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Using a multigene phylogenetic analysis to assess generic delineation and character evolution in *Verrucariaceae* (*Verrucariales*, *Ascomycota*)

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ABSTRACT

Verrucariaceae are a family of mostly crustose lichenized ascomycetes colonizing various habitats ranging from marine and fresh water to arid environments. Phylogenetic relationships among members of the *Verrucariaceae* are mostly unknown and the current morphology-based classification has never been confronted to molecular data. A multilocus phylogeny (nuLSU, nuSSU and *RPB1*) was reconstructed for 83 taxa representing all main genera of this family to provide a molecular phylogenetic framework necessary to assess the current morphology-based classification. Four main well-supported monophyletic groups were recovered, one of which contains seven robust monophyletic subgroups. Most genera, as traditionally delimited, were not monophyletic. A few taxonomic changes are proposed here to reconcile the morphology-based classification with the molecular phylogeny (*Endocarpon diffractellum* comb. nov., *Heteroplacidium fusculum* comb. nov., and *Bagliettoa marmorea* comb. nov.). Ancestral state reconstructions show that the most recent common ancestor of the *Verrucariaceae* was most likely crustose with a weakly differentiated upper cortex, simple ascospores, and hymenium free of algae. As shown in this study, the use of symplesiomorphic traits to define *Verrucaria*, the largest and type genus for the *Verrucariaceae*, as well as the non monophyly of the genera *Polyblastia*, *Staurothele* and *Thelidium*, explain most of the discrepancies between the current classification based on morphological similarity and a classification using monophyly as a grouping criterion.

Introduction

Verrucariaceae Zenker is a group of mainly lichenized ascomycetes comprising widely diverse habits. The structure of the thallus varies greatly in this family, with sizes ranging from a few millimeters to more than 10 cm diam, and shapes from granulose or crustose for the smallest thalli to squamulose or foliose umbilicate for the largest ones. Although vegetatively quite variable, members of *Verrucariaceae* are easy to recognize as their ascomata present good diagnostic features for the family. The perithecial ascomata are characterized by the presence of an apical ostiole and of short pseudoparaphyses (or periphysoids, but see Roux and Triebel 1994) bordering the upper part of the perithecial cavity and hanging into this cavity without or only barely reaching the hymenium (Janex-Favre 1970, 1975; Wagner 1987). Asci are typically bitunicate (Janex-Favre 1970, 1975; Wagner 1987), and their dehiscence was shown in some species to occur by a gelification of

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the apical part of the outer wall (Grube 1999). The lack, at least at maturity, of long, interascal, sterile hyphae and the positive reaction of the hymenial gel to potassium–iodine are also typical of this family (Henssen & Jahns 1973). The perithecium of some species of *Verrucariaceae* has been the subject of anatomical and ontogenetical studies (Doppelbauer 1959; Janex-Favre 1970, 1975; Wagner 1987). Because the ascoma development is quite variable within this family, the recognition of *Verrucariaceae* as either ascohymenial or ascolocular fungi has long been debated (Janex-Favre 1970, 1975).

Species classified within *Verrucariaceae* grow mainly on rocks, either epilithically or endolithically within the superficial layer of the rock. Members of this family can also colonize other types of substrates: soils (Breuss 1996), wood or bark (Breuss 1993a, 1994a, 1998a; Orange 1989), mosses (Döbbeler 1997; Döbbeler & Triebel 1985), and other lichens (Zehetleitner 1978). Saxicolous species of this family grow mostly in dry environments, but some species are also found in aquatic habitats, such as boulders located in rivers (Keller 1995, 2000; Thüs 2002), or marine intertidal and supralittoral zones of rocky shores (Brodo & Santesson 1997; Flenniken & Gibson 2003; Harada 2004; Sanders *et al.* 2004). Although saxicolous members of *Verrucariaceae* are particularly diverse on calcareous substrates, they can also colonize siliceous rocks, especially in aquatic or semi-aquatic conditions. Members of this family are found worldwide, from polar regions to the tropics (Aptroot 1998, 2002; Aptroot & Seaward 1999; Aptroot & Sipman 2001; Breuss 1993b, 1994b, 1998b; Brodo *et al.* 2001; Clauzade & Roux 1985; Harada 1993a,b; McCarthy 2001; Thomson 1997; Vězda 1973).

Eschweiler (1824) first attempted to classify genera of *Verrucariaceae*. For these taxa, he created two 'cohors' *Verrucariae* and *Dermatocarpeae* (Table 1), based mainly on the structure of the thallus. He attributed the crustose taxa to the *Verrucariae* and the squamulose and foliose taxa to the *Dermatocarpeae*. Unfortunately, Eschweiler (1824) also included many non-related genera within these two groups (Table 1). In 1827, Zenker validated the family *Verrucariaceae* (at the time called *Verrucariae*), in which he included the genus *Verrucaria* and some other unrelated crustose taxa (Table 1). This author also separated the squamulose and foliose taxa from the crustose *Verrucariae*, and placed them within the *Endocarpa*. It was only almost one century later that the first extensive monographic work on the family *Verrucariaceae* was published (Zschacke 1913, 1914, 1918, 1921, 1924, 1927). In 1933–1934, Zschacke recognized the two families *Verrucariaceae* and *Dermatocarpaceae* (Eschw.) Stizenb., as did Zahlbruckner (1921–22) in an earlier publication. Servít worked on the *Verrucariaceae* in the late 1940s and 1950s (Servít 1946, 1950a,b, 1952, 1953, 1954), and published a classification for the entire group in 1955. In this work, he also considered the family *Dermatocarpaceae* as a separate taxon, and recognized four additional families (*Staurothelaceae Servít*, *Microglaenaceae Servít*, *Pyrenidiaceae Zahlbr.*, and *Bagliettoaceae Servít*). However, two of these families are currently recognized in other orders as synonyms (*Microglaenaceae* is a synonym of *Thelenellaceae H. Mayrhofer*, and *Pyrenidiaceae* is a synonym of *Dacampiaceae Körb.*) and the validity of the two other families has always been questioned (Hale 1961; Henssen & Jahns 1973; Poelt 1973), and excluded from subsequent classifications (Eriksson 1983; Henssen & Jahns 1973; Poelt 1973). Currently, almost all genera belonging to the order *Verrucariales* are included in the family *Verrucariaceae* (Eriksson 2006). Two other genera, also included in *Verrucariales* and characterized by their long and persistent interascal elements (paraphysoides according to Triebel 1993) in addition to their short pseudoparaphyses, and by their lichenicolous habits, are classified in the second family of this order, the *Adelococcaceae* Triebel. Today, the family *Verrucariaceae* includes 45 genera (Eriksson 2006) and approximately 750 species (Hawksworth *et al.* 1995).

In the classifications of Servít (1955), Zahlbruckner (1921–22), and Zschacke (1933–34), taxa within *Verrucariales* were mainly circumscribed based on thallus structure, ascospore morphology, and the presence or absence of hymenial algae. However, the phylogenetic value of these characters was thought to be doubtful and in need of further investigation. Although Servít (1955) accepted the separation of the families *Verrucariaceae* and *Dermatocarpaceae* based on thallus morphology, early on he suggested the limitations of this character (1946: 49): 'In my opinion the distinction made between these two families on the basis of the degrees of the development of the thallus cannot be maintained, if we want to replace the present artificial system by one which is at least a little more natural.' Recently, molecular studies showed that, in other groups of ascomycetes (Miller & Huhndorf 2004, for the genus *Lasiosphaeria*; Staiger 2002, for the family *Graphidaceae*), ascospore septation was not always a reliable character to delineate monophyletic groups at the genus and higher taxonomical ranks. Because the generic delimitation within *Verrucariaceae* is mainly based on ascospore septation and thallus structure, molecular data are needed to assess the current morphology-based classification. To date, only a few molecular studies have been carried out on members of this family, either at the infrageneric level (Amtoft 2006; Amtoft *et al.* 2008; Heiömarsson 2003, on *Dermatocarpon*) or in the context of large-scale molecular phylogenies (Del Prado *et al.* 2006; Geiser *et al.* 2006; James *et al.* 2006; Liu & Hall 2004; Lumbsch *et al.* 2002, 2004, 2005; Lutzoni *et al.* 2001, 2004; Spatafora *et al.* 2006). However, the family *Verrucariaceae* was never the focus of a phylogenetic study and, before this study, only a few DNA sequences were available in GenBank for this family. This study aims to provide a multigene phylogeny for the main genera of *Verrucariaceae* in order to confront the current morphology-based classification with molecular data. Selected morphological traits and ecological aspects were studied to characterize inferred monophyletic groups. Generic delineation is discussed based on both molecular and morphological data, and a few taxonomic changes were undertaken. Ancestral state reconstructions were carried out to better understand: (1) the discrepancy between a morpho-similarity based classification and a classification based on a monophyletic grouping criterion, as well as, (2) the evolutionary history of the *Verrucariaceae*.

Materials and methods

Taxon sampling

Because of the relatively high number of taxa in the family *Verrucariaceae* and the difficulty in obtaining material for