American Journal of Botany

American Journal of Botany 97(5): 738–752. 2010.

Symbiotic lifestyle and phylogenetic relationships of the bionts of *Mastodia tessellata* (Ascomycota, *incertae sedis*)[1]

Sergio Pérez-Ortega[2,3,4], Asunción de los Ríos[3], Ana Crespo[2], and Leopoldo G. Sancho[2]

[2]Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense, E-28040 Madrid, Spain; and [3]Instituto de Recursos Naturales, Centro de Ciencias Medioambientales CSIC, E-28006 Madrid, Spain

The biological nature of some symbioses is unclear because it is often not easy to discern whether the symbionts obtain any benefits from the association. *Mastodia tessellata*, a symbiosis between a leafy green alga and a fungus of uncertain phylogenetic position, is among the most investigated, controversial, and poorly understood associations. Because it has been difficult to determine whether this association is mutually beneficial or parasitic, not all scientists accept *M. tessellata* as a true lichen symbiosis. *Mastodia tessellata* is thus an interesting model to illustrate the interactions and processes that occur in fungal–algal symbioses. To improve our understanding of this association, we address the phylogenetic positions of the bionts involved and examine their interactions at the ultrastructural level. Examining the nuLSU and nuSSU gene regions of the mycobiont and the *rbc*L gene region of the photobiont, we found the fungus to be related to a group of marine species in the genus *Verrucaria*, family Verrucariaceae, despite its present ascription to the family Mastodiaceae. In addition, the photobiont of the symbiosis emerged as closely related to the North American species *Prasiola borealis*. Our electron microscopy observations provide new information on the process of fungal colonization of the algal thalli, as well as on relationships between the symbionts during different stages of colonization. The special features of this lichen symbiosis are discussed and compared with other examples of fungal symbioses in nature.

Key words: fungal symbiosis; marine lichens; mycophycobiosis; photobionts; Prasiolales; pyrenomycetes; symbiotic continuum; symbiont interactions; ultrastructure; Verrucariaceae.

Lichens are often considered the paradigms of symbiosis. In its broadest sense, the term "symbiosis" refers to a prolonged close association between different species (de Bary, 1879). The common denominator of symbiotic associations may be the acquisition of a novel metabolic capacity by one or more of the symbionts (Douglas, 1994). Some authors use the term "symbiosis" to refer only to associations of mutual benefit to all partners. However, it is often impossible to establish whether an organism benefits from an association or not, even when there is proof that the species interact, for example in dealing with environmental conditions or in matching developmental cycles (Douglas, 1994; Rodriguez and Redman, 2008). Fungal symbiotic associations display a wide range of lifestyles, spanning from mutualism to parasitism, depending on the fitness benefits obtained by their symbionts, and this range has been referred to as the "symbiotic continuum" (Carroll, 1988; Saikkonen et al., 1998; Rodriguez and Redman, 2008).

The capacity of fungi to live symbiotically with algae or cyanobacteria has been independently acquired over time by different taxa (Lutzoni et al., 2001, Gueidan et al., 2008, Schoch et al., 2009). The term "lichen" is applied to a wide variety of associations among fungi, algae, and cyanobacteria that involve life strategies that are often difficult to distinguish. Lichenization is a complex process that is better defined in functional terms than in terms of basic morphology or anatomy. Hawksworth and Honegger (1994) provided the most commonly accepted definition of " lichen": "an ecologically obligate, stable mutualism between an exhabitant fungal partner—the mycobiont—and inhabitant population of extracellularly located unicellular or filamentous algal or cyanobacterial cells—the photobiont." There are many examples of fungus–alga relationships described as "primitive lichens" or "loose symbioses," in which the mycobiont does not act as the exhabitant of true lichen associations (Hawksworth and Honegger, 1994). For instance, some fungal partners inhabit the unmodified algae or cyanobacteria, as in the case of mycophycobioses (Kohlmeyer and Kohlmeyer, 1979; Hawksworth, 1987). *Mastodia tessellata* and *Collemopsidium halodytes* are classified as "borderline lichens" because they lack a true lichen thallus, although they display certain modifications of the algal thallus and the fungus shows some specialization (Kohlmeyer et al., 2004). The case of *M. tessellata* has generated controversy between authors who consider it a true lichen association (e.g., Brodo, 1976; Kappen et al., 1987; Kováčik and Pereira, 2001; Lud et al., 2001; Kohlmeyer et al., 2004) and those who are unwilling to accept this. For instance, Ahmadjian (1967) refers to *Mastodia* as a "lichen-like" association, and others have

[1] Manuscript received 20 October 2009; revision accepted 10 March 2010.

This work was supported by the Spanish Ministry of Science and Innovation (Projects: ANT-COMPLEX–CGL2006-12179-CO2-01, CTM2009-12838-C04, GEOBIO–CGL2006-04658 and CGL2007-64652 (MEC, Spain). The authors thank G. Kantvilas (Tasmania, Australia) for providing the Tasmanian specimens of *M. tessellata*, O. Blanco (Madrid, Spain) and C. Gueidan (CBS, Utrecht, The Netherlands) for their helpful comments and information, H. Thüs (London, UK) for his kind help with the identification of marine *Verrucarias*, and the Fundación Omora (Chile) for assistance and collaboration during field work on Isla Navarino. We also thank A. Ferrero and A. Burton for editing the English text. Gene sequencing was performed at the Centro de Genómica y Proteómica del Parque Científico, Madrid.

[4] Author for correspondence (e-mail: sperezortega@ccma.csic.es)

doi:10.3732/ajb.0900323