Bulletin de la Société entomologique de France, **126** (4), 2021 : 513-519. ISSN 0037-928X
https://doi.org/10.32475/bsef_2221 eISSN 2540-2641

Characterizing the taxonomic status of *Sphingonotus caerulans* in the upper Rhine Valley of Alsace (France) (Orthoptera, Acrididae, Oedipodinae)

Jean-Pierre Vacher[1,2], Roberto D'Agostino[2] & Sylvain Ursenbacher[1,3]

[1] Department of Environmental Sciences, Section of Conservation Biology, University of Basel, St. Johanns-Vorstadt 10, CH – 4056 Basel, Suisse <jpvacher@gmail.com>
[2] Association Imago, 8 rue Adèle-Riton, F – 67000 Strasbourg, France <rougegorge68@yahoo.fr>
[3] Info fauna, Université de Neuchâtel, avenue de Bellevaux 51, CH – 2000 Neuchâtel, Suisse <s.ursenbacher@unibas.ch>

(Accepté le 20.IX.2021 ; publié le 7.XII.2021)

Abstract. – *Sphingonotus* is a genus of grasshoppers that contains species groups with several closely related species, among which *Sphingonotus caerulans* and an unnamed *Sphingonotus* that are found in continental France. The exact distribution of both species is still under investigation, but it is believed that *S. caerulans* might be restricted to the northern part of the country, and that *Sphingonotus* sp. occurs in the southern half and might reach the north east. We explored the genetic identity of *Sphingonotus* grasshoppers in the upper Rhine Valley of Alsace (north-eastern France) using combined fragments of mtDNA ND5 and cytb genes included with other available samples in ML and Bayesian phylogenetic analyses. The results indicate that the five specimens sampled within this region belong to *S. caerulans*. The actual distribution of *Sphingonotus* sp. in France remains to be investigated with wider sampling, especially to get a better knowledge on its northern limit.

Résumé. – **Caractérisation du statut taxinomique de *Sphingonotus caerulans* dans la basse vallée du Rhin en Alsace (France) (Orthoptera, Acrididae, Oedipodinae)**. Les criquets du genre *Sphingonotus* constituent des groupes d'espèces dont certaines sont très proches. *Sphingonotus caerulans* et une espèce inédite sont présents en France continentale. Leur répartition exacte nécessite des investigations complémentaires, mais il est admis que *S. caerulans* est restreint au nord du pays alors que *Sphingonotus* sp. serait méridional et attendrait le nord-est. Nous avons exploré l'identité génétique des criquets *Sphingonotus* dans la basse vallée du Rhin en Alsace (France) à l'aide de deux fragments de gènes DN5 et cytb de l'ADN mitochondrial, intégrés avec d'autres échantillons déjà disponibles dans des analyses phylogénétiques de maximum de vraisemblance et bayésiennes. Les résultats indiquent que les cinq échantillons prélevés dans cinq localités réparties du sud au nord de la région appartiennent à l'espèce *S. caerulans*. La répartition de *Sphingonotus* sp., une espèce non décrite et présente dans le sud de la France, reste à étudier à l'aide d'un échantillonnage plus large, en particulier pour comprendre la limite septentrionale de son aire.

Keywords. – Genetic analysis, distribution, contact zone.

The blue-winged grasshopper *Sphingonotus caerulans* (Linnaeus, 1767) is widely distributed throughout Europe. A recent phylogenetic study based on two mitochondrial markers (ND5 and 12S) retrieved the grasshoppers attributed to *S. caerulans* as a paraphyletic group, this name being used for at least three well-differentiated mitochondrial lineages, two of which occur in France and would correspond to two separate species, *S. caerulans* and *Sphingonotus sp.* (Husemann *et al.*, 2013). Although it is speculated that *S. caerulans* would occur in the northern margin of France, while *Sphingonotus* sp. would occupy a larger area, basically consisting of the southern two-thirds of the country (Defaut, 2014), up to now, *Sphingonotus* sp. has only been confirmed in four localities in the Allier, Bouches-du-Rhône, Drôme, and Gard departments (Husemann *et al*., 2013, A. Hochkirch, pers. comm.), as well as in Spain in Barcelona and Mallorca (Husemann *et al.*, 2013). The northeastern limit of *Sphingonotus sp*. is not known, but should reach the region Bourgogne-Franche-Comté up to the southern margin

of Alsace, in the upper Rhine Valley (Defaut, 2014). According to Defaut & Morichon (2015), the distinction between the two species seems to rely on three morphological characters, which are the total length, the tegmen length, and the ratio between posterior tibia and tegmen, mainly measured on males. However, a recent study aimed at identifying which species of *Sphingonotus* occurred in Alsace based on the characters proposed by Defaut & Morichon (2015) showed that taxonomic assignment only based on these three characters lacks accuracy as more than 50% of individuals displayed overlapping values for the measurement of several characters and could not be assigned to one or the other species (D'Agostino & Vacher, 2020). Another character, the shape of the anal plate, seems to be more reliable (A. Hochkirch, pers. comm.) and could be used for future similar studies.

As the Rhine Valley in France harbours insect species with southern affinities [e.g., *Mantis religiosa* (Linnaeus, 1758), *Phaneroptera nana* Fieber, 1853, *Ruspolia nitidula* (Scopoli, 1786)], and as doubt persists on the correct taxonomic assignment of most individuals from Alsace, we used genetic markers to assess the taxonomic status of the *Sphingonotus* grasshoppers found in Alsace, and also to verify whether *Sphingonotus* sp. was present in the region, as speculated by Defaut & Morichon (2015) and D'Agostino & Vacher (2020) or not. We expected to find this species in the southern part of the region and *Sphingonotus caerulans* in the northern part (Defaut & Morichon, 2015 ; D'Agostino & Vacher, 2020). Therefore, we implemented a sampling design that included localities throughout the whole region, from south to north, and collected DNA material for subsequent molecular analysis.

Material and methods

Study area and sampling design. – We collected five femora of putative *Sphingonotus caerulans* in five different localities (one femur per locality) throughout Alsace (fig. 1, table I). Each sample belonged to populations that were included in the morphometric study performed previously by D'Agostino & Vacher (2020).

Genetic analysis. – We extracted genomic DNA from five femora of *S. caerulans* using the Qiagen DNeasy Blood & Tissue Kit (QIAGEN, Hombrechtikon, Switzerland) following the protocol provided by the manufacturer. We amplified mitochondrial 12S and ND5 by PCR with two fragments of respectively about 300 and about 1000 bp using 12S ai and 12S bi (Kocher *et al.*, 1989), and ND V-His and ND V-Phe (Su *et al*., 1998) primers based on primer sequences provided by Hochkirch (2001). Amplifications were performed following Husemann *et al*. (2013) and sequencing was performed by Macrogen (Amsterdam, the Netherlands). The retrieved sequences were then blasted in GenBank to confirm their taxonomic assignment.

We combined the resulting sequences with previously published sequences of *S. caerulans* (Husemann *et al.*, 2013), and this new batch of sequences was aligned in MEGA v.7.0.16 (Kumar *et al.*, 2016). GenBank accession numbers of all the sequences used for this analysis are provided

Table I. – List of localities and samples. The numbers in the first column correspond to the numbers reported on the map in fig. 1. F = France; latitudes and longitudes in decimal degrees WGS84.

Voucher number	Genbank accession number (12S, ND5)	Locality	Latitude	Longitude
SPH01	MW939464, MW939469	Oberentzen (Alsace, F)	47.9326	7.3789
SPH03	MW939465, MW939470	Haguenau (Alsace, F)	48.8181	7.8236
SPH04	MW939466, MW939471	Ensisheim (Alsace, F)	47.8601	7.3996
SPH05	MW939467, MW939472	Bernolsheim (Alsace, F)	48.7469	7.6752
SPH07	MW939468, MW939473	Hettenschlag (Alsace, F)	48.0019	7.4654

in table I. We then concatenated the two fragments with the program FASconCAT v. 1.0 (Kück & Meusemann, 2010). The resulting alignment comprised 1362 bp in total length composed of two partitions (12S: 1–342, ND5: 343–1362). We inferred the best-fitting model of molecular evolution with PartitionFinder v.1.1.1 (Lanfear *et al.*, 2012) with BIC, and conducted a ML analysis with RAxML v.8.2.4 using the GTR+G model. Support of nodes was investigated with 1000 bootstrap replicates using the fast bootstrapping algorithm. We also conducted a

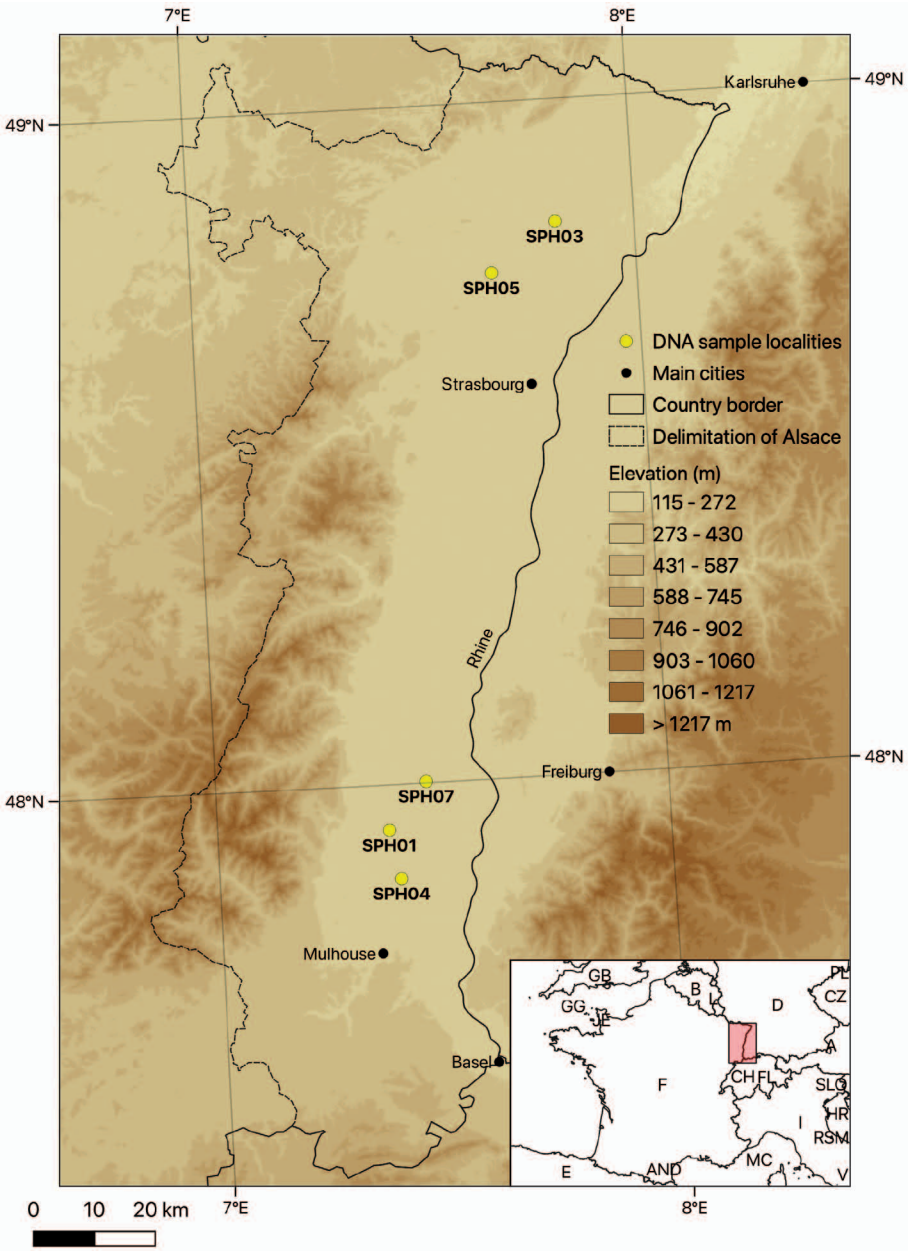

Fig. 1. – Map of the sample localities for *Sphingonotus caerulans* DNA in Alsace, France. The numbers provided under each DNA sample sites correspond to the specimen voucher numbers given in table I and fig. 2.

Bayesian analysis implemented in MrBayes v.3.2.6 (RONQUIST & HUELSENBECK, 2003). We ran the MCMC chain for ten million generations, sampling every 100 generations. We checked for stationary and convergence of the chains after discarding the 1000 first trees in Tracer v.1.6 (RAMBAUT *et al.*, 2014). A consensus tree was retrieved using PAUP v.4.0 (SWOFFORD, 2002). We used *Sphingonotus candidus* as outgroup for both analyses (HUSEMANN *et al.*, 2013). We calculated pairwise genetic p-distances with the *ape* package (PARADIS *et al.*, 2004) implemented in R (R CORE TEAM, 2016).

RESULTS

We retrieved a Maximum Likelihood tree topology that is similar to the one provided by HUSEMANN *et al*. (2013). In both ML and Bayesian analyses, the five Alsacian *Sphingonotus* cluster with the German/Swedish clade retrieved by HUSEMANN *et al*. (2013) (fig. 2-3), confirming their assignment to *Sphingonotus caerulans s. str.* (fig. 4). As the type locality of this species is in Northern Europe, specimens from Central and Northern Europe (Alsace, Germany, Sweden) should be assigned to that species (HUSEMANN *et al.*, 2013). The mean genetic p-distance between specimens from this clade is 0.002 [0.001-0.004], whereas the mean p-distance with the other taxa is 0.011 [0.006-0.017] (Suppl. table I).

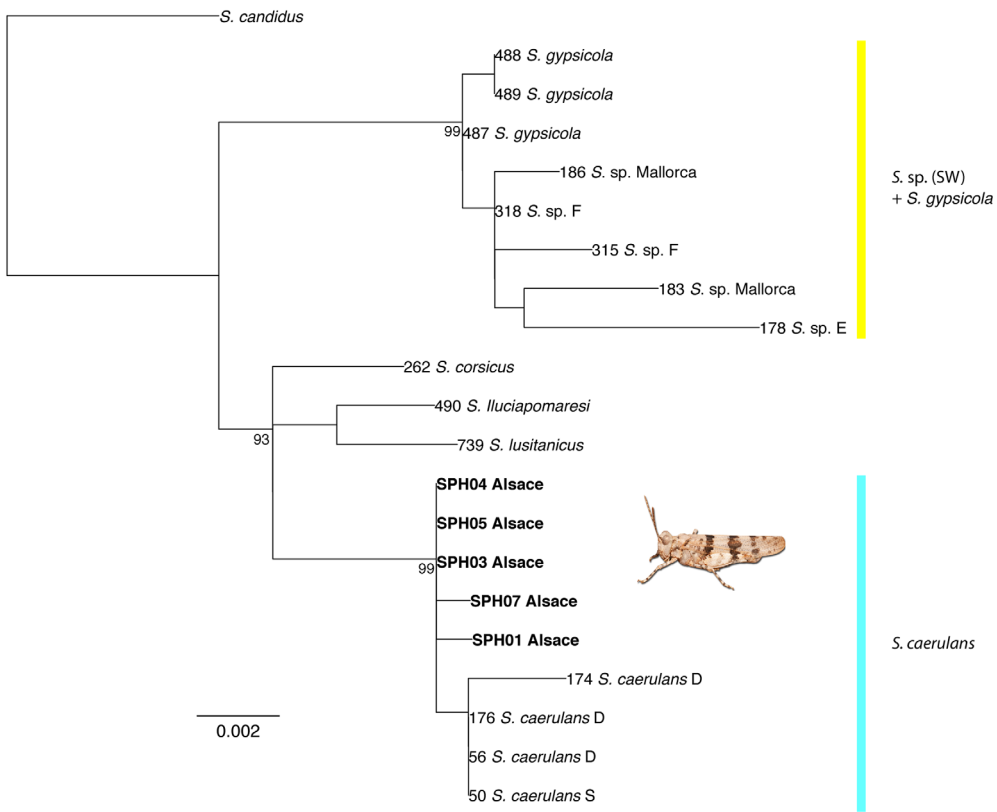

Fig. 2. – Phylogram inferred from Maximum Likelihood analysis of 1362 bp of concatenated 12S and ND5 genes of *Sphingonotus* grasshoppers. The new sequences are represented in bold. Bootstraps values are not represented when > 80. SW= Southwest; D = Germany; S = Sweden; E = Spain; F = France. The labels of the new sequences correspond to the specimen voucher numbers (table I and fig. 1) and the name of the locality.

Discussion

In *Sphingonotus* grasshoppers, p-distances based on mitochondrial DNA between species are usually low, <0.01, and the values we retrieved for our specimens are in accordance with what is usually observed for these insects (Husemann *et al.*, 2013). The low resolution of phylogenetic relationships within this group might be a result of such low p-distances (Husemann *et al.*, 2013). Still, the mean p-distances value between *S. caerulans* and *Sphingonotus* sp. is 0.013 [0.007 – 0.017], which is well above the values we found between specimens of Alsace and other *S. caerulans* from Germany and Sweden (Suppl. table I). Therefore, based on these results, we can confirm that the *Sphingonotus* grasshoppers found in the upper Rhine Valley of Alsace belong to *S. caerulans*.

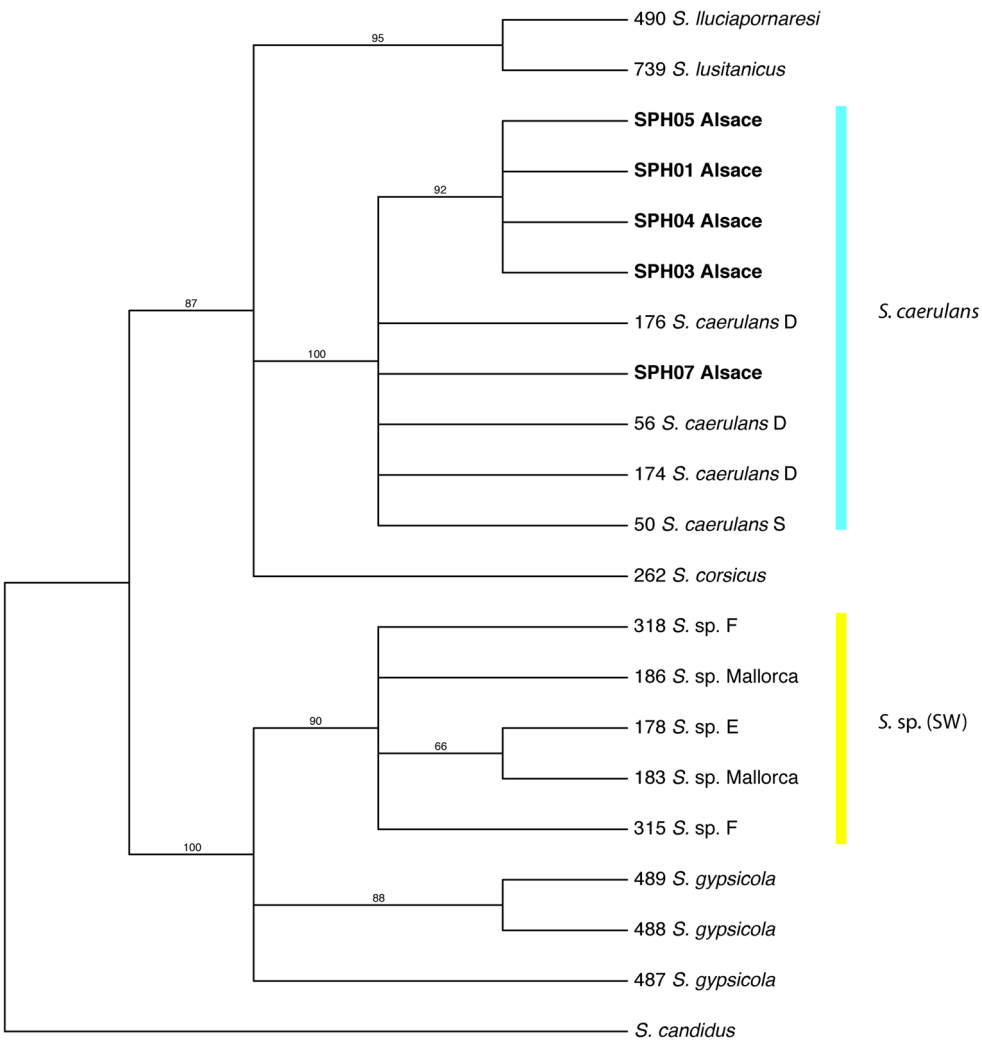

Fig. 3. – Bayesian consensus tree of 1362 bp of concatenated 12S and ND5 genes of *Sphingonotus* grasshoppers. The new sequences are represented in bold. Percentage posterior probabilities values are not represented when > 60. SW= Southwest; D = Germany; S = Sweden; E = Spain; F = France. The labels of the new sequences correspond to the specimen voucher numbers (table I and fig. 1) and the name of the locality.

Sphingonotus caerulans harbours at least seven subspecies, among which *S. caerulans cyanopterus* (Charpentier, 1825) is found in northern Europe and can be distinguished by a faint smoky band on the hind wings. The taxonomic status of this subspecies requires further investigation as the tree presented in HUSEMANN *et al*. (2013) displayed it as paraphyletic. The phenotype assigned to this subspecies, with a dark bar on the hind wings, has been observed in four specimens of three localities in Alsace (D'AGOSTINO & VACHER 2020) among populations that otherwise displayed the phenotype of the nominotypical subspecies.

The knowledge on distribution of *Sphingonotus* sp. in France is little known and the northern limit of the species is still unknown (fig. 4). Our results seem to indicate that this species does not currently reach the Rhine Valley, so that its northern limit might occur more to the south. Further sampling should be performed at different localities in France, especially in the north of the Auvergne-Rhône-Alpes region, as well as throughout the Bourgogne-Franche-Comté region, to better understand the distribution of both species.

Also, a previous study on *Sphingonotus* grasshoppers in Alsace based on three morphological characters cited above indicated that there was no clear distinction between phenotypes and that it was not possible to discriminate species based solely on these characters (D'AGOSTINO & VACHER, 2020); thus, these characters should be used with caution. However, the shape of the anal plate might be a better character to distinguish both species (A. Hochkirch, pers. comm.). Therefore, if further sampling of genetic material should occur throughout France, taking measurements of these four characters would be useful to incorporate them in an integrative approach with the genetic assignments. As both species are quite similar in shape, such an integrative sampling design would enable to evaluate how these characters perform for species identification.

Finally, we cannot rule out the existence of a hybrid zone in France. Hybridization within European Orthoptera is presently rather unknown, at least at the genetic level, even though

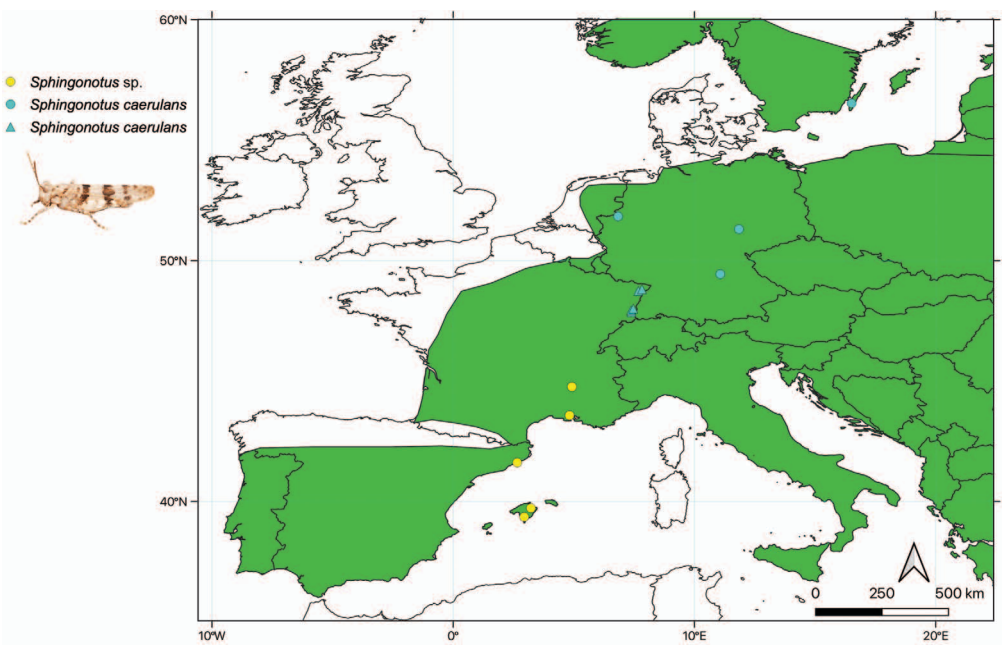

Fig. 4. – Distribution map of *Sphingonotus caerulans sensu lato* (in green), with localities for genetic samples used in this study. Turquoise markers denote *S. caerulans sensu stricto*, whereas yellow markers denote *Sphingonotus sp*. Circles denote samples from GenBank (HUSEMANN *et al.*, 2013), triangles correspond to newly collected samples (this study).

evidence exists that closely-related species or taxa hybridize in the wild (Bridle *et al.*, 2006 ; Hochkirch & Lemke, 2011). Such a study, involving both mtDNA and nuDNA markers, would require extensive sampling of *Sphingonotus* grasshoppers throughout their whole range in France. It might help to better understand the biogeography of both species and therefore refine our understanding of the taxonomy of this group.

Acknowledgements. – We would like to thank Axel Hochkirch, Martin Husemann, and Sigfrid Ingrisch for their useful comments on the first draft of the manuscript. We would also like to thank Raynald Moratin for his help on the field.

References

Bridle J. R., Saldamando C. I., Koning W. & Butlin R. K., 2006. – Assortative preferences and discrimination by females against hybrid male song in the grasshoppers *Chorthippus brunneus* and *Chorthippus jacobsi* (Orthoptera: Acrididae). *Journal of Evolutionary Biology,* **19** (4) : 1248-1256. https://doi.org/10.1111/j.1420-9101.2006.01080.x

D'Agostino R. & Vacher J.-P., 2020. – Quelle espèce de *Sphingonotus* Fieber, 1852 (Insecta, Orthoptera, Acrididae) se trouve dans la plaine du Rhin (Alsace, Grand Est) ? *Bulletin de la Société d'Histoire naturelle et d'Ethnographie de Colmar*, **76** (5) : 18-24.

Defaut B., 2014. – Notes de lecture concernant l'étude de Husemann & al. (2013) sur les Sphingonotini ibériques (Acrididae, Locustinae). *Matériaux Orthoptériques et Entomocénotiques*, **19** : 115-120.

Defaut B. & Morichon D., 2015. – *Criquet de France (Orthoptera, Caelifera). Faune de France 97. Volume 1, fascicule b*. Fédération Française des Sociétés de Sciences Naturelles, 687 p.

Hochkirch A., 2001. – *A phylogenetic analysis of the East African grasshopper genus* Afrophlaeoba *Jago, 1983 (Orthoptera: Acridoidea: Acrididae)*. PhD dissertation, University of Bremen, 192 p.

Hochkirch A. & Lemke I., 2011. – Asymmetric mate choice, hybridization, and hybrid fitness in two sympatric grasshopper species. *Behavioral Ecology and Sociobiology*, **65** : 1637-1645. https://doi.org/10.1007/s00265-011-1174-6

Husemann M., Lucià-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** (1) : 29-60. https://doi.org/10.1111/zoj.12023

Kocher T. D., Thomas W. K., Meyer A., Edwards S. V., Pääbo S., Villablanca F. X. & Wilson A. C., 1989. – Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, **86** (16) : 6196-6200. https://doi.org/10.1073/pnas.86.16.6196

Kück P. & Meusemann K., 2010. – FASconCAT, Version 1.0, Zoologisches Forschungsmuseum A. Koenig,Germany.

Kumar S., Stecher G. & Tamura K., 2016. – MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33** : 1870-1874. https://doi.org/10.1093/molbev/msw054

Lanfear R., Calcott B., Ho S. Y. W. & Guindon S., 2012. – PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29** (6) : 1695-1701. https://doi.org/10.1093/molbev/mss020

Paradis E., Claude J. & Strimmer K., 2004. – APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*, **20** (2) : 289-290. https://doi.org/10.1093/bioinformatics/btg412

R Core Team., 2016. – *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rambaut A., Suchard M. A., Xie D. & Drummond A. J., 2014. – Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer

Ronquist F. & Huelsenbeck J. P., 2003. – MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19** (12) : 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Su Z. H., Tominaga O., Okamoto M. & Osawa S., 1998. – Origin and diversification of hindwingless *Damaster* ground beetles within the Japanese islands deduced from mitochondrial ND5 gene sequences (Coleoptera, Carabidae). *Molecular Biology and Evolution*, **15** (8) : 1026-1039. https://doi.org/10.1093/oxfordjournals.molbev.a026001

Swofford D. L., 2002. – Paup*. PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0 Beta 10. Sinauer Associates, Sunderland.