Addressing inconsistencies in Cyperaceae and Juncaceae taxonomy: Comment on Brožová et al. (2022)

Tammy L. Elliott^{a,*}, Isabel Larridon^{b,c}, Russell L. Barrett^d, Jeremy J. Bruhl^e, Suzana M. Costa^f, Marcial Escudero^g, Andrew L. Hipp^{h,i}, Pedro Jim´enez-Mejías^{j,k}, Jan Kirschner^l, Modesto Lucen˜o^m, Jos´e Ignacio Ma´rquez-Corro^{b,m}, Santiago Martín-Bravo^m, Eric H. Roalsonⁿ, Ilias Semmouri^o, Daniel Spalink^p, William Wayt Thomas^q, Tamara Villaverde^r, Karen L. Wilson^d, A. Muthama Muasya^s

^a Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic ^b Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, United Kingdom

^c Systematic and Evolutionary Botany Lab, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

^d National Herbarium of New South Wales, Australian Institute of Botanical Science, Australian Botanic Garden, Locked Bag 6002, Mount Annan, New South Wales 2567, Australia

^e Botany and N.C.W. Beadle Herbarium, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia

^f Departamento de Biologia, Instituto de Cièncias Naturais, Universidade Federal de Lavras, Caixa Postal 3037, Lavras, Minas Gerais, CEP 37200-000, Brasil

^g Department of Plant Biology and Ecology, University of Seville, Reina Mercedes 6, 41012 Seville, Spain

^h The Morton Arboretum, 4100 Illinois Route 53, Lisle, IL 60532-1293, the United States of America

ⁱ The Field Museum, 1400S. Lake Shore Dr., Chicago, IL 60605, the United States of America

^j Departamento de Biología (Botánica), Facultad de Ciencias Biológicas, Universidad Autónoma de Madrid, C/Darwin, 2, 28049 Madrid, Spain

^k Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad Autónoma de Madrid, 28049 Madrid, Spain

¹ Institute of Botany of the Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic

^m Botany Area, Department of Molecular Biology and Biochemical Engineering, Universidad Pablo de Olavide, ctra. de Utrera km. 1, 41013, Seville, Spain

ⁿ School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, the United States of America

^o Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, Faculty of Bioscience Engineering, 9000 Ghent, Belgium

^p Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843 the United States of America

^q New York Botanical Garden, Bronx, NY 10458-5126, the United States of America

^r Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, C/Tulipán s/n, 28933 Móstoles, Madrid, Spain

^s Bolus Herbarium, Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch, Cape Town 7701, South Africa

* Corresponding author.

E-mail address: tammy.elliott@mail.mcgill.ca (T.L. Elliott).

1. Introduction

Brožová et al. (2022) present a study, "*Toward finally unraveling the phylogenetic relationships of Juncaceae with respect to another cyperid family, Cyperaceae*", with the premise of revising the phylogenetic relationships in Juncaceae and Cyperaceae based on Sanger sequencing of one nuclear rDNA (ITS) and two plastid regions (the gene *rbcL* and the spacer *trnL*-F). While the paper highlights the need for ongoing taxonomic revision in Juncaceae, we are concerned that their analyses and conclusions could lead to nomenclatural instability and unnecessary confusion among researchers, particularly those working in cyperids (Cyperaceae, Juncaceae and Thurniaceae) and closely allied monocot lineages. Our concerns stem from the use of incomplete datasets and the failure to integrate recently published taxonomic literature. As a result, the systematic conclusions drawn by the authors are outdated and conflict with previous studies based on more complete phylogenomic datasets (e.g., Larridon et al., 2021), leading to unjustifiable (i.e., proposed recognition of non-monophyletic genera) and superfluous (i.e., already completed nomenclatural changes) recommendations.

While we acknowledge that Brožová et al. (2022) correctly show that Juncaceae is in need of further scientific attention, their conclusions about the larger clade containing both Cyperaceae and Juncaceae are based on a limited understanding of Cyperaceae taxonomy. As the authors accurately highlight in their Introduction, Cyperaceae and Juncaceae are both well established as monophyletic families; the only studies that confused this issue were based on biased/incomplete sequence data, which was clarified nearly 20 years ago (e.g., Kristiansen et al., 2005; Drábková and Vlček, 2007). However, it is somewhat unclear from the outset whether Brožová et al. (2022) are more focused on Juncaceae (cf. Abstract, and the aims in the Introduction), or on cyperids (cf. Title, and the Introduction). By including Cyperaceae in their study, the authors risk taking the understanding of Cyperaceae taxonomy backwards since the paper presents outdated conclusions, potentially confounding readers.

2. Review of Cyperaceae systematics

Since the Cyperaceae classification of Goetghebeur (1998), the circumscription of genera in this family has been altered rather extensively, mostly in tribes Abildgaardieae, Cariceae, Cryptangieae, Cypereae, Eleocharideae, Schoeneae and Scirpeae, and in the Fuireneae s.l. grade (tribes Pseudoschoeneae and Schoenoplecteae). Larridon (2022) provides an overview of the main rearrangements that have been made in the last c. 25 years, which include publication of a range of new genera (e.g., Calliscirpus C.N.Gilmour, J.R.Starr & Naczi, Krenakia S.M. Costa, Zulustylis Muasya), new tribes (e.g., Khaosokieae, Pseudoschoeneae, Sumatroscirpeae), recircumscription of genera and tribes (e.g., *Carex* L., *Cyperus* L., *Schoenus* L.; tribe Fuireneae) or synonymising smaller genera into more broadly circumscribed genera (e.g., former genera *Kyllinga* Rottb., *Nemum* Desv., *Pycreus* P.Beauv., *Schoenoxiphium* Nees, *Uncinia* Pers.). These changes culminated in the publication of a new tribal, subtribal and generic classification of Cyperaceae (Larridon et al., 2021) based on targeted sequencing data, which includes up

to 353 nuclear genes.

Unfortunately, the Introduction of Brožová et al. (2022) misrepresents the current state of knowledge in Cyperaceae systematics. Brožová et al. (2022) refer to Larridon et al. (2021), thus giving the impression that the most recent studies focusing on Cyperaceae systematics were incorporated into their work. However, the authors overlook these changes and make statements about the Cyperaceae, such as "Finer segmentation into tribes is even more complicated and still ongoing" (Brožová et al., 2022). Importantly, Brožová et al. (2022) fail to acknowledge that Larridon et al. (2021) presents a well-supported, phylogeny-informed classification for the family with broad support from the Cyperaceae research community (as shown by the extensive authorship).

Brožová et al. (2022) go on to state, "Recently, molecular phylogenetic studies on Cyperaceae have relied heavily on relatively few loci, such as a selection of plastid markers and the nuclear markers ITS and ETS (Semmouri et al., 2019; Léveillé-Bourret et al., 2018; Larridon et al., 2020; Starr et al., 2021; Villaverde et al., 2020a, 2020b, 2021)." However, the last four studies listed (five in their list, as Villaverde et al., 2020a, 2020b refer to the same paper, Villaverde et al., 2020) are based on genome-scale high-throughput sequencing data, using either targeted sequencing (HybSeq) or restriction-site associated DNA sequencing (RAD-seq), with the latter study (Villaverde et al., 2021) not even focused on systematics. Furthermore, Brožová et al. (2022) state: "The most recent study by Larridon et al. (2021) presented a comprehensive family-wide phylogenomic study of this family based on targeted sequencing using the Angiosperms353 probe kit sampling 311 accessions. They also provided a taxonomic treatment including five new subtribes." This statement is particularly close in wording to what is written in the abstract of Larridon et al. (2021) and overlooks important information provided in the article, such as the full classification of Cyperaceae at tribal and generic levels.

3. Misrepresentation of existing taxonomic knowledge

We are concerned that the misrepresentation of the current state of taxonomic knowledge of the Cyperaceae will result in unnecessary confusion and incorrect inferences. Spurious conclusions are manifest in Brožová et al. (2022); for example, they devote a paragraph of section

4.2. of the Discussion (*Notes on the Cyperaceae*) to explaining the phylogenetic and morphological relationships of three *Schoenoplectus* species, eventually concluding that these species should be transferred to *Schoenoplectiella*. However, the three proposed changes suggested by Brožová et al. (2022) were published a decade ago (Hayasaka, 2012) and have already been implemented in the Plants of the World Online (POWO, 2022) database. Thus, these recommendations from Brožová et al. (2022) do not represent new information and might lead to confusion in the scientific community.

The authors state in the final paragraph of section 4.2. of the Discussion, "Our analyses revealed a few more cases of species that did not align well and might have been represented by a problematic sequence: *Amphiscirpus nevadensis* (ITS sequence; AF190618.1) and *Trichophorum cespitosum* (*rbcL* sequence; Y12969.1). Resampling

of these taxa might shed light on this problem." Importantly, this research has already been done (e.g., Léveillé-Bourret et al., 2015, 2020). Thus, Brožová et al. (2022) again propose a confusing step backwards in our knowledge of the systematics and classification of the Cyperaceae family.

4. Limited representation of available sequence data

The choice of the DNA regions sequenced in Brožová et al. (2022) has not been properly justified. While the authors state, "As the amount of accessible molecular data is still increasing, the analysis of these data is easily obtainable, and there are promising means of resolving intra- family relationships", several widely sequenced DNA regions for the study group (e.g., ETS, *rps16*, *matK*) were excluded. Given that previous studies on the Juncaceae used DNA regions not included in this study (*psbA-trnH*, *atp1*), the specific DNA regions chosen by the authors have likely influenced their results. The authors also fail to address the relative merits of Sanger versus genomic-scale datasets for estimating evolutionary relationships and phylogeny-based classifications. While both approaches have yielded insights into the systematics of angio- sperms, the current scientific consensus is that genome-scale datasets are better able to accurately determine evolutionary patterns and processes. Although single-to-few DNA region phylogenetic analyses remain valuable, in this case the three DNA region analysis of Cyperaceae of Brožová et al. (2022) provides far less resolution than the available targeted enrichment dataset published in Larridon et al. (2021).

5. Future directions for the Juncaceae

Brožová et al. (2022) should be commended for attempting to tackle important questions related to Juncaceae taxonomy, a family that has received relatively little taxonomic attention in the last 20 years compared to Cyperaceae. However, we are concerned about the methods, results, interpretations and taxonomic framework presented by the authors for Juncaceae. The taxonomic framework relies heavily on that presented in Kirschner et al. (1999) and Kirschner (2002), which was acknowledged by Kirschner et al. as being a pragmatic, status quo treatment and is not necessarily congruent with the findings from the molecular analyses presented by Brožová et al. (2022). We consider that current data (molecular combined with morphological) should direct any novel taxonomic proposals, instead of relying so heavily on an older framework that was based on interpretations of primarily morphological and geographical data.

In their Abstract, Brožová et al. (2022) state that the six new genera that they propose recover monophyly in Juncus. The reservations voiced by Kirschner et al. (1999, page 379) about the circumscriptions of several sections in subgenus *Juncus* (e.g., sect. *Graminifolii*, sect. *Stygiopsis*, sect. *Ozophyllum*) appear not to have been fully addressed by Brožová et al. (2022), and instead previous non-monophyletic groupings have been maintained. After examining Table 3, it is clear that the suggested changes in *Juncus* taxonomy almost directly follow the taxonomic

framework proposed by Kirschner et al. (1999), with two changes: sect. *Graminifolii* is split into the genera 'Australojuncus' and 'Boreojuncus', whereas sect. *Iridifolii* and sect. *Ozophyllum* are combined to form genus 'Verojuncus'. Despite the authors' statement that they aimed to create monophyletic genera, the proposed new genus 'Boreojuncus' is shown to be nested within 'Verojuncus' (Brožová et al. 2022, Figs. 1–3). In addition, the proposed genus 'Juncinella' composed of species from sect. *Caespitosi* is polyphyletic, which is worrying because only two out of the 16 species were included in the phylogeny, and the two samples of *Juncus capitatus* appear to be evolutionarily distant from each other (Brožová et al. 2022, Fig. 3).

Additionally, we found discrepancies in the number of sequences included in Table S1 and the Results section, and when multiple tips are included in the phylogenetic reconstructions for a single species, it is not possible to distinguish which sequences correspond to each tip (e.g., *Juncus capitatus*, *Trichophorum cespitosum*). Many branches of their phylogenies also appear to have little support based on maximum likelihood and Bayesian inference analyses (Brožová et al. 2022, Figs. 2,3). Furthermore, the odd position of several *Juncus* species in the phylogenetic trees might be a consequence of the high level of polyploidy (possibly allopolyploidy) reported in the genus (Drábková, 2013). The case of *J. capitatus* also brings up the possibility that sequences from distinct species were included to represent a single species in the phylogeny (which might explain the evolutionary distance between the two conspecific tips), as the accession numbers listed in Table S1 show that the sequences used for *J. capitatus* were from material collected from three very distant locations (United States, Czechia and the Azores). These issues raise challenges to interpreting the support for taxonomic proposals in the paper. We are, moreover, concerned that the updated framework presented, based on three DNA regions with relatively low coverage across taxa, might conflict with genomic-scale studies in the not-so-distant future. Such repeated taxonomic changes might lead to unfortunate or unnecessary disruptions to names in a widely distributed and ecologically important group of monocots.

The number of proposed nomenclatural changes, if effected in future papers, also seems disruptive. The main justification for the proposed generic rearrangement of Juncaceae seems to be that five austral genera, each composed of relatively few species, are nested within the larger genus *Juncus: Rostkovia* (2 spp.), *Marsippospermum* (4 spp.), *Distichia* (3 spp.), *Patosia* (1 sp.) and *Oxychloe* (5 spp.). Beyond the Sanger-based monophyly of *Juncus*, this paper does not discuss whether these genera deserve to be maintained because of their apomorphies, or whether they could be considered outliers within the plesiomorphic morphology of a more broadly conceived circumscription of *Juncus*. Whatever the rationale for recognizing them, the number of nomenclatural changes to be accepted to merge these five austral genera within *Juncus* would be far less than the number of combinations necessary to split the genus. A similar debate was addressed for large non-monophyletic genera of Cyperaceae, and the different possible nomenclatural solutions thoroughly debated prior to making taxonomic changes. This, for example, led to the merging of small to medium-sized satellite genera within a broadly conceived *Carex* (Global Carex Group, 2015) and *Cyperus* (Larridon et al., 2011, 2014, Bauters et al., 2014; Pereira-Silva et al., 2020) as the preferred choice among several possible solutions. While taxonomic decisions of this type are in

part stylistic, we would urge the coupling of strong evidence with robust community dialogue before proposing such a dramatic taxonomic rearrangement.

Brožová et al. (2022) includes several nomenclatural problems: (1) The taxonomic rank of 'supragenus' introduced by Brožová et al. (2022) is not recognized under the *International Code of Nomenclature for algae, fungi and plants* (ICN: Turland et al., 2018). While this new rank could be proposed for the ICN with appropriate justification (including appropriate suffix to denote the rank), it is currently invalid. In addition, the form of the rank would more correctly be 'supergenus'. Further, it would be incorrect to have 'Juncus' as a 'supergenus' name, as it is already a recognized generic name and should not be repeated in that same form at a higher rank without the appropriate suffix. The authors could, of course, use 'Supergenus' is illegitimate, since *Cephaloxys* Desv. (a legitimate and valid name) is listed in synonymy and shares the same type (*Juncus repens* Michx.). (3) The name of the genus 'Agathryon' is legitimate but superfluous because it includes the legitimate name Tenageia Rchb.

The taxonomy, character evolution, biogeography and evolution of Juncaceae merits more study before adopting any taxonomic changes, as we think there is likely to be a more parsimonious framework that would be easier for all to adopt. If the recovered topology proves to be supported by more extensive studies, it is, for example, important to reassess the merits of splitting *Juncus* into several segregate genera versus expanding the genus so it includes the five strictly austral genera. It is our view that whichever choice is made, the overall goal of future studies on Juncaceae systematics should be to propose a framework that will be relatively straight-forward to implement by other researchers and those working in the field.

6. Concluding remarks

Contributions from diverse voices is key in scientific discovery and progress, with the caveat that the work is scientifically rigorous and passes peer-review. This review process is a powerful step in the sharing of knowledge among scientists. However, it appears peer-review failed to ensure that Brožová et al. (2022) integrated recent taxonomic knowledge and more rigorous approaches into their work. In the case of Juncaceae taxonomy, we caution against taking up their taxonomic proposals and recommend further research involving several different approaches before adapting a new nomenclatural framework.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper. However, the authors share a scientific interest and publication record with regards to Cyperaceae systematics and classification and a similar, but more limited, record with Juncaceae, which could be considered a competing interest. However, we emphasize that we welcome all researchers globally to work on Cyperaceae. To promote collaboration, we have recently brought together the international community studying Cyperaceae under the umbrella of the International Sedge Society that is open to anyone with an interest in the family to join. We are very happy for anyone to enhance knowledge of the evo- lution and diversity of the Cyperaceae, whether or not in collaboration with us, and we encourage the development of similar networks to enhance understanding of the Juncaceae.

Data availability

No data was used for the research described in the article.

Acknowledgements

We thank John McNeill, Nicholas Turland and John Wiersema for nomenclatural advice.

References

Bauters, K., Larridon, I., Reynders, M., Huygh, W., Asselman, P., Vrijdaghs, A., Muasya, A.M., Goetghebeur, P., 2014. A new classification for *Lipocarpha* and *Volkiella* as infrageneric taxa of *Cyperus* s.l. (Cypereae, Cyperoideae, Cyperaceae): in- sights from species tree reconstruction supplemented with morphological and floral developmental data. *Phytotaxa* 166, 1–32. https://doi.org/10.11646/phytotax a.166.1.1.

Brožová, V., Pro'cko'w, J., et al., 2022. Toward finally unraveling the phylogenetic relationships of Juncaceae with respect to another cyperid family. Cyperaceae. *Mol. Phylogenet. Evol.* 177, 107588 https://doi.org/10.1016/j.ympev.2022.107588.

Drábková, L.Z., 2013. A Survey of karyological phenomena in the Juncaceae with emphasis on chromosome number variation and evolution. Bot. Rev. 79, 401–446. https://doi.org/10.1007/s12229-013-9127-6.

Drábková, L.Z., and Vlček, C., 2007. The phylogenetic position of *Oxychloe* (Juncaceae): evidence from morphology, nuclear and plastid DNA regions. Taxon 56, 95–102. https://doi.org/10.2307/25065739.

Global Carex Group, 2015. Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): a new broader circumscription. Botanical Journal of the Linnaean Society 179, 1–42.

Goetghebeur, P., 1998. Cyperaceae. In: Kubitzki, K. (Ed.), Flowering Plants Monocotyledons. The Families and Genera of Vascular Plants, vol 4. Springer, Berlin, Heidelberg.

Hayasaka, E., 2012. Delineation of *Schoenoplectiella* Lye (Cyperaceae), a genus newly segregated from *Schoenoplectus* (Rchb.) Palla. *J. Jap. Bot.* 87, 169–186.

Kirschner, J. (Ed.), 2002. Juncaceae 1–3, Species Plantarum: Flora of the World Parts 6–8. Australian Biological Resources Study, Canberra, Australia.

Kirschner, J., Novara, L.J., et al., 1999. Supraspecific division of the genus *Juncus* (Juncaceae). *Folia Geobot*. 34, 377–390. https://doi.org/10.1007/BF02912822.

Kristiansen, K.A., Cilieborg, M., et al., 2005. DNA taxonomy: the riddle of *Oxychloe* (Juncaceae). Syst. Bot. 30, 284–289. https://doi.org/10.1600/0363644054223710.

Larridon, I., 2022. A linear classification of Cyperaceae. *Kew Bull.* 77, 309–315. https:// doi.org/10.1007/s12225-022-10010-x.

Larridon, I., Reynders, M., et al., 2011. Taxonomic changes in C3 *Cyperus* (Cyperaceae) supported by molecular phylogenetic data, morphology, embryology, ontogeny and anatomy. *Plant Ecol. Evol.* 144, 327–356. https://doi.org/10.5091/ plecevo.2011.653.

Larridon, I., Bauters, K., et al., 2014. Taxonomic changes in C4 *Cyperus* (Cypereae, Cyperoideae, Cyperaceae): combining the sedge genera *Ascolepis*, *Kyllinga* and *Pycreus* into *Cyperus* s.l. *Phytotaxa* 166, 33–48. https://doi.org/10.11646/ phytotaxa.166.1.2.

Larridon, I., Villaverde, T., et al., 2020. Tackling rapid radiations with targeted sequencing. *Fron. Pl. Sci.* 10, 1655. https://doi.org/10.3389/fpls.2019.01655.

Larridon, I., Zuntini, A., et al., 2021. A new classification of Cyperaceae (Poales) supported by phylogenomic data. *J. Syst. Evol.* 59, 852–895. https://doi.org/10.1111/jse.12757.

Léveillé-Bourret, E., Donadío, S., Gilmour, C.N., Starr, J.R., 2015. *Rhodoscirpus* (Cyperaceae: Scirpeae), a new South American sedge genus supported by molecular, morphological, anatomical and embryological data. *Taxon* 64, 931–944. https://doi.org/10.12705/645.4.

Léveillé-Bourret, E´, Starr, J.R., et al., 2018. Why are there so many sedges? Sumatroscirpeae, a missing piece in the evolutionary puzzle of the giant genus *Carex* (Cyperaceae). *Mol. Phylogenet. Evol.* 119, 93–104. https://doi.org/10.1016/j. ympev.2017.10.025.

Léveillé-Bourret, E., Chen, B.-H., et al., 2020. RAD sequencing resolves the phylogeny, taxonomy and biogeography of Trichophoreae despite a recent rapid radiation (Cyperaceae). *Mol. Phylogenet. Evol.* 145, 106727 https://doi.org/10.1016/j. ympev.2019.106727.

Pereira-Silva, L., Trevisan, R., et al., 2020. Combining the small South American genus *Androtrichum* in *Cyperus* (Cyperaceae). *Plant Ecol. Evol.* 153, 446–454. https://doi.org/10.5091/plecevo.2020.1746.

Semmouri, I., Bauters, K., et al., 2019. Phylogeny and systematics of Cyperaceae, the evolution and importance of embryo morphology. *Bot. Rev.* 85, 1–39. https://doi.org/10.1007/s12229-018-9202-0.

Starr, J.R., Jiménez-Mejías, P., et al., 2021. Targeted sequencing supports morphology and embryo features in resolving the classification of Cyperaceae tribe Fuireneae s.l. *J. Syst. Evol.* 59, 809–832. https://doi.org/10.1111/jse.12721.

POWO (2022). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 18 November 2022.

Turland, N.J., Wiersema, J.H., Barrie, et al. (eds.), 2018. International Code of Nomen- clature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. https://doi.org/10.12705/Code.2018. Villaverde, T., Jim´enez-Mejías, P., et al., & The Global Carex Group, 2020. A new classification of *Carex* (Cyperaceae) subgenera supported by a HybSeq backbone phylogenetic tree. Bot. J. Linn. Soc., 194(2), 141–163. https://doi.org/10.1093/ botlinnean/boaa042.

Villaverde, T., Maguilla, E., Luceño, M., Hipp, A.L., 2021. Assessing the sensitivity of divergence time estimates to locus sampling, calibration points, and model priors in a RAD-seq phylogeny of *Carex* section *Schoenoxiphium*.
J. Syst. Evol. 59 (4), 687–697. https://doi.org/10.1111/jse.12724.