al. 2008) but molecular evidence suggests in the case of *Chorioactis geaster* that distantly disjunt populations are reflective of real longterm separations (Peterson et al. 2004).

Placement of the genus has not been resolved in our molecular phylogenetic analysis. On morphological grounds there are similarities with the *Chorioactidaceae*. Ascus bases are similar to some of those found in the *Chorioactidaceae* and the encrustation of walls of the excipulum is similar to that observed in that families. Cytological work might provide some further details that would aid in placement. In the *Chorioactidaceae* the ascospores are multinucleate as are the cells of the paraphyses. In the *Sarcosomataceae* ascospores are multinucleate but the cells of the paraphyses are uninucleate. As is the case in the *Chorioactidaceae*, species of *Strobiloscypha* lack gelatinous tissues in the apothecia that is the case in *Sarcosomataceae* where all species have gel to a greater or lesser degree. We have been successful in growing *S. cupressina* in culture. The asexual state resembles *Verticicladium* and is similar to those states found in in some of the *Chorioactidaceae*. Pending further study we tentatively place *Strobiloscypha* in the *Chorioactidaceae*.

Acknowledgement

We express our gratitude to Jelena Lazarević for help in making the culture of the fungi.

Literature Cited

Baral H.-O., 1992. – Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* 44 : 333-390.

Hansen K., LoBuglio K.F. & Pfister D.H., 2005. – Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, b-tublin, and LSU rDNA. *Molecular Phylogenetics and Evolution* 36: 1-23.

Harrington F.A., Pfister D.H., Potter D., & Donoghue M.J., 1999. – Phylogenetic studies within the Pezizales. I. 18S rRNA sequence data and classification. *Mycologia* 91: 41-50.

Landvik S., 1996. – *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA sequences. *Mycological Research* 100: 199–202.

Leslie A.B., Beaulieu J.M., Ral H.S., Crane P.R., Donoghue M.J. & Mathews S., 2012. – Hemisphere-scale differences in confier evolutionary dynamics. PNAS 109(40): 16217-16221.

Liu Y., Whelen S. & Hall B., 1999. – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.

Mao K., Milne R.I., Zhang R. I., Peng Y., Lie J., Thomas P., Mill R.R & Renner SS., 2012. – Distribution of living *Cupressaceae* reflects the breakup of Pangea. PNAS 109: 7793-7798.

Marx D.H., 1969 –The influence of ectotrophic mycorrhyzal fungi on the resistance on pine roots to patagonic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopatology* 59: 159-163.

Miller M.A., Holder M.T., Vos R., Midford P.E., Liebowitz T., Chan L., Hoover P. & Warnow T. – The CIPRES Portals. CIPRES. URL: http: //www.phylo.org/sub_sections/portal. 2009-08-04. (Archived by WebCite(r) at http: //www.webcitation.org/5imQlJeQa).

Moncalvo J.-M., Lutzoni F.M., Rehner S.A., Johnson J. & Vilgalys R., 2000. – Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* 49: 278–305.

Perry B. A., Hansen K. & Pfister D.H., 2007. – A phylogenetic overview of the family *Pyronemataceae* (Ascomycota, Pezizales). *Mycological Research* 111: 549-571.

Peterson K.R., Bell C.D., Kurogi S. & Pfister D.H., 2004. – Phylogeny and biogeography of *Chorioactis geaster* (Pezizales, Ascomycota) inferred from nuclear ribosomal DNA sequences. *Harvard Papers in Botany* 8 (2): 141–52.

Pfister D.H., Slater C. & Hansen K., 2008. – *Chorioactidaceae*: a new family in the Pezizales (Ascomycota) with four genera. *Mycological Research* 112: 513-527.

Rambaut A., 2002. – Se-Al v2.0a11. University of Oxford, UK. http://tree.bio.ed.ac.uk/software/seal/.

Swofford D.L., 2002. – PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland.

Weber N.S. & Denison W.C., 1995. – Western American Pezizales. *Strobiloscypha keliae* gen. and sp. nov. (Pezizales, *Sarcosomataceae*) from Oregon. *Mycotaxon* 54: 129-135.

White T., Bruns J.T., S. Lee & Taylor J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in PCR Protocols: A Guide to Methods and Applications, eds. M.A. Innis, D.H. Gelfard, J.J. Sninsky and T.J. White, eds. San Diego: Academic Press.