Resurrection of Boudier's generic name *Urceolella* for *Excipula aspera* Moug. ex Fr. (*Helotiales*)

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Abstract: The author proposes a reconsideration of the placement of *Excipula aspera* Moug. ex Fr., a *Osmunda regalis* inhabiting species, in the genera *Urceolella* Boud. and *Olla* Velen. based on its hair chemistry and genetics.
Keywords: Ascomycota, hair chemistry, *Hyaloscyphaceae*, nomenclature, *Olla*, *Osmunda*, taxonomy.

Introduction

Based on molecular and micro-chemical data, the genus *Urceolella* Boud. is showed to be used for *Excipula aspera* Moug. ex Fr. (Fries, 1823) over *Olla* Velen., after over 100 years. Although the current name of this species is, according to Index Fungorum and MycoBank, still *Unguicularia aspera* (Fr.) Nannf. over the more recent placement in *Olla* by Raitviir (2004).

Material and methods

The ITS dataset was created in MEGA version 7 (Kumar *et al.*, 2016), using GenBank and newly generated sequences (Table 1) derived from strains obtained from fresh fruitbodies by myself and colleagues (sequencing made by Alvalab, Spain). Alignment was done with Mafft online (Katoh *et al.*, 2013). The final Maximum Likelihood tree was obtained with MEGA X (Kumar *et al.*, 2018), using the GTR + G model, determined with its own model search option, with 1000 bootstrap replications. Gaps are treated as missing data. The tree was rooted with *Hymenoscyphus fructigenus* (Bull.) Gray and *Cyathicula cyathoidea* (Bull.) Thüm. The collapsed *Helotiales* ITS tree was conducted from a dataset of 318 sequences aligned by Mafft using the FFT-NS-i method and conversed in the Phylip format by the online ALTER program (Glez-Peña *et al.*, 2010). From this dataset with 1138 sites a tree is calculated by PhyML online (Guindon *et al.*, 2010) using the GTR + G + I method chosen by the build-in Smart Model Selection program (Lefort *et al.*, 2017).

Microscopical mounts were made in tap-water, 10% KOH and Congo-Red in NH_4OH or Congo-Red SDS (CR) mounts are used for testing the hair chemistry, Lugol's solution (IKI) was added to a water mount for testing the reaction of asci pore-walls and hairs, * = in living state for microscopical measurements.

Taxonomy

Urceolella aspera (Moug. ex Fr.) Boud., *Hist. class. Discom. Eur.*: 130 (1907).

Basionym: *Excipula aspera* Moug. ex Fr., *Syst. mycol.*, 2 (2): 597 (1823).

≡ *Unguicularia aspera* (Moug. ex Fr.) Nannf., *Nova Acta R. Soc. Scient. upsal., Ser. 4*, 8 (2): 278 (1932); *Hyalopeziza aspera* (Moug. ex Fr.) Raitv., *Akad. Nauk Estonskoi S.S.R., Inst. Zool. Bot., Tartu*: 33 (1970); *Olla aspera* (Moug. ex Fr.) Raitv., *Scripta Mycol.*, 20: 87 (2004).

Urceolella aspera is a seldom recorded hairy discomycete with a strong host-specificity that can only be found on the dead still standing fertile stems of *Osmunda regalis* L. (Royal Fern) in early spring when leaves start to grow, not seldom found on this host.

Description: The fruitbodies are growing gregarious, with hundreds specimens on a single stem. **Apothecia** light brown in sunny places to hyaline in shady places, Ø 0.2–0.45 mm, in dry state appearing as tiny hairy pyrenomycetes. When rehydrated the hairs are bundled together in vertical rims on the flanks and the hymenium is surrounded with a collar which is covered on the inside with short hyaline thin-walled hairs, up to ± 100 µm high, depending on the age. The **ectal excipulum** in the lower flanks is made of a thick-walled long-celled *textura angularis* tending to a *textura oblita*; on the higher flanks and margin the excipulum is changing into a *textura angularis*; both textures are CR positive. The **hairs** are made of a massive glassy matter with a lumen only visible at the base; on the flanks merely pointed, 20–45 µm long, sometimes curved at the apex; on the margin and the inside of the collar 7–15 µm long, often undulating and apically branched. The glassy matter is not reacting in CR, reddish-purplish grey in IKI and completely dissolving in 5–10% KOH, only leaving inconspicuous wall parts. **Asci** arising from croziers, * 45–69 × 7.5–8.5 µm, the apical pore wall of *Calycina* type

Table 1 – Newly generated ITS sequences

Species	Voucher number	GenBank accession number
Urceolella aspera	SBRH827	MH221523
Cistella graminicola	SBRH915	MT017661
Psilachnum sp.	MH 50815	Not deposited
Incrupila aspidii	SBRH909	MT040757
Hyalopeziza raripila	SBRH897	MT017660
Hyalopeziza sp.	SBRH932	Not deposited
Hyalopeziza sp.	SBRH934	Not deposited

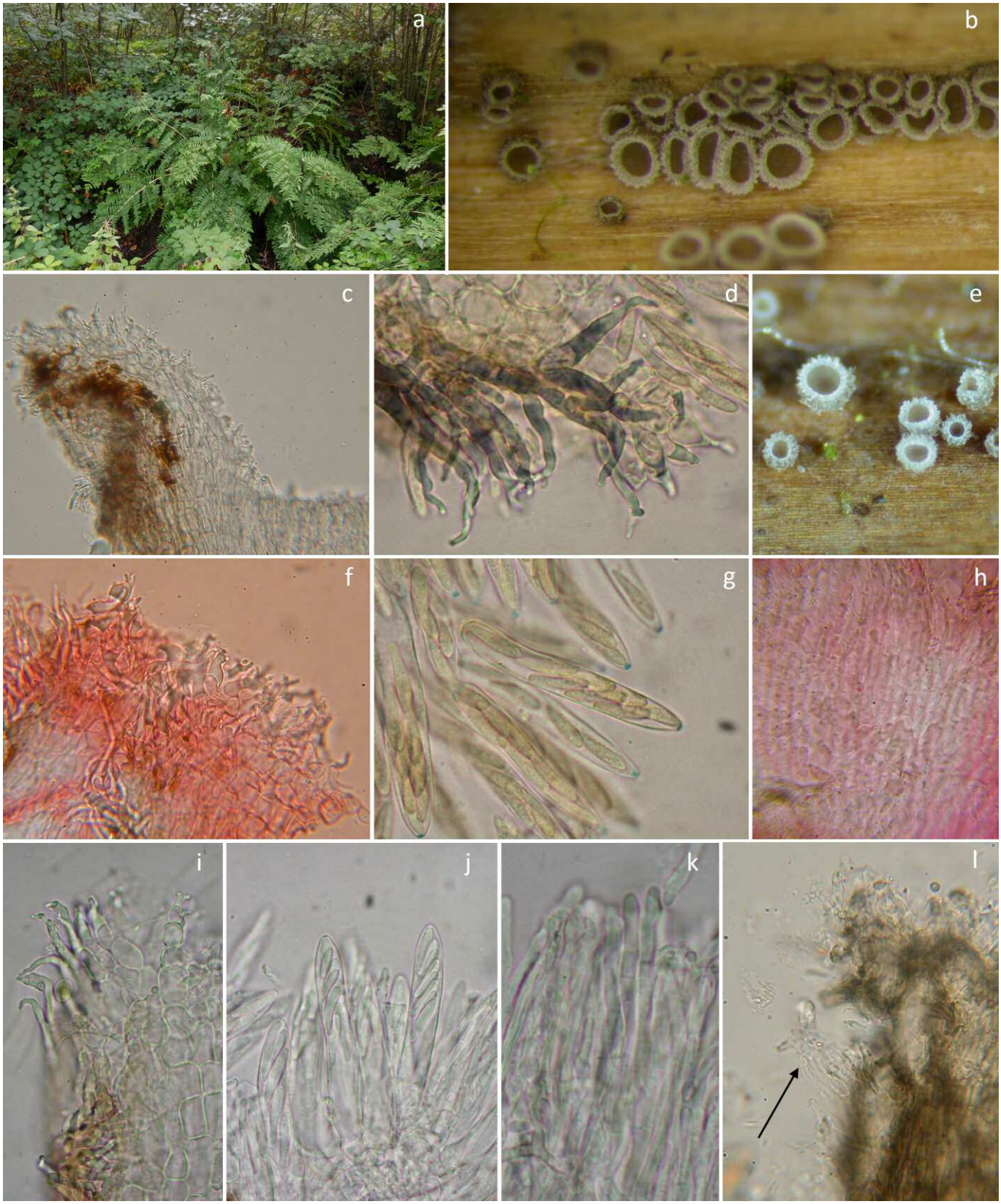

Plate 1 – *Urceolella aspera*. a: *Osmunda regalis* in summer. b: Fruitbodies from SBRH 720. c: Marginal collar in square view. d: Hairs in IKI. e: Fruitbodies from MH 50713. f: Marginal hairs in CR. g: Asci in IKI. h: Excipulum in surface view. i: Hairs in H_2O. j: Asci in H_2O. k: Paraphyses in H_2O. l: Hairs destroyed with 10% KOH.

becomes bright blue in IKI. **Paraphyses** cylindrical, * 2–2,5 µm wide, 3–4 septate in the lower three-quarters, without any contents. **Ascospores** * 10.5–14 (16) × 2.8–3.5 (3.8) µm, elongate clavate, aseptate, sometimes slightly curved without any contents.

Culture results: Culture attempts on Malt Extract Agar failed, from five attempts. Only in one case germinating and rapid growth was seen for 2.5 days. After this period, all growth stopped. The other four attempts did not germinate at all, perhaps caused by the strict host-specificity of the species.

Discussion

The genus concept as it is these days for the delimitation of the glassy haired genus *Urceolella* Boud is as follows: The hairs are losing their glassiness in KOH as described by Huhtinen (1987) and also the absence of a CR reaction of the hairs connected with a positive CR reaction of the excipulum (Raitviir, 2004). These characters allow us to resurrect the generic name given by Boudier (1907) for this fungus according to the histochemical reactions of the hairs. Raschle (1977) used, for the generic placements in *Unguicularia* Höhn., the absence of a reaction using a 5% KOH solution and a dextrinoid reaction in Melzer's reagent. Although on this particular species the KOH reaction is not stated, perhaps Raschle himself was uncertain at that time. My personal observations with an old 5% KOH solution were also negative at the first time, but with a fresh prepared 5% KOH solution the dissolving of the glassy matter of the hairs was clear. So the reaction may be doubtful for a ± 5% concentration but moreover in a 10% KOH solution the glassiness is immediately dissolved as found out by H.-O. Baral (pers. comm.) when checking one of my collections.

Raitviir (2004) placed the species in the genus *Olla*, based on the work of Raschle (1977). As the result from an electronic correspondence with A. Raitviir and myself between October 2005 and February 2006, Raitviir had tested Bøhler's specimen from Oslo, and answered: "You were right on *Excipula aspera* Fr. on *Osmunda*. I have examined good collections of it by Bøhler and found that unfortunately I had misread the description by Raschle. It is really a good *Urceolella*, not *Olla*."

His intention to include a correction in a new edition of his "Revised Synopsis of the *Haloscyphaceae*" (Raitviir, 2004) became unfortunately no reality, so we feel allowed to settle this matter.

This species is well placed in *Urceolella* because of excipular and hair morphology together with its micro-chemical characteristics: the positive reaction of the excipulum and the negative reaction of the hairs in CR (Plate1 f + h). A striking but yet unrecorded feature should be noticed: in living state, it is easily observed that the margin of the ectal excipulum is raising as a white collar above the hymenium in mature apothecia (Plate 1b). Microscopical mounts show that this collar is clothed with the same short irregularly shaped, thin-walled, hyaline hairs on the inside as on the margin (Plate 1c). It is evident that this marginal outgrowth serves as a protection for the hymenial layer during drought. This is an effective survival strategy, the fruiting bodies remain vital in a very dry environment for a long period. This character shows a similarity with the genus *Solenopezia* Sacc., a genus placed in the *Lachnaceae* Raitviir, which possesses firm to thick-walled, roughly granulate hairs on the ectal excipulum.

Phylogenetic results

In the past few years, more sequences of glassy haired *Helotiales* became available including this species. It gives the possibility to build some phylogenetic trees. So far in all genetic ITS results, *Urceolella aspera* shows a high similarity to other *Urceolella* species together with *Cistella* Quél. and *Psilachnum* Höhn. (although a sharp generic delimitation based on the available molecular data is yet

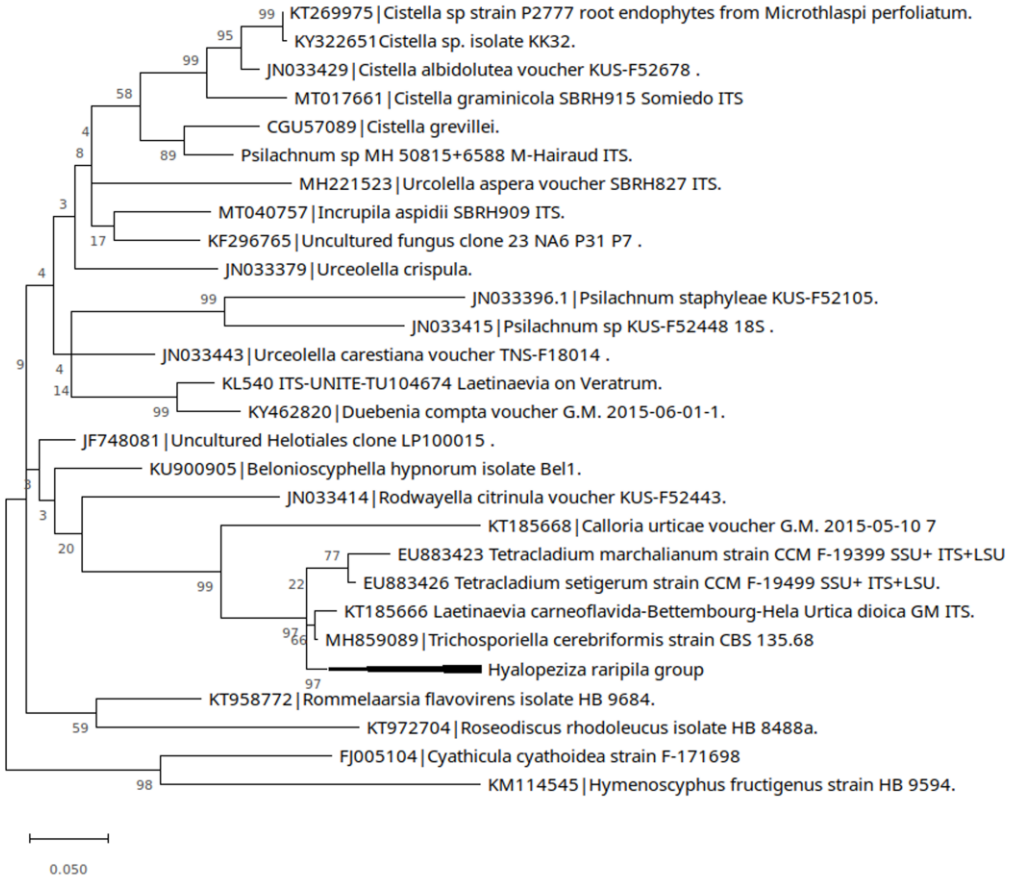

Fig. 1 – The Maximum Likelihood GTR +G ITS-tree (1000 bootstrap iterations), of the *Cistella* / *Urceolella* / *Calloriaceae* clade rooted with *Hymenoscyphus* and *Cyathicula*. The numbers left to the nodes show the bootstrap support percentage.

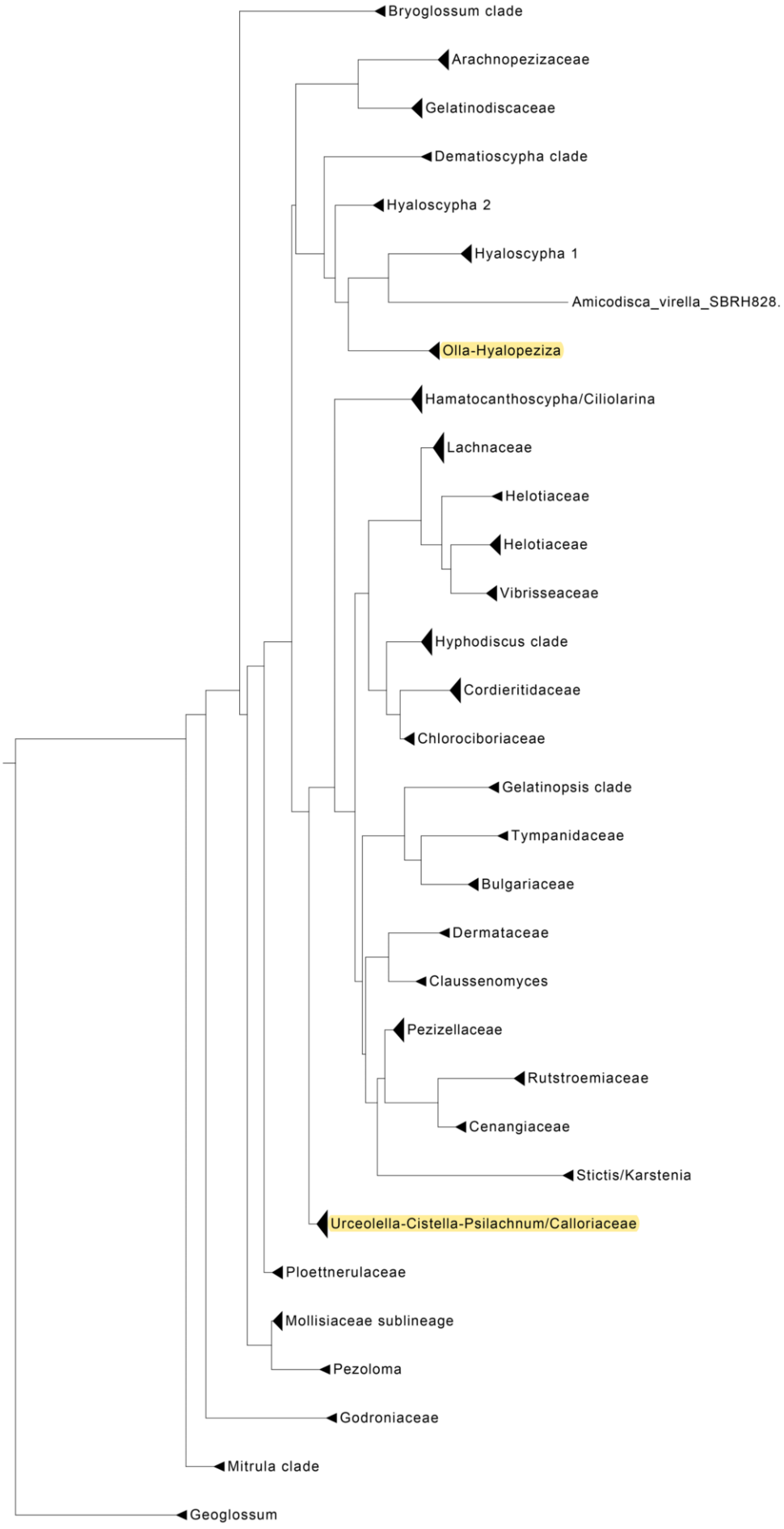

Fig. 2 – The PhyML GTR + G + I ITS-tree of the *Helotiales* rooted with *Geoglossum* to show the distance between the *Hyalopeziza* / *Olla* and *Cistella* / *Urceolella* / *Calloriaceae* clades.

unclear) in the *Cistella/Calloriellaceae* clade, leaving *Olla* together with *Hyaloscypha* Boud. and *Hyalopeziza* Fuckel *p.p.* in the *Hyaloscyphaceae* (Nannf.) Raitv. This is shown in the yet unpublished *Helotiales* ITS tree, here presented as a collapsed tree (Fig. 2), the distance between the *Cistella* and *Olla* clades are visualised by highlightning of the clades. The names of the clades used here are merely derived from Baral in Jaklitsch *et al*. (2016).

The Maximum likelihood ITS bootstrap-tree (Fig. 1) shows the superclade of the *Cistella/Psilachnum* and *Calloriaceae* Baral & G. Marson. The low bootstrap support for *Urceolella* demonstrates that more sequences are needed to explain the natural origin of the species and the concepts of the genera based on hair morphology, as already shown in Han *et al.* (2014).

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