The Lichenologist (2021), **53**, 231–232
doi:10.1017/S0024282921000189

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Response

Response to Clerc & Naciri (2021) *Usnea dasopoga* (Ach.) Nyl. and *U. barbata* (L.) F. H. Wigg. (Ascomycetes, *Parmeliaceae*) are two different species: a plea for reliable identifications in molecular studies

During the last thirty years phylogenetic analyses based on molecular characters have developed from simple single-locus studies into complicated surveys containing multi-locus phylogenies, species trees and possibilities to evaluate the evolutionary history of characters. This has been an exciting era for systematists, including fungal taxonomists. The majority of lichenized taxa have originally been described using morphological characters s. lat. (i.e. traits related to morphology, anatomy and chemistry), and thus the congruence between traditional species description and species delimitation based on their molecular evolutionary history remains a challenge. The use of morphological characters has not been abandoned, as predicted or advocated by some researchers (Lumbsch & Leavitt 2011; Hibbett *et al.* 2016). However, we now know that the morphology-based approach to species recognition has also been demonstrated in several cases to substantially misrepresent diversity, as it either underestimates the occurrence of cryptic species (Altermann *et al.* 2014; Boluda *et al.* 2016) or, on the contrary, overestimates the true diversity due to high levels of intra-specific morphological and chemical variation (Leavitt *et al.* 2011; Velmala *et al.* 2014). Therefore, morphological characters continue to be useful for the delimitation of species, but only if their discriminative ability has been verified using phylogenetic analyses.

Phenotypic species recognition in the genus *Usnea* is particularly complicated; the species are delimited by distinctive combinations of diagnostic morphological traits (Clerc 1998, 2011) which may, however, in certain cases be poorly developed or even absent (Clerc 2011). This is aggravated by the fact that there are a great number of *Usnea* species and high intra-specific variation, leading to a situation where most lichenologists are not able to identify *Usnea* species or do not undertake the task at all. This drives researchers to find other solutions. An alternative and modern way for the identification of species is DNA barcoding (Schindel & Miller 2005). A test of the success of DNA barcoding with ITS as the barcoding marker in a case study of 112 *Usnea* specimens from the British Isles (Kelly *et al.* 2011) was encouraging as the method assigned a high percentage of samples to correct species. Recent thorough analysis (Lücking *et al.* 2020) found usage of ITS to be a good first approximation to assess species delimitation and recognition in *Usnea*; however, species boundaries can be reliably established using several markers and different phylogenetic tools.

Our main interest in the paper by Mark *et al.* (2016) focused on phylogenetic issues as we attempted to reconstruct evolutionary relationships in sect. *Usnea* using DNA data from six markers of 144 specimens, and to determine evolutionarily independent lineages using multiple coalescent-based species delimitation approaches. To perform these tasks, we also followed a traditional approach using morphological characters to identify the samples. Clerc & Naciri (2021) revise the traditional identification of 35 samples used in our analyses (table 1 in Clerc & Naciri (2021)) and present the details of their morphological and chemical characters. Of these, 11 samples appeared to be misidentified in Mark *et al.* (2016). The main disparity arose from our identification of nine *U. dasopoga* specimens as *U. barbata*. Indeed, the distinction between the two species caused difficulties for us, partly because some of the samples used appeared to be atypical or young. It is encouraging to learn that a new, previously unused character, the ratio of medulla/cortex (M/C), has proved to be the most useful discriminant in separating *U. barbata* and *U. dasopoga* (fig. 2 in Clerc & Naciri (2021)).

Accepting the new morphological identifications, the interpretation of two clades (viz. *barbata-chaetophora-dasypoga-diplotypus* clade and *barbata-intermedia-lapponica-substerilis* clade) on our phylogenetic tree (fig. 1b in Mark *et al.* (2016)) must be reconsidered. The first of the two clades, now the *dasopoga* clade in Clerc & Naciri (2021), contains only *U. dasopoga* specimens. However, the clade has low support on the Bayesian and maximum likelihood consensus tree, on account of which the *U. dasopoga* monophyly is not statistically supported and its sister relationships are unresolved in our analyses. The composition of species in the second, strongly supported clade remains variable, containing samples of *U. barbata, U. intermedia, U. perplexans* (= *U. lapponica*) and *U. substerilis*. The subclades within this clade do not have strong support and morphological species are intermixed between them. We want to point out that the synonymization of *U. substerilis* under *U. perplexans* (= *U. lapponica*) proposed by us was not based merely on the well-supported sister relationship of two samples (SBS15 and LAP5), and thus the reidentification of the latter does not refute the synonymization. This synonymy was also reasonably supported by Lücking *et al.* (2020). It can be inferred from our phylogenetic tree with new expert identifications based on

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Cite this article: Randlane T and Mark K (2021) Response to Clerc & Naciri (2021) *Usnea dasopoga* (Ach.) Nyl. and *U. barbata* (L.) F. H. Wigg. (Ascomycetes, *Parmeliaceae*) are two different species: a plea for reliable identifications in molecular studies. *Lichenologist* **53**, 231–232. https://doi.org/10.1017/S0024282921000189

morphological characters (fig. 1 in Clerc & Naciri (2021)) that the phylogenetic distinction and relationships between *U. barbata*, *U. intermedia*, *U. perplexans* (= *U. lapponica*) and *U. substerilis* remain unclear. This group needs new, improved evaluation with molecular tools of higher refinement.

Clerc & Naciri (2021) conclude that *Usnea barbata* and *U. dasopoga* are morphologically and anatomically truly distinct species for which molecular data do not support their conspecificity; however, phylogenetic analyses of molecular data (including the analyses performed in Mark *et al.* (2016) and re-evaluated by Clerc & Naciri (2021)) have so far not corroborated the monophyletic origin of either species. Rapid diversification and the occurrence of young species, reflected by a high morphological divergence but a low genetic variation, are probably the processes responsible for this in sect. *Usnea*, the conclusion that was presented in our original paper and is not questioned by Clerc & Naciri (2021) either.

Lessons learnt:

1. We thank Philippe Clerc and Yamama Naciri for pointing out the misidentifications of the analyzed *Usnea* specimens, and are very pleased that the problem of the confusing species composition within the *barbata-chaetophora-dasypoga-diplotypus* clade of fig. 1b (Mark *et al.* 2016), now the *dasopoga* clade, seems to be solved. Moreover, this case demonstrates that phylogenetic analyses, if appropriate and correctly performed, are usable and useful also after morphological reidentifications.
2. The final recommendations given by Clerc & Naciri (2021) are relevant and constructive, and should be taken into account, if possible. It is generally considered that morphological identification of lichen species is easier, cheaper and less time-consuming than identification based on DNA sequences. The present case vividly questions this presumption. Phenotypic species recognition has also been viewed as an occupation comparable to art. With *Usnea* it is truly a very complicated art and, as demonstrated by Clerc & Naciri (2021), in solitary cases the traditional morphological identification could not even be confirmed by the discriminant *a posteriori* analysis based on anatomical measures (table 1 in Clerc & Naciri (2021)). A future undertaking will be to delimit several species in this genus on a phylogenetic basis and to search for the best phenotypic characters for the recognition of these species.

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