INTEGRATED STUDIES IN THE CLASSIFICATION OF THE PEZIZACEAE. RE-EVALUATION OF THE GENUS PACHYELLA WITH A NEW SEGREGATE GENUS ADELPHELLEA

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Summary
A new genus Adelpella is proposed for Pachyella babingtonii. Molecular, morphological, histochemical and cytological studies, including data obtained through detailed study of living material employing vital taxonomic methods, have shown that it is closely related to Pachyella and Boudiera, but that it can be sufficiently differentiated from them to warrant recognition of a new genus. Adelpella, Pachyella, and Boudiera are similar in ecology, occurring on water-soaked wood or on soil/silt that is periodically flooded.

INTRODUCTION

In recent years reliable molecular phylogenetic work has been done on the Pezizaceae by Hansen and co-authors. This work has given insight into the relationships among members of this large and diverse family but it has not provided a useful classification. Many lineages are delimited but few of these have names. This is the first in a series of papers that will undertake an ordering of the family so that lineages and species can be appropriately named. Of particular importance in these studies are the observations of living material using a variety of histochemical methods that has been undertaken by two of us, N.M. and I.K.

The senior author’s (D.H.P.) personal history with the Pezizaceae, and especially with the
genus *Pachyella* Boud., dates from 1967 when he began in the graduate program at Cornell University studying with Richard P. Korf. The second author (N.M.) began his long-term study of *Pezizales* in 1989, and was joined in 2002 by the junior author (I.K.). They concentrated on studying living material and were particularly concerned with the application of such research to ascomycete taxonomy. Shortly after Baral (1992) introduced the concept of vital taxonomy. Our independent long-term investigations based on employing techniques of vital taxonomy have focused on *Pezizaceae*.

Initially work by DHP centered on the genus *Pachyella* about which there was controversy regarding the application of the name and its delimitation. Questions were raised particularly in regard to *Peziza* Fr. and *Psilopezia* Berk. *Peziza* was, and remains, ill-defined and in its broadest sense is composed of a number of discordant elements (Hansen et al. 2001, 2005). *Pachyella* and *Psilopezia* species are similar macroscopically and occupy similar habitats—wet or water-soaked wood often directly in moving fresh water—thus boundaries between the two were blurred. Indeed, several epithets were combined in both genera. A consequence of this confusion was that the common and widespread *Pachyella babingtonii* (Berk.) Boud. was reported both in *Psilopezia* and *Pachyella*. No comparative or type studies of the species of *Pachyella* had been undertaken and the seemingly erratic reaction of some species in iodine solutions was particular problematic. The paper that resulted from these earlier studies by the senior author set *Pachyella* on firm footing (Pfister 1973c) and subsequently most workers have recognized it. Furthermore, employing Korf's (1972, 1973) then new classification of the *Pezizales* it was possible to place the two genera in separate families, *Pachyella* in the *Pezizaceae* (Pfister 1973c) and *Psilopezia* in the *Pyronemataceae* (Pfister 1973b), family positions that are held today. A series of papers added to or further clarified some of the concepts and characters (Pfister 1973a, 1974, 1975, 1981, 1995, 2001). In the present account we review the genus *Pachyella* in light of recently published phylogenetic work in the *Pezizaceae* (Hansen et al. 2001, 2005).

**Material and methods**

Specimen handling and microscopical procedures on living material follow earlier papers (Baral 1992, Matoćec 2000). Additionally, we applied Congo red dye, as outlined below, to the living material in order to test walls of various living cell types for differential staining (especially asci). Vertical-median sections of living apothecia were first placed in tap water at the centre of a microscope slide. A minute drop of aqueous Congo red solution (3% weight solution, without ammonia) was placed on the slide close to the sections. The sections were gently nudged one by one into the Congo red drop using a horse eyelash, allowed to stain for a minute, and then they were returned in new drop of tap water in the same way. Observations were made on the staining mode and structures at the ascal apices using only fully ripe asci (often observed discharging spores). Orientation of the asci is important. *Pezizalean* asci are usually bent in apical portions and are fairly voluminous and are three dimensional. The ascus is observed in profile in order to view the apical apparatus. Ascal amyloid reaction (using Lugol's solution, after Baral, 1992) obtained on the living cell was described only in asci with undisturbed periascal gel.
RESULTS AND DISCUSSION

As characterized by PFISTER (1973c) Pachyella included species with pulvinate apothecia as well as those with flat, appressed, broadly attached apothecia. All have hyploid hairs that originate from the globose to angular cells of outer excipulum and they often form a palisade over the outer surface. The hyploid hairs are often embedded in a gelatinous matrix and gel may be present to greater or lesser degree within the excipular tissues. Ascospores are smooth or ornamented and prominently biguttulate. Asci are diffusely J+ over their entire length. In the majority of the species the margin is formed of hyploid hairs that are morphologically similar to paraphyses. Species occur on water-soaked wood or occasionally on soil. Conceived in this way Pachyella represents a morphologically and ecologically coherent group. In phylogenetic studies using rDNA LSU (HANSEN et al. 2001) these species form a well-supported monophyletic group along with Boudiera Cooke. Using combined LSU, RPB2 and β-tubulin (HANSEN et al. 2005) a Boudiera-Pachyella clade also is recovered but Pachyella is not monophyletic, rather Pachyella babingtonii groups with two species of Boudiera; the remaining species of Pachyella (which include the type species) are sister to the Boudiera – P. babingtonii group.

These findings counter the view that the genus or the constituent species should be treated in Peziza (SEAVAR 1928, ECKBLAD 1968) or that Pachyella should be treated as a subgenus of Peziza as stated by DONADINI (1980). The phylogenetic studies show that the genus Peziza as viewed in modern treatments is not monophyletic but rather represents several distinct morphological and phylogenetic lineages.

HANSEN et al. (2001) discussed the extent and intensity of ascus reactions in iodine solutions within the family Pezizaceae. Not all species have asci that become blue in iodine but in those that do there are four types of reactions: 1) faint bluing over the entire ascus but often more strongly so at the apex; 2) faint bluing over the entire ascus but with a distinct ring zone at the apex; 3) those that are strongly amylloid over the entire ascus; and 4) faint bluing over the entire ascus, sometimes associated with hymenial gel. Species of Boudiera and Pachyella have asci and associated hymenial gel that blue diffusely, that is, they exhibit the type 4 bluing reaction. PFISTER (1973c) and TRIMBACH (1990) comment specifically on the bluing reaction in Pachyella. The lack of reaction in some collections of P. babingtonii seems to be associated with the dilution or dissolution of the gel in the hymenium perhaps being related to the submersion of the ascomata in water.

Boudiera species are morphologically reminiscent of P. babingtonii but have globose, ornamented spores. Ascomata of Boudiera, like those of P. babingtonii, are turbinate to pulvinate with indistinct margins and in species of Boudiera and in P. babingtonii the ectal excipulum is composed of globose to angular cells. Boudiera species do not produce excipular gel whereas the gel in P. babingtonii is copious in the medullary excipulum and imbeds the hairs on the outer surface of the apothecia (Fig. 1). Boudiera species are not completely out of place ecologically in this larger clade since they occur on soil that has been flooded or inundated. DISSING and SCHUMACHER (1979) discuss some species of Boudiera and their special habitats. HIRSCH (1980, 1983) also gives a summary of the genus. As mentioned above globose spores are characteristically found in Boudiera but globose spores are also found in

the recently described *Pachyella globispora* K. Maruy. & Hosoya. This further draws attention to similarities within the clade.

With phylogenetic, morphological, histochemical and cytological information in hand we can make recommendations about generic limits in this group.

**ONE GENUS OR TWO GENERA?**

In the combined data set analyses (Hansen *et al.* 2005) *Pachyella babingtonii* is supported with 100% bootstrap. It is sister to *Boudiera* rather than to the remainder of the species of the genus *Pachyella*; *P. babingtonii* differs from the other *Pachyella* species in that it lacks an organized palisade layer of hyphoid hairs running from the margin along the base of the apothecium (Figs. 1, 2) and thus in its cellular constructions it seems more like *Boudiera*. The species also differs from the other species of *Pachyella* in microstructure of the apical apparatus of mature living asci (Fig. 3) and their staining mode in Congo-red. The apical indentation ring is much more pronounced ("Z" shaped in profile) in "true" *Pachyella* species than in *P. babingtonii*. The opercular wall is much thinner than the subapical wall at the inflexion point in those species, while it is of same thickness in *P. babingtonii*. The globose ectal excipular cells are thick and bilayered with the inner layer being more strongly stained than the outer layer by congo-red (Fig. 4); three tested species of *Pachyella* have thin-walled cells homogenously and weakly stained in the same dye. Furthermore, apical cells of living

*Fig. 1. Pachyella dypeata*, left and *Adelphella babingtonii*. Paraffin imbedded sections stained specifically to show gelatinous materials.

Fig. 2. Vertical median section of the living apothecia. Left: *Adelphella babingtonii* (CNF 2/7481), right: *Pachyella pseudosuccosa* (CNF 2/6435)

Fig. 3. Apical apparati of the living asci. Left: *Adelphella babingtonii* (CNF 2/7481), centre: *Pachyella peltata* (CNF 2/7651), right: *P. clypeata* (CNF 2/7652). IR - indentation ring, OW - opercular wall, PM - perisal mucus.

Fig. 4. Living ectoexcipular cells in *Adelphella babingtonii* (CNF 2/7651) stained with Congo red.

Fig. 5. Apical portions of the living paraphyses. Left: *Adelphella babingtonii* (CNF 2/7481), right: *P. elypeata* (CNF 2/7652). HG - hyaline hymenial gel, PG - hymenial gel with pigment deposits, PV - pigmented non-refractive vacuoles, VB - vacuolar bodies.

Unstained paraphyses in *P. babingtonii* are either filled with highly refractive granular sub-hyaline vacuolar bodies or are filled with few very large non-refractive, isabelline-ochraceous vacuoles (Fig. 5). On the other hand, all apical cells of living paraphyses in five tested species of *Pachyella*, including the type species, contain non-refractive vacuoles filled with yellow, greenish-yellow or cinnamomeous-ochraceous pigment. The living ascospores of *P. babingtonii* have only an inner wall layer that is stainable in Cresyl-blue and the two prominent polar lipid bodies are accompanied by numerous minute lipid bodies that are dispersed between them (Figs. 6, 7); the other tested species of *Pachyella* have both wall layers stainable in Cresyl-blue and only two prominent polar lipid bodies (Fig. 7). If some additional small refractive elements occur in their sporoplasm, they are stainable in the dye and disintegrate in dying spores and thus cannot be termed lipid structures. All microscopical differencies are summarized in the table 1.

As the lengthy synonymy suggests (see Pfister 1973), *P. babingtonii* has been described under several specific epithets and has been treated in several genera. One of the synonymous names, *Peltidium oocardii* Kalchb., is the type species of the genus *Peltidium* Kalchb., and also of *Pulvinaria* Velen. Unfortunately, both generic names have earlier applications and are not available.

In an attempt to sort and name morphological groups Haffner (1992) included *P. babingtonii* as the sole member of the section *Babingtoniae* under an emended and highly hetero-
Fig. 6. Living ascospores in Cresyl blue. Left: Adelphella babingtonii (CNF 2/7481), right: P. clypeata (CNF 2/7652). MLB - main lipid body, N - nucleus, PW - primary wall, SW - secondary wall.

Fig. 7. Living ascospores. a - Adelphella babingtonii (CNF 2/7481), b - Pachyella clypeata (CNF 2/7652), c - P. peltata (CNF 2/7651), d - P. punctispora (CNF 2/1803), e - P. violaceonigra (CNF 2/3306) and f - P. pseudosuccosa (CNF 2/6435).

gendeous concept of the genus *Pachyella*. Given the morphological, histochemical, cytological and comparative phylogenetic information available we believe *P. babingtonii* is sufficiently distinct from both *Pachyella* and *Boudiera* to warrant its segregation as a new genus.

<table>
<thead>
<tr>
<th></th>
<th><strong>Adelpahella</strong> (single species)</th>
<th><strong>Pachyella</strong></th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>apical apparatus (<em>mature ascis</em>)</td>
<td>opercular lense weakly differentiated, convex-concave, central thickness equal or less than subapical wall, slightly pre-delimited by shallow indentation</td>
<td>opercular lense sharply differentiated, convex-flat, central thickness greater than subapical wall, sharply pre-delimited by «Z» shaped indentation</td>
<td>3</td>
</tr>
<tr>
<td>ascal congo-red staining (*)</td>
<td>homogenous</td>
<td>indentation ring area lighter stained</td>
<td>3</td>
</tr>
<tr>
<td>ascogenous cell configuration (*)</td>
<td>compact, densely packed, croziers not perforated</td>
<td>loosely woven, «H» and «W» shaped cells frequent, croziers when present perforated</td>
<td>3</td>
</tr>
<tr>
<td>cresyl-blue spore wall staining (*)</td>
<td>outer wall layer not stained, inner wall layer staining blue</td>
<td>both wall layers of some shade of blue</td>
<td>3</td>
</tr>
<tr>
<td>cresyl-blue sporoplasm staining (*)</td>
<td>Whole extranuclear/extralipid sporoplasm pale blue</td>
<td>none or dot-like structures encircling each lipid body greenish-cyan</td>
<td>3</td>
</tr>
<tr>
<td>spore shape (*+/+)</td>
<td>oblong-ellipsoid, ends blunt</td>
<td>ellipsoid to subslusiform, end tapered</td>
<td>5</td>
</tr>
<tr>
<td>sporoplasmic lipid configuration (*)</td>
<td>two large polar lipid bodies together with many smaller lipid bodies dispersed among them</td>
<td>only two large polar lipid bodies present</td>
<td>5</td>
</tr>
<tr>
<td>paraphysal heteromorphy (*)</td>
<td>apical cells of paraphyses of two types: (a) filled with highly refractive granular isabelline-ochraceous vacuolar bodies; (b) filled with few very large non-refractive subhyaline vacuoles</td>
<td>all apical cells of paraphyses contain low refractive vacuoles filled with yellow, greenish-yellow or cinnamon ochraceous pigment</td>
<td>5</td>
</tr>
<tr>
<td>paraphysal / hymenial exudate (*+/+)</td>
<td>hyaline</td>
<td>pigmented and/or bearing pigmented granules</td>
<td>5</td>
</tr>
<tr>
<td>ectoxicipar texture type (*+/+)</td>
<td>continuous layer of textura globulosa, on the outside some free cylindric hyphoid terminal cells</td>
<td>continuous layer of textura globulosa-prismatic oriented perpendicularly to the surface, forming a hymeniform cell layer</td>
<td>5</td>
</tr>
<tr>
<td>ectoxicipar cell wall (*)</td>
<td>thick, bilayered with inner layer strongly stained with the congo-red and outer layer weakly stained in the congo-red</td>
<td>thin, not stratified, homogenously weakly stained with the congo-red</td>
<td>3</td>
</tr>
</tbody>
</table>

* refers to the living state of the cell bearing given character
+ refers to the dead state of the cell bearing given character

Table 1. Comparative table: *Adelpahella* vs. *Pachyella*

*Adelpahella* Pfister, Matočec et I. Kušan, **gen. nov.**

*Pachyella* similars sed gelatina copiosa interna, apothecia mina et pulvinata. Sporae cum duabus gutulis prominentibus gutulisisque multis minutis. Apothecia carens stratum exterius pilorum valli.


**Etymology**: Adelphe, sister, and –ella, the diminutive, little sister, referring both to the small size of the apothecia as compared to other *Pachyella* species and to the phylogenetic sister relationship it and *Boudiera* hold to *Pachyella*.

We note further that in the analyses by Hansen et al. (2001, 2005) the two sequences of *P. babingtonii* are highly divergent. This may suggest that this cosmopolitan taxon should be further investigated at a finer level including comparative studies of the living cells and tissues. Pfister (1973c) had earlier illustrated and described both smooth and punctate ascospores in this species.

THE RESTRICTED GENUS PACHYELLA

As now defined Pachyella species are characterized by forming a distinct palisade layer of hyphaloid hairs at the margin and outer surface of the apothecia; these are embedded in a gelatinous matrix. Gel is often also present in the medullary excipulum. The hairs arise from globose or ovoid cells with two wall layers that stain in Cresyl-blue. Ascospores are biguttulate and lack minute lipid bodies.

Further necessary detailed morphological, cytological and phylogenetic studies on this genus are in the progress since conflicting interpretations have been presented for some of these taxa. The type of the genus is Peziza barlaeana (=Pachyella barlaeana), the older name Peziza violaceonigra Rehm (=Pachyella violaceonigra) we consider the proper name for this species. The following species should be included in this genus; taxonomic synonyms are included where critical:

Pachyella adnata (Berk. & M.A. Curtis) Pfister
Pachyella clypeata (Schwein.) Le Gal
Pachyella globispora K. Maruy. & Hosoya
Pachyella babrospora Pfister
Pachyella hydropilosa (Sacc.) Pfister
Pachyella laazzariana Trimbach
Pachyella megulosperma (Le Gal) Pfister
Pachyella pellata Pfister & Cand. [BARAL et al. 1981, described this taxon under the name Pachyella clypeata from a German collection]
Pachyella pseudosuccosa (Le Gal) Pfister = Pachyella aquatilis (Berthet & Donadini) Donadini
Pachyella punctispora Pfister
Pachyella violaceonigra (Rehm) Pfister = Pachyella barlaeana (Bres.) Boud.

DISCORDANT ELEMENTS INCLUDED IN THE GENUS PACHYELLA

Some authors have included species in Pachyella that do not fit the present circumscription. The broadest and least tenable view of the genus is that presented by HÄFFNER (1992). Here 13 species are described in three sections, Babingtoniae, Castaneae and Clypeatae. Section Babingtoniae, with only Pachyella babingtonii, is now recognized as the genus Adelphella. Species in the section Clypeatae and also P. punctispora of section Castaneae form the genus Pachyella. The section Castaneae represents divergent elements as shown by molecular phylogenetic studies. Peziza depressa was included in Pachyella (section Castaneae) by HÄFFNER (1992). While such an inclusion might be defended bases on the repand form of the fruit body this fungus fails to meet other criteria. It occurs on soil, it lacks gelatinous material, the asci blue intensely in the upper half rather than diffusely, and in phylogenetic analysis it falls within a distinct and distant clade (HANSEN et al. 2005).

Several species have been included because of the form of the fruitbodies. Smaller repand Pezizaceae with differentiated excipula have been mistakenly included in Pachyella. One such taxon for which evidence from phylogenetic studies is available is Peziza subisabellina. This fungus has been confused in the past with Pachyella species and

indeed when the senior author first studied the genus *Pachyella* he looked at material of it. The apothecia are broadly reprod and the flesh is thick. It occurs on plant debris and soil and it lacks gelatinous material and the distinctive hypothalline hairs of *Pachyella* species. It possesses ascosporas that are thin-walled, eguttulate and with two polar non-lipid groups of small globules of medium refractivity, stainable in Cresyl-blue and ascus apparatus (thinned wall of the opercular wall) completely different both from *Pachyella* species and *Adelphella babingtonii*. In molecular phylogenetic studies this species is only distantly related to the Boudieria-*Pachyella* clade.

Other taxa that have been included in *Pachyella* similarly fall within *Peziza* sensu lato:  
*Pachyella castanea* Häfner (= *Peziza depressa*)  
*Pachyella celtica* (Boud.) Häfner  
*Pachyella coquandii* (Donadini) Häfner & Schopfer  
*Pachyella subisabellina* (Le Gal) Trimbach (= *Peziza subisabellina*)  
*Pachyella subisabellina* var. ianthina (Grelet ex Le Gal) Trimbach (= *Peziza isabellina* var. ianthina)  
*Pachyella subisabellina* var. subisabellina  
*Pachyella subuliginosa* Donadini

**Conclusion**

This case study serves as a reminder that classifications need to be reviewed and reinterpreted as additional data comes to light. This is particularly important in cases where molecular phylogenetic analyses have been performed and can be used to better interpret morphological and the other types of relevant data. The Pezizales now have more detailed phylogenetic data applied to them than most other groups yet the classification has not been modified and updated to take phylogeny into account. The *Pezizaceae* is a family badly in need of a new classification might serve to remind us of the work we face.

**Acknowledgements**

Genevieve Lewis-Genry of the Farlow Herbarium staff, Harvard University, Cambridge, MA, kindly provided the Latin translation of the diagnosis. We also thank Vladimir Hršak of the Dept. of Botany, Faculty of Science, Zagreb, Croatia for useful suggestions.

**Literature cited**


