Improved propagation method, rapid molecular identification and ultrastructural characterization as a multidisciplinary approach for *Trebouxia* species delimitation

Patricia MOYA[1], Salvador CHIVA[1], Arantxa MOLINS[1], Francisco GARCÍA-BREIJO[2], José REIG-ARMIÑANA[1], Eva BARRENO[1]

[1] Dpto. Botánica, ICBIBE, Fac. CC. Biológicas, Universitat de València, C/ Dr. Moliner 50. 46100-Burjassot, Valencia, Spain.
[2] Dpto. Ecosistemas Agroforestales, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.

The detection of new *Trebouxia* lineages is increasing dramatically, arising the need for an overall revision of taxonomic concepts to identify and delimit species boundaries. Multidisciplinary standardized approaches should be established in the lichenological community to avoid duplicating names.
An improved method for rapid propagation and molecular identification was designed to easily separate and identify the predominant microalgal diversity. Moreover, the coexistence of several phycobionts needs to be studied using comparative ultrastructural analyses in symbiotic and culture states, as some taxonomic features remain fairly stable both within the lichen thalli and in culture, and this would allow us to identify and distinguish them from other co-occurring species. The ability to correlate molecular and ultrastructural data represents a necessary improvement in clarifying the taxonomy of *Trebouxia* diversity both in the symbiotic and culture states.
Buellia zoharyi can be considered a model lichen for setting the boundaries when several *Trebouxia* lineages coexist. We have selected populations covering the entire range (from the Canary Islands, Mediterranean surrounding areas, and western Asia) of the species in the field. Phycobiont phylogenetic analyses were made using both chloroplast (LSU rDNA) and nuclear (nrDNA ITS) molecular markers. In addition, ultrastructural microscopic techniques were used to characterize each of the microalgae found. Our results evidence the presence throughout all populations of at least four different *Trebouxia* lineages. Furthermore, phycobiont coexistence events recovered in all the analyzed populations is strengthened by the presence of at least three lineages of co-occuring microalgae.
The integration of diverse techniques has led to considering symbiotic interactions more complex events, and to deal with lichen symbioses from a multifaceted point of view to efficiently reveal the hidden diversity of intrathalline microalgae inside a single thallus (phycobiont coexistence). (GVA, PROMETEOII/2013/021; MINECO, CGL2012-40058-C02-01; FEDER)