Zootaxa 5481 (2): 297–300
https://www.mapress.com/zt/

Correspondence

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5481.2.8
http://zoobank.org/urn:lsid:zoobank.org:pub:5F85DD95-F6EA-42F5-8E7C-837591451487

Splitters versus Lumpers? Subspecies designations must rely on robust morphological and/or genetic data—a response to Nabholz *et al.* (2024)

AXEL HOCHKIRCH[1], LARA-SOPHIE DEY[2] & MARTIN HUSEMANN[3]
[1]*Musée national d'histoire naturelle Luxembourg, 25 rue Münster, 2160 Luxembourg, Luxembourg.*
[2]*Senckenberg Deutsches Entomologisches Institut, Eberswalder Straße 90, 15374 Müncheberg, Germany.*
https://orcid.org/0000-0003-1182-0162
[3]*Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstraße 13, 76133 Karlsruhe, Germany.*
https://orcid.org/0000-0001-5536-6681
Corresponding Author. *https://orcid.org/0000-0002-4475-0394*

Nabholz *et al.* (2024) published an important comment on our study on the phylogeography of *Oedipoda* Latreille, 1829, reflecting some discussions we also had internally when writing the paper. While they agree with our conclusions of raising *Oedipoda mauritanica* Lucas, 1847, *Oedipoda meridionalis* Ramme, 1913 and *Oedipoda pentagonalis* Jannone, 1937 to species level, they challenge our synonymization of a subspecies described by the senior author (*Oedipoda fuscocincta morini* Defaut, 2006). Nabholz *et al.* (2024) argue that numerous cases exist in which mitochondrial phylogenies are not in line with valid species designations. We completely agree with this and we have documented such unresolved species groups before, such as the *Sphingonotus caerulans* group (Husemann *et al.* 2014), or the various species in the tribe Bryodemini (Kock *et al.* 2024). For the same reason, we have refrained from taxonomic conclusions on the species status of *Oedipoda germanica* (Latreille, 1804) and *Oedipoda fuscocincta* Lucas, 1847. However, in these cases robust morphological data (and in the case of *Sphingonotus* Fieber, 1852 also bioacoustic data) support the species status of the taxa in question. In the case of subspecies, the situation is way more complex as subspecies definitions lack objective criteria and frequently fail to reflect distinct evolutionary units (Braby *et al.* 2012).

We agree that our dataset is dominated by mitochondrial data, which is not unusual due to the slower mutation rates of nuclear DNA. For the same reason, a separate analysis of the ITS2 dataset does not provide substantial insights into the phylogeny of the genus. The ITS2 phylogeny presented by Nabholz *et al.* (2024) does not include any branch support indicators, but based upon our own analyses we can confirm that in addition to poor resolution, bootstrap support values and posterior probabilities of the ITS2 dataset alone were not acceptable to be publishable. Adding more comprehensive nuclear genetic data would be desirable, but due to their large genomes, genomic studies have long been challenging in Orthoptera, particularly in Oedipodinae (Hawlitschek *et al.* 2023). Meanwhile such issues can be overcome by sampling genome wide data using transcriptomics or genome reduction techniques such as hybrid capture or ddRAD to create more extensive SNP datasets (see for example Schmidt *et al.* 2024).

We agree that taxonomic conclusions from genetic data alone must be undertaken with care and all evidence must be considered. This is particularly true for mitochondrial genes, which may be affected by introgression, incomplete lineage sorting or NUMTs as correctly stated by Nabholz *et al.* (2024). When we started sequencing mitochondrial genes for inferring phylogenies in Orthoptera (Hochkirch 2001), we soon realized that COI (the "barcoding gene") is particularly difficult to apply in Orthoptera phylogenies, as this gene appears to be frequently copied into the nuclear genome, leading to unresolved phylogenies (see for example Hawlitschek *et al.* 2017; Kock *et al.* 2024). For this reason, we relied on more stable gene fragments, including NDS, which is a combination of 16S rDNA, tRNA-Leu and NADH-Dehydrogenase subunit 1 (and therefore unlikely to be copied as a whole into the nuclear genome) as well as 12S rDNA, which is known to be quite stable in Orthoptera. This, however, does not prevent any erroneous conclusions from mitochondrial introgression or incomplete lineage sorting.

Nabholz *et al.* (2024) argue with the *Chorthippus biguttulus* group as an example of a species group that cannot be separated with mitochondrial data alone (note that we follow the globally accepted taxonomy here (Cigliano *et al.* 2024) and still refer to *Chorthippus* Fieber, 1852 rather than *Gomphocerippus* Roberts, 1941). We do not doubt the validity of *Chorthippus biguttulus* (Linnaeus, 1758), *Chorthippus mollis* (Charpentier, 1825) and *Chorthippus brunneus* (Thunberg,

Accepted by H. Song: 6 Jun. 2024; published: 16 Jul. 2024

1815), as these differ substantially in their songs, which are known to be important premating barriers (Gottsberger & Mayer 2007). Nevertheless, hybridization between these species in nature (see Table 1 in Gottsberger 2007) is well documented and even gave rise to the evolution of a hybrid species, *Chorthippus jutlandica* Fogh Nielsen, 2003. We have shown in our own studies widespread hybridization in the sister genus *Pseudochorthippus* Defaut, 2012 (Rohde *et al.* 2015), confirming the hypothesis that hybridization is more common in Orthoptera than expected based upon the assumption that songs act as effective premating barriers. Hybridization and subsequent mitochondrial introgression is one of the main reasons for inconsistencies between mitochondrial and nuclear datasets. There even exist documented cases in which the complete mitochondrial gene pool appears to have been displaced by multiple introgression events (Babik *et al.* 2005). Furthermore, incomplete lineage sorting is a second important reason for inconsistencies between mitochondrial phylogenies and taxonomic designation (Maddison & Knowles 2006) as correctly stated by Nabholz *et al.* (2024). Despite these obvious issues with introgression and incomplete lineage sorting in the genus *Chorthippus*, the senior author of Nabholz *et al.* (2024) transferred the species of the *Chorthippus biguttulus* group into the genus *Gomphocerippus* (Defaut 2022) by exploiting an incomplete mitochondrial phylogeny of other researchers (Nolen *et al.* 2020), a practice which has been criticized by other colleagues (Fontana *et al.* 2024).

We completely agree with the statement by Nabholz *et al.* (2024) that phenotypic distinct taxa should not be synonymized based upon genetic data alone and we would not come to such taxonomic conclusions carelessly. In the case of *Chorthippus* and *Sphingonotus*, the species have very distinct songs, acting as premating barriers - even though they may not be 100% effective. However, the status of the *Oedipoda* subspecies is not based on robust data. Nabholz *et al.* (2024) argue that *O. fuscocincta morini* Defaut, 2006 is slightly smaller and has a blue-green hind wing color. Body size is known to be quite plastic in many Orthoptera species (e.g. Berggren 2008) and island populations of other Orthoptera species are also smaller than mainland populations (e.g. *Sphingonotus rubescens* on the Baleares are much smaller than on the mainland, even though they possess the typical sinuous intercalary vein and produce the typical song of that species; Husemann *et al.* 2013). We argued in our publication (Hochkirch *et al.* 2023) that hind wing color is strongly variable in many *Oedipoda* species, e.g. *O. charpentieri* Fieber, 1853 has red winged and blue winged specimens in sympatric populations, *O. caerulescens* (Linnaeus, 1758) has blue, yellow or reddish hind wings, *O. schochii* Brunner von Wattenwyl, 1884 has blue or purple hind wings, *O. miniata* (Pallas, 1771) has a rose-winged and a yellow-winged morph. In some cases, these may represent valid subspecies, but wing color alone is not a sufficient character to justify such a status. The determination of wing coloration of Orthoptera remains unknown, but it may well be that it is just based upon a single mutation in a single gene, or may even be caused by phenotypic plasticity as has been shown for the fore wing color of the Oedipodinae genus *Sphingonotus* (Edelaar *et al.* 2017) and other Orthoptera species (Hochkirch *et al.* 2008). There is currently also no evidence that hind wing color plays any significant role in mate choice of *Oedipoda* species.

We here follow the subspecies approach by Braby *et al.* (2012), who recommend that the "definition of subspecies [should] be restricted to extant animal groups that comprise evolving populations representing partially isolated lineages of a species that are allopatric, phenotypically distinct, and have at least one fixed diagnosable character state, and that these character differences are (or are assumed to be) correlated with evolutionary independence according to population genetic structure." We currently do not see any evidence for any independent population genetic structure in *O. fuscocincta morini* and subsequently synonymized this subspecies. It is unlikely that the lack of any mitochondrial differences is caused by mitochondrial introgression as the taxon is endemic to two islands, a situation very different from *O. germanica*, which has a hybrid zone with *O. coerulea* in the Pyrenees. While incomplete lineage sorting cannot be excluded, this would also suggest that any separation must be very recent.

We have kept the subspecies rank of *Oedipoda caerulescens sardeti*. Similar to *O. fuscocincta morini*, morphological differentiation of Corsican specimens is weak and based upon color pattern and morphometrics. The phylogeny places it in a distinct clade together with specimens from the Iberian Peninsula and southern France. It clearly fulfills the requirements of an evolutionary independent unit if those specimens are included. Assigning subspecies based upon a few morphological characters is always associated with the risk that these characters reflect the human perception of species recognition (e.g. colors), while more complex patterns of differentiation in other traits (e.g. pheromones, genitalia, courtship behavior) remain unstudied.

Nabholz *et al.* (2024) finally provide some comments on the position of *O. coerulea* and potential hybridization with *O. germanica*. As we have stressed in our publication (Hochkirch *et al.* 2023), this is indeed a case that requires further study and we agree that the pattern is most likely influenced by introgression. This also requires a critical evaluation of the types of *O. germanica pyrenaica* Uvarov, 1936, which may in fact represent our *O. coerulea* clade, but is currently treated as a synonym of *O. germanica*. In this case, *O. coerulea* may not be valid and most of the blue-winged individuals from the Iberian Peninsula would be moved back to *O. fuscocincta*. However, any conclusions on this group are currently premature and highly speculative.

In conclusion, this discussion might deserve to be placed in the splitters vs. lumpers context. With the data at hand, we do not see sufficient support for keeping *O. fuscocincta morini* as a valid subspecies, but future studies may falsify our conclusion. In the end, taxonomy remains dynamic - different species concepts and new methodological approaches have always fostered taxonomic re-arrangements and this will continue to keep taxonomists busy.

References

Babik, W., Branicki, W., Crnobrnja-Isailović, Cogălniceanu, D., Sas, I., Olgun, K., Poyarkov, N.A., Garcia-París, M. & Arntzen, J.W. (2005) Phylogeography of two European newt species - discordance between mtDNA and morphology. *Molecular Ecology*, 14, 2475–2491.
https://doi.org/10.1111/j.1365-294X.2005.02605.x

Berggren, Å (2008) The effects of population and landscape ecology on body size in orthopterans. *Journal of Orthoptera Research*, 17, 183–188.
https://doi.org/10.1665/1082-6467-17.2.183

Braby, M.F., Eastwood, R. & Murray, N. (2012) The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? *Biological Journal of the Linnean Society*, 106, 699–716.
https://doi.org/10.1111/j.1095-8312.2012.01909.x

Cigliano, M.M., Braun, H., Eades, D.C. & Otte, D. (2024) Orthoptera Species File. Available from: http://orthoptera.speciesfile.org (accessed 4 June 2024)

Defaut, B. (2022) Notes de lecture phylogénétique sur les Orthoptères. 4. L'étude de NOLEN & al. (2020) sur la radiation des espèces européennes de «*Chorthippus*». *Matériaux orthoptériques et entomocénotiques*, 27, 13–15.

Edelaar, P., Baños-Villalba, A., Escudero, G. & Rodríguez-Bernal, C. (2017) Background colour matching increases with risk of predation in a colour-changing grasshopper. *Behavioral Ecology*, 28, 698–705.
https://doi.org/10.1093/beheco/arx016

Fontana, P., Odé, B., Willemse, L. & Massa, B. (2024) In favor of the principle of nomenclatural stability: the case of the Gomphocerini (Orthoptera: Acrididae). *Zootaxa*, 5406 (3), 495–496.
https://doi.org/10.11646/zootaxa.5406.3.10

Gottsberger, B. (2007) *Interspecific hybridization between the grasshoppers Chorthippus biguttulus and C. brunneus (Acrididae; Gomphocerinae)*. PhD thesis, Friedrich-Alexander-Universität Erlangen-Nürnberg. Avaialble from: https://open.fau.de/handle/openfau/596 (accessed 1 July 2024)

Gottsberger, B. & Mayer, F. (2007) Behavioral sterility of hybrid males in acoustically communicating grasshoppers (Acrididae, Gomphocerinae). *Journal of Comparative Physiology A*, 193, 703–714.
https://doi.org/10.1007/s00359-007-0225-y

Hawlitschek, O., Morinière, J., Lehmann, G.U.C., Lehmann, A.W., Kropf, M., Dunz, A., Glaw, F., Detcharoen, M., Schmidt, S., Hausmann, A., Szucsich, N.U., Caetano-Wyler, S.A. & Haszprunar, G. (2016) DNA barcoding of crickets, katydids and grasshoppers (Orthoptera) from Central Europe with focus on Austria, Germany and Switzerland. *Molecular Ecology Resources*, 17, 1037–1053.
https://doi.org/10.1111/1755-0998.12638

Hawlitschek, O., Sadilek, D., Dey, L.-S., Buchholz, K., Noori, S., Baez, I.L., Wehrt, T., Brozio, J., Trávníček, P., Seidel, M. & Husemann, M. (2023) New estimates of genome size in Orthoptera and their evolutionary implications. *PLoS One*, 18, e0275551
https://doi.org/10.1371/journal.pone.0275551

Hochkirch, A. (2001) *A phylogenetic analysis of the East African grasshopper genus Afrophlaeoba Jago, 1983 (Orthoptera: Acridoidea: Acridinae)*. PhD thesis, University of Bremen. Cuvillier Verlag, Göttingen, 192 pp.

Hochkirch, A., Deppermann, J. & Gröning, J. (2008) Phenotypic plasticity in insects: the effects of substrate color on the coloration of two ground-hopper species. *Evolution & Development*, 10, 350–359.
https://doi.org/10.1111/j.1525-142X.2008.00243.x

Husemann, M., Deppermann, J. & Hochkirch, A. (2014) Multiple independent colonization of the Canary Islands by the winged grasshopper genus *Sphingonotus* Fieber, 1852. *Molecular Phylogenetics and Evolution*, 81, 174–181.
https://doi.org/10.1016/j.ympev.2014.09.017

Husemann, M., Llucià-Pomares, D. & Hochkirch, A. (2013) A review of the Iberian Sphingonotini with description of two novel species (Orthoptera: Acrididae: Oedipodinae). *Zoological Journal of the Linnean Society*, 168, 29–60.
https://doi.org/10.1111/zoj.12023

Kock, L.-S., Körs, E., Husemann, M., Davaa, L. & Dey, L.-S. (2024) Barcoding Fails to Delimit Species in Mongolian Oedipodinae (Orthoptera, Acrididae). *Insects*, 15, 128.
https://doi.org/10.3390/insects15020128

Maddison, W.P. & Knowles, L.L. (2006) Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology*, 55, 21–

30.
https://doi.org/10.1080/10635150500354928

Nabholz, B., Puissant, S. & Defaut, B. (2024) A cautionary note on synonymization based on mitochondrial data in Orthoptera: a comment of Hochkirch *et al.* 2023. *Zootaxa*, in press.

Nolen, Z.J., Yildirim, B., Irisarri, I., Liu, S., Groot Crego, C., Buchvaldt Amby, D., Mayer, F., Gilbert, T.P. & Pereira, R.J. (2020) Historical isolation facilitates species radiation by sexual selection: Insights from *Chorthippus* grasshoppers. *Molecular Ecology*, 29, 4985–5002.
https://doi.org/10.1111/mec.15695

Rohde, K., Hau, Y., Weyer, J. & Hochkirch, A. (2015) Wide prevalence of hybridization in two sympatric grasshopper species may be shaped by their relative abundances. *BMC Evolutionary Biology*, 15, 191.
https://doi.org/10.1186/s12862-015-0460-8

Schmidt, R., Dufresnes, C., Krištín, A., Künzel, S., Vences, M. & Hawlitschek, O. (2024) Phylogenetic insights into Central European *Chorthippus* and *Pseudochorthippus* (Orthoptera: Acrididae) species using ddRADseq data. *Molecular Phylogenetics and Evolution*, 193, 108012. [published online]
https://doi.org/10.1016/j.ympev.2024.108012