Integrative taxonomy in the classification of neotropical *Lecanora* species (lichenized fungi) from Bolivia

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Introduction

Proper species identification is fundamental for the protection of biodiversity. Species delimitation is sometimes challenging in lichens taxonomy. Two main adversities in the classification of many species are unclear limits of variability and cryptic biodiversity. Integrative taxonomy is very helpful in clarifying doubts. It is currently widely used as a tool in species delimitation (Frisch et al. 2020). Lichens are symbiotic organisms. They consist of fungal (mycobiont) and algal (photobiont) cells. The latest research on lichen structures has proven the presence of the third component, specific basidiomycetes yeast embedded in the cortex (Spiribille et al. 2016).

Lecanora Ach. represents one of the largest and the most diverse genus in the lichen world. It is represented in all climate zone and biogeographical regions by about 600 species (Bungartz et al. 2020). Until 2000, delimitation within that genus was based on morphological and chemical data. The inclusion of molecular data better resolves the phylogeny. Despite many attempts made towards *Lecanora* classification, some are inconclusive (Grube et al. 2004, Perez-Ortega et al. 2010)

Bolivia is one of the largest regions of great biodiversity (Ibisch and Merida 2004). The ecosystem is rich in lichen variety (Flakus et al. 2012). The lichenological research conducted so far in this area has recorded hundreds of new species (Flakus ana Kukwa 2012). Even new species for South America was discovered (Śliwa et al. 2012). However, there are still various taxonomic groups not well recognized in Bolivia. The best example of it is the genus, *Lecanora* Ach. So far, a total of 27 species of this genus have been recorded in Bolivia (Śliwa et al. 2012, Rodriguez de Flakus et al. 2014, Śliwa et al. 2014).

Species delimitation of the genus *Lecanora* Ach.

In the lichen world, it is important to use all possibilities to create a classification that will reflect the actual name of the species as closely as possible. For this purpose, the classification is supported by three pieces of evidence: morphology, chemistry, and phylogenetic position.

Morphology and anatomy

Lecanora is characterized by lecanorine apothecia. It has simple, hyaline ascospores produced in *Lecanora* type asci (Burgantz et al. 2020). In morphological tests, a 25% KOH (K) solution is used. In K ascospores are measured. To observe the solubility of the crystals, apart from K, a 65% nitric acid (N) solution is used. Granulation is observed in polarized light (Śliwa et al. 2012)

Chemistry

Species of the genus *Lecanora* have a characteristic composition of secondary metabolites. Thin-layer chromatography (TLC) is used to determine secondary metabolites in lichens, according to the methodology of Culberson and Kristionson 1970 and Orange et al. 2001. Fragments of the lichen thallus are placed in acetone. The solution is applied to silicate plates. Separation is recommended in three eluents for accurate determination of secondary metabolites.

Phylogenetic position

Molecular studies help determine species of the genus Lecanora. ITS, mtSSU rDNA, nucLSU, RPB1, RPB2, and MCM7 genes are used for phylogenetic analyzes. The multi-gene concept usually suggests new, more supported classifications within lacanoroid lichens. It allows a better understanding of the evolution of this group of lichens. The phylogenesis is reconstructed with the Maximum Likelihood (ML) method and supported also by Bayesian analyzes. Well supported clades are those with bootstrap support ≥70% under ML (Zhao et al. 2016).

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Bibliography

1. Bungartz F., Elix J.A. & Printzen C. (2020) Lecanoroid lichens in the Galapagos Islands: the genera *Lecanora*, *Protoparmeliopsis*, and *Vainionora* (Lecanoraceae, Lecanoromycetes). Phytotaxa 431(1): 001–085.
2. Culberson C.F., Kristinsson H. (1970) A standardized method for the identification of lichen products. Jour. Chromatogr. 46: 85–93.
3. Flakus A., Kukwa M. 2012. New species of lichenicolous fungi from Bolivia. Lichenologist 44: 469–477.
4. Flakus A., Rodriguez Saavedra P., Kukwa M. 2012. A new species, new combinations and new records of *Hypotrachyna* and *Remototrachyna* from Bolivia. Mycotaxon 119: 157–166.
5. Frisch A., Moen V.S., Grube M., Bendiksby M. (2020) Integrative taxonomy confirms three species of *Coniocarpon* (Arthoniaceae) in Norway. MycoKeys 62: 27–51.
6. Grube M., Baloch E., Arup U. (2004) A phylogenetic study of the *Lecanora* rupicola group (Lecanoraceae, Ascomycota). Mycological Research 108 (5): 506–514
7. Ibisch P.L., Mérida G. (2004) Biodiversity: The richness of Bolivia. State of knowledge and conservation. Editorial FAN, Santa Cruz de la Sierra, Bolivia
8. Lumbsch H.T., Leavitt S.D. (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. Fungal Diversity 50: 59–72

9. Orange A., James P.W., White FJ. (2001) Microchemical methods for the identification of lichens. London, British Lichen Society

10. Pérez-Ortega S., Spribille T., Palice Z., Elix J.A., Printzen C. (2010) A molecular phylogeny of the *Lecanora varia* group, including a new species from western North America. Mycological Progress 9 (4): 523–535

11. Rodriguez Flakus P., Flakus A., Kukwa M., Lücking R., Meneses R.I., Rivas Plata E., Stanton D., Truong C., Vargas R. (2012) Preliminary catalogue of lichens and lichenicolous lichens from Bolivia. Version 1.2. W. Szafer Institute of Botany, Polish Academy of Sciences, Krakow http://botan.botany.pl/lichens-bolivia

12. Spribille T., Tuovinen V., Resl P., Vanderpool D., Wolinski H., Aime M.C., Schneider K., Stabentheiner E., Toome-Heller M., Thor G., et al. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science 353, 488–492.

13. Śliwa L., Wilk K., Rodriguez Flakus P., Flakus A. (2012) New records of *Lecanora* for Bolivia. Mycotaxon 121: 385–392.

14. Śliwa L., Rodriguez Flakus P., Wilk K., Flakus A. (2014) New records of *Lecanora* for Bolivia. II. Journal article : Polish Botanical Journl 59 (1): 97-103

15. Zhao, X., Leavitt, S.D., Zhao, Z.T., Zhang, L.L., Arup, U., Grube, M., Pérez- Ortega, S., Printzen, C., Śliwa, L., Kraichak, E., Divakar, P.K., Crespo, A. & Lumbsch, H.T. (2016) Towards a revised generic classification of lecanoroid lichens (Lecanoraceae, Ascomycota) based on molecular, morphological and chemical evidence. Fungal Diversity 78 (1): 293–304