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Are species-pairs diverging lineages? A nine-locus analysis uncovers speciation among species-pairs of the *Lobaria meridionalis*-group (Ascomycota)

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ABSTRACT

In spite of considerable effort to verify the theory of species-pairs, uncertainty still exists about the relationship between sexually or vegetatively reproducing populations of morphologically indistinguishable, sympatric lichen species. The current paper studies putative species-pairs within the Asian *Lobaria meridionalis*-group, using a nine-locus and time calibrated species-tree approach. Analyses demonstrate that pairs of sexually or vegetatively reproducing lineages split into highly supported monophyletic clades—confirming molecularly the species-pair concept for the *L. meridionalis*-group. In the broader context of evolution and speciation dynamics in lichenized fungi, this paper attempts to synthesize molecular findings from the last two decades to promote a more modern perception of the species-pair concept. Taxonomically, eight species were found to currently conform to the *L. meridionalis*-group, which differentiated during the Pliocene and Pleistocene. The coincidence of paleoclimatic events with estimated dates of divergence support a bioclimatic hypothesis for the evolution of species in the *L. meridionalis*-group, which also explains their current eco-geographic distribution patterns. Greater recognition for species with a long and independent evolutionary history, which merit high conservation priority, will be especially critical for preserving geographically restricted endemics from Southeast Asia, where habitat loss is driving rapid declines.

1. Introduction

Over the past twenty years, phylogenies have led to substantial shifts in the taxonomy of lichen-forming fungi based on criteria such as reciprocal monophyly or the degree of genetic clustering (Lumbsch et al., 2000; Spatafora et al., 2006; Lumbsch and Leavitt, 2011; Lücking et al., 2014). However, in spite of considerable phylogenetic research effort, conflict continues over the legitimacy of the species-pair concept (hereinafter SPC). According to Poelt (1970), this concept goes back to the idea of a sexual and an asexual *Sippe* of lichens that are indistinguishable except for their mode of reproduction (Fig. 1). *Sippe* is a German term that describes a group of organisms of common descent that is not restricted to any taxonomic rank. While *Sippe* can be translated as *taxon* in a strict taxonomical sense, in the present study, we consider the evolutionary term *lineage* to be more appropriate. To clarify, here the term lineage refers to a series, in this case, of populations connected by a continuous line of ancestor–descendent relationships (de Queiroz, 2005, 2007). Lineage, as is the case for taxon, is not restricted to any taxonomic rank. Poelt (1970) set the idea of a *Sippenpaar* of sexual and asexual lineages but suggested calling them *species-pair* because lichenologists agreed at that time to treat both partners as distinct species. Additionally, Poelt (1970) granted that asexual lineages produce mainly soredia or isidia (small propagules that contain both fungal and algal cells; Fig. 1) but can produce fruiting bodies rarely, under ecologically favorable conditions, although apothecia and their ascospores may be poorly developed.

The two main ideas of the SPC in Poelt's sense have been intensively discussed since the seventies (Tehler, 1982; Mattsson and Lumbsch, 1989; Crespo and Perez-Ortega, 2009). The first idea, which suggests that for each sexual lineage there is a corresponding derived, asexual lineage that is sentenced to disappear earlier or later due to its inability to recombine, is known as the *dead-end theory*. However, genetic variation has been documented in several asexual lineages, and sexual taxa have been shown to have arisen from asexual ancestors (Buschbom and Barker, 2006; Cornejo et al., 2009; Lendemer and Harris, 2014; Widhelm et al., 2016). Tripp (2016), based on a model-based reconstruction of transitions

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Abbreviations: SPC, species-pair concept; ML, maximum likelihood; PSC, phylogenetic species concept

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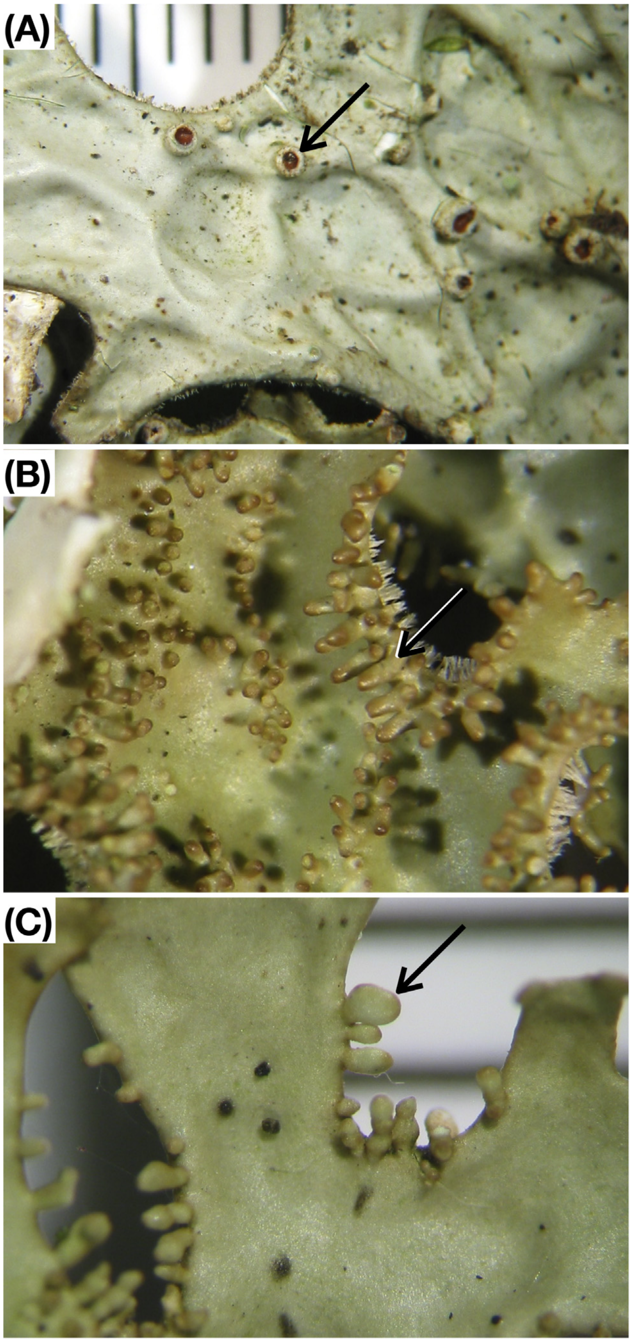

Fig. 1. Reproductive structures in the *Lobaria meridionalis*-group. (A) *L. gyrophorica*, showing several young apothecia (arrow). An apothecium is a bowl-shaped ascocarp, which typically contains the sexual ascospores. (B) *L. isidiophora*, covered with cylindrical isidia. (C) *L. spathulata*, with lobulate, flattened isidia along the thallus margins (arrow). (B) and (C) Isidia are corticated, vegetative propagules of lichens, consisting of both fungal hyphae and algal cells. They are fragile structures and may break off and be distributed by wind, animals, and splashing raindrops.

between sexual and asexual reproduction of 23 phylogenetic studies, recently demonstrated that primarily asexual lineages are able to undergo speciation, to give rise to primarily sexual lineages, and are likely to be evolutionarily old. Hence, regarding the SPC question, the idea of dead-end asexual lineages must be rejected. As discussed in a previous study (Cornejo et al., 2009), we basically assume multiple transitions from asexual lineages, which have the potential to form fruiting bodies although they do it rarely, towards sexual lineages that produce no vegetative propagules—and *vice versa* over a long-term time-scale.

Things look different for the second underlying idea of the SPC that sexual and asexual lineages are closely related but distinct species. A considerable amount of molecular research has tested the monophyly of paired species, resulting in conflicting conclusions. Out of 25 closely examined studies (Supplemental Table S1), 19 rejected the SPC because specimens with different reproductive modes were intermingled within one single monophyletic clade. Several studies that rejected the SPC (n = 8) were based on the application of the nuclear ribosomal locus ITS, while others (n = 11) applied up to four loci. Together, these studies question the validity of the SPC and it has been claimed to be obsolete. Consequently, some negative findings led to taxonomical revisions synonymizing names for both species-pair counterparts (e.g. Tehler et al., 2013; Messuti et al., 2016).

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Conversely, the SPC was supported in other studies because strong support was found for reciprocal monophyly between sexual and asexual counterparts. While Lendemer and Harris (2014) detected the species-pair *Porpidia degelii* (H. Magn.) Lendemer vs. *P. albocaerulescens* (Wulfen) Hertel & Knoph in each single-locus tree, Miadlikowska et al. (2011) applied two concatenated loci in *Hypogymnia* (Nyl.) Nyl. and Saag et al. (2014) five concatenated loci in *Vulpicida* J.-E. Mattsson & M.J. Lai to resolve reciprocal monophyly. In Cornejo and Scheidegger (2015), a three-loci phylogeny unraveled the species-pairs *L. kurokawae* Yoshim. (sexual) vs. *L. retigera* (Bory) Trevis. (asexual), and *L. sachalinensis* Asah. (sexual) vs. *L. kazawaensis* (Asah.) Yoshim. (asexual), although three other putative pairs within the *L. meridionalis*-group showed only a weak indication of reciprocal monophyly similarly to results in Wei and collaborators (2016). In Widhelm et al. (2016), the morphology and chemistry of two species-pairs were concordant with monophyletic clades of a seven-loci phylogeny.

Together, these examples indicate a simple point. The fact that in a particular case (Lendemer and Harris, 2014) monophyly of paired species was reflected in each analyzed locus in contrast to the concatenation of several loci—which were needed to unravel species-pairs in other cases (Miadlikowska et al., 2011; Altermann et al., 2014; Saag et al., 2014; Cornejo and Scheidegger, 2015; Wei et al., 2016)—indicates that some pairs are well sorted, while others may not be. Most studies have relied on one or a few loci to recognize species, i.e., on a phylogenetic tree-oriented approach, which requires gene-tree monophyly of completely sorted lineages. However, the time required for the development of different characteristics and properties during speciation processes varies, and may or may not involve morphology, physiology, phenology, ecology or molecular heritage (de Queiroz, 2007). Consequently, this paper argues that most species-pairs may be molecularly incompletely sorted due to a young age in geological terms and that an accurate picture of recent speciation history will only be possible when integrating the information available from multiple independent gene histories (Knowles and Carstens, 2007).

The *Lobaria meridionalis*-group is a complex of taxa that serves as an excellent model for the controversial topic of the SPC. We refer to this taxa as the *L. meridionalis*-group because it contains several described species that were separated from *L. meridionalis* or merged with this species (Yoshimura, 1969, 1971; for a related discussion, see Cornejo and Scheidegger, 2015). This group includes valuable indicator species for conservation-relevant habitats because they are distributed in old-growth forests. They grow on tree-bark in deciduous forests in Southeast Asian mountainous regions, temperate rainforests in Northeast Asia, and Himalayan subalpine forests. All lineages within the *L. meridionalis*-group exhibit low morphological divergence forming conspicuous, foliose thalli. Asexual populations produce cylindrical isidia (e.g. *L. pindarensis*; Fig. 2) or lobulate isidia (*L. spathulata*), and rarely fruiting bodies (apothecia), but sexual populations are assumed to reproduce exclusively by ascospores (e.g. *L. orientalis*; Fig. 2). Consequently, the species of the *L. meridionalis*-group were historically described as species-pairs based on the main reproductive mode and based