

Methods

Study species. The anostracan genus *Branchinecta* comprises around 50 species. Its members are found on all continents except Australia^{77–79}. The genus is represented by five species in the Palearctic, with only two being present in the study area: *Branchinecta ferox* and *Branchinecta orientalis*. *B. orientalis* inhabits mineral-rich temporary waters and has a disjunct distribution ranging between 27° and 55° N in Europe and Asia (Fig. 1a). Active populations generally occur in spring, but they have also been recorded in autumn or winter^{80–82}. *B. ferox* has a circum-Mediterranean and Central European distribution (Fig. 1b). It is the only *Branchinecta* species occurring in Africa, being present in the north-western part of the continent (Morocco, Algeria and Tunisia⁷⁸). In Europe, it occurs in Spain and Central Europe (Pannonian Plain), and its range extends further east across South Ukraine to the west of Russia^{83,84}. This species has also been reported in the Middle-East (i.e. Jordan, Israel and Turkey^{85–88}). *B. ferox* is a halotolerant species, occurring both in freshwater rain pools in the circum-Mediterranean area⁸⁵ and saline pans in the Pannonian Plain⁶⁸. Active populations mostly occur in late winter and early spring^{68,78,81}. The geographic distribution of these two *Branchinecta* species overlaps in the Pannonian Plain, Iberian Peninsula and Turkey^{68,87,89}. On the Iberian Peninsula and the Pannonian Plain, the two species are found almost exclusively in large and shallow saline pans, and represent a preferred food source for waterbirds on their seasonal migration routes⁷⁰.

Species distribution maps. We compiled a list of known occurrences of both species based on the above listed samples and literature data^{47,67,68,78,83–87,89–100}. The literature sources mentioning distribution and ecology of *B. ferox* and *B. orientalis* were searched via Google Scholar and Web of Science. Sources that did not report precise habitat coordinates of populations and/or are older than 50 years are not included, hence the actual distribution of the species is probably underrepresented (e.g., the actual distribution of *B. orientalis* in Asia is most likely underrepresented here). To account for this, we built species distribution maps with the ‘dismo’ package¹⁰¹ of R v. 4.0.3¹⁰². Here, we used all available bioclimatic variables from the WorldClim database (<http://www.worldclim.org>)¹⁰³, and predicted the probability of occurrence for each species. Although these variables do not include the presence of suitable habitats (i.e., shallow temporary waters, for which there is no publicly available database yet), they should provide a reliable indication for the climatic conditions where suitable habitats are likely to occur. According to the probability maps, our general coverage of sequenced samples was in a good agreement with the overall distribution of both species, including samples from the Mediterranean, the Pannonian Plain in Central Europe (both species), and Middle to Central Asia (*B. orientalis*). Even though our model predicted the possible occurrence of *B. ferox* in Italy and Southern France (Fig. 1b), we can mostly exclude these latter regions given that both are very well covered by previous Anostraca studies that have never reported the species there^{104,105}.

Sampling procedure. We collected *Branchinecta orientalis* specimens from 29 temporary pools, ponds and shallow lakes in Europe and Asia (Table A1). *Branchinecta ferox* specimens were collected from 16 habitats in Europe, North Africa, and Asia (Table A1). Specimens were collected between 1971 and 2018 and fixed in ethanol (of various concentrations). Once the samples arrived at the lab, animals were transferred immediately to pure ethanol until further processing. All specimens were dissected to obtain phyllopod tissue for DNA extraction. For the molecular laboratory procedures to acquire the DNA sequences for the targeted gene regions, see Appendix B.

Reconstructions of phylogeny based on mitochondrial COI and nuclear ITS2 DNA region. All generated *B. ferox* and *B. orientalis* sequences were assembled and visually checked for quality in SeqScape v3. We checked the COI alignment for indels and internal stop codons that would indicate unintentional amplification of nuclear pseudogenes¹⁰⁶. The produced sequences were edited in BioEdit¹⁰⁷. The newly produced sequences were aligned together with the existing sequences in GenBank (for *B. ferox* and *B. orientalis* see Table A1 in Appendix 1A; *Branchinecta lynchi* MF037649; *B. lindahli* MF037694-5; *B. tolli* HG797695; *B. paludosa* HG797672, HG797699 and JN233828)^{47,51,89,96,108,109} and one outgroup taxon (for COI, we used *Branchipus schaefferi* MK449416⁴³ and for ITS2, *Chirocephalus diaphanus* LT860206⁸⁹) by using CLUSTALW multiple alignment tool in BioEdit for the COI gene region and MUSCLE for the ITS2 DNA region. The most likely evolutionary model for the COI marker was determined in PartitionFinder2¹¹⁰ and for the ITS2 in MEGA X¹¹¹ based on the Akaike Information Criterion (AIC). For the COI gene region, the AIC selected a General Time Reversible model (GTR), which was used to reconstruct ML and BI tree. For the ITS2 DNA region, the AIC selected for GTR model with a gamma shape parameter (+G, $\gamma = 1.22$), which was used to reconstruct ML and BI tree.

ML analyses were performed in MEGA X with 1000 bootstrap replicates. Bayesian inference was performed in BEAST v2.6.4¹¹² in case of the COI gene region. The settings included the strict molecular clock, Yule model and a lognormal prior distribution for the taxon set of the *Branchinecta paludosa* samples (set as monophyletic; mean \pm standard deviation: 1.25 ± 0.15 as in Lindholm et al.⁵¹). The analyses were run for 10 million generations. Molecular evolutionary rates of 2% divergence per million years were applied by Lindholm et al.⁵¹ on the closely related *B. paludosa*, and were thus here applied to get a tentative temporal frame for the main cladogenetic events observed within our study taxa. We used TreeAnnotator v. 2.6.4 to construct a single tree by discarding 25% of the compiled trees as a burn-in. As molecular clock is not available for the ITS2 DNA region, we used MrBayes^{113–115} to an ITS2 phylogenetic tree using BI. We applied the Markov Chain Monte Carlo (MCMC) method for 10⁶ generations (standard deviation of split frequencies reached < 0.01) while the trees were sampled every 1000 generations. The initial 25% of produced trees were discarded as burn-in.

For the *B. ferox* and *B. orientalis* COI gene fragments, we built a median-joining haplotype network for each species ($\epsilon = 0$; Bandelt et al., 1999) using PopART v 1.7¹¹⁷; <http://popart.otago.ac.nz>). The sites containing missing

bases at the end and the beginning of the alignment, as well as ambiguous bases, were masked leaving 479 (*B. ferox*) and 304 (*B. orientalis*) sites for further network analysis.

Analysis of genetic diversity. Substitution saturation was tested in DAMBE v. 7.0.28¹¹⁸, using the default settings and including all sites. The index of substitution saturation (Iss) was significantly smaller than the critical index of substitution saturation (Iss c), indicating little saturation^{119,120} for both markers. Pairwise genetic K2P distances between all generated sequences and the mean genetic distances within and among the main groups in the phylogeny of *B. ferox* and *B. orientalis* were calculated in MEGA X¹²¹ with partial deletion of 90% (515 positions in the final data set for COI and 574 positions for ITS2). The haplotype number was determined in DnaSP 6¹²².

In both *B. ferox* and *B. orientalis*, we tested for the dispersal limitation based on the relationship between pairwise genetic differences on the mitochondrial COI gene region and geographic distances. To do so, we exported pairwise genetic distances from MEGA X in a form of a data matrix and applied Hellinger transformation. We calculated pairwise geographic distances between all sampling sites as orthodromic distance. To reveal effective dispersal over distinct distance classes, we used the computed pairwise genetic distances and log + 0.1 transformed spatial distances to perform a Mantel test with 999 permutations and calculate Mantel correlation coefficients. In addition to the full dataset, separate Mantel tests were performed within two main *B. orientalis* clades (Clade A and Clade B). Mantel correlation coefficients were calculated between pairwise genetic distances within eight distance classes for all COI sequences of *B. orientalis* and repeated separately for the two main clades to detect positive autocorrelation as signs of effective dispersal. For *B. ferox*, we calculated Mantel correlation coefficients between pairwise genetic distances within seven distance classes as the highest spatial distance between *B. ferox* populations was lower than between individual *B. orientalis* populations. Calculation of pairwise spatial distances, Mantel tests and Mantel correlation coefficients were performed in R software, with the 'fields'¹²³ and 'vegan'¹²⁴ packages.

Data accessibility

The DNA sequence data supporting the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, accession numbers are listed in the Appendix A, Table A1.

Received: 20 July 2021; Accepted: 3 November 2021

Published online: 24 November 2021

References

- Paillard, D. The timing of Pleistocene glaciations from a simple multiple-state climate model. *Nature* **391**, 378–381 (1998).
- Hewitt, G. M. Genetic consequences of climatic oscillations in the Quaternary. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **359**, 183–195 (2004).
- Hewitt, G. The genetic legacy of the quaternary ice ages. *Nature* **405**, 907–913 (2000).
- Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.-F. Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.* **7**, 453–464 (1998).
- Incagnone, G., Marrone, F., Barone, R., Robba, L. & Naselli-Flores, L. How do freshwater organisms cross the 'dry ocean'? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Hydrobiologia* **750**, 103–123 (2015).
- Schmitt, T. & Varga, Z. Extra-Mediterranean refugia: The rule and not the exception?. *Front Zool* **9**, 22 (2012).
- Hewitt, G. M. Speciation, hybrid zones and phylogeography—Or seeing genes in space and time. *Mol. Ecol.* **10**, 537–549 (2001).
- Habel, J. C., Drees, C., Schmitt, T. & Assmann, T. Review refugial areas and postglacial colonizations in the Western Palearctic. In *Relict Species* (eds Habel, J. C. & Assmann, T.) 189–197 (Springer, 2010).
- Hewitt, G. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Lin. Soc.* **58**, 247–276 (1996).
- Marrone, F., Lo Brutto, S. & Arculeo, M. Molecular evidence for the presence of cryptic evolutionary lineages in the freshwater copepod genus *Hemidiaptomus* G.O. Sars, 1903 (Calanoida, Diaptomidae). *Hydrobiologia* **644**, 115–125 (2010).
- Husemann, M., Schmitt, T., Zachos, F. E., Ulrich, W. & Habel, J. C. Palaeartic biogeography revisited: Evidence for the existence of a North African refugium for Western Palearctic biota. *J. Biogeogr.* **41**, 81–94 (2014).
- García-Vázquez, D., Bilton, D. T., Foster, G. N. & Ribera, I. Pleistocene range shifts, refugia and the origin of widespread species in western Palearctic water beetles. *Mol. Phylogenet. Evol.* **114**, 122–136 (2017).
- Perktas, U., Barrowclough, G. F. & Groth, J. G. Phylogeography and species limits in the green woodpecker complex (Aves: Picidae): Multiple Pleistocene refugia and range expansion across Europe and the Near East. *Biol. J. Lin. Soc.* **104**, 710–723 (2011).
- Stewart, J. R. & Lister, A. M. Cryptic northern refugia and the origins of the modern biota. *Trends Ecol. Evol.* **16**, 608–613 (2001).
- Stewart, J. R., Lister, A. M., Barnes, I. & Dalén, L. Refugia revisited: Individualistic responses of species in space and time. *Proc. R. Soc. B Biol. Sci.* **277**, 661–671 (2010).
- Sworobowicz, L., Mamos, T., Grabowski, M. & Wysocka, A. Lasting through the ice age: The role of the proglacial refugia in the maintenance of genetic diversity, population growth, and high dispersal rate in a widespread freshwater crustacean. *Freshw. Biol.* **65**, 1028–1046 (2020).
- Provan, J. & Bennett, K. D. Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* **23**, 564–571 (2008).
- Antal, L. *et al.* Phylogenetic evidence for a new species of *Barbus* in the Danube River basin. *Mol. Phylogenet. Evol.* **96**, 187–194 (2016).
- Copilaş-Ciocianu, D., Fişer, C., Borza, P. & Petrussek, A. Is subterranean lifestyle reversible? Independent and recent large-scale dispersal into surface waters by two species of the groundwater amphipod genus *Niphargus*. *Mol. Phylogenet. Evol.* **119**, 37–49 (2018).
- Říčanová, Š *et al.* Multilocus phylogeography of the European ground squirrel: Cryptic interglacial refugia of continental climate in Europe. *Mol. Ecol.* **22**, 4256–4269 (2013).
- Vörös, J., Mikulíček, P., Major, Á., Recuero, E. & Arntzen, J. W. Phylogeographic analysis reveals northerly refugia for the riverine amphibian *Triturus dobrogicus* (Caudata: Salamandridae). *Biol. J. Lin. Soc.* **119**, 974–991 (2016).
- Wielstra, B. *et al.* Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. *Front. Zool.* **10**, 13 (2013).

23. Hutchison, D. W. & Templeton, A. R. Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898–1914 (1999).
24. Schmitt, T. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. *Front. Zool.* **4**, 11 (2007).
25. Ewart, K. M. *et al.* Phylogeography of the iconic Australian red-tailed black-cockatoo (*Calyptorhynchus banksii*) and implications for its conservation. *Heredity* **125**, 85–100 (2020).
26. Hutama, A. *et al.* Identifying spatially concordant evolutionary significant units across multiple species through DNA barcodes: Application to the conservation genetics of the freshwater fishes of Java and Bali. *Glob. Ecol. Conserv.* **12**, 170–187 (2017).
27. Médail, F. & Baumel, A. Using phylogeography to define conservation priorities: The case of narrow endemic plants in the Mediterranean Basin hotspot. *Biol. Cons.* **224**, 258–266 (2018).
28. Previšić, A., Walton, C., Kučinić, M., Mitrakeski, P. T. & Kerovec, M. Pleistocene divergence of Dinaric *Drusus* endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. *Mol. Ecol.* **18**, 634–647 (2009).
29. Brendonck, L. & Riddoch, B. J. Wind-borne short-range egg dispersal in anostracans (Crustacea: Branchiopoda). *Biol. J. Linn. Soc.* **67**, 87–95 (1999).
30. Horváth, Z., Vad, C. F. & Ptacnik, R. Wind dispersal results in a gradient of dispersal limitation and environmental match among discrete aquatic habitats. *Ecography* **39**, 726–732 (2016).
31. Brochet, A. L. *et al.* Field evidence of dispersal of branchiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France). *Hydrobiologia* **637**, 255 (2009).
32. Figuerola, J. & Green, A. J. Dispersal of aquatic organisms by waterbirds: A review of past research and priorities for future studies. *Freshw. Biol.* **47**, 483–494 (2002).
33. Vanschoenwinkel, B. *et al.* Dispersal of freshwater invertebrates by large terrestrial mammals: A case study with wild boar (*Sus scrofa*) in Mediterranean wetlands. *Freshw. Biol.* **53**, 2264–2273 (2008).
34. Brendonck, L., Rogers, D. C., Olesen, J., Weeks, S. & Hoeh, W. R. Global diversity of large branchiopods (Crustacea: Branchiopoda) in freshwater. *Hydrobiologia* **595**, 167–176 (2008).
35. Dumont, H. J. & Negrea, S. V. *Introduction to the Class Branchiopoda*. (Backhuys Publishers, 2002).
36. Belk, D. Global status and trends in ephemeral pool invertebrate conservation: Implications for Californian fairy shrimp. In *Ecology, Conservation, and Management of Vernal Pool Ecosystems—Proceedings from a 1996 conference* 147–150 (California Native Plant Society, 1998).
37. Jocque, M., Vanschoenwinkel, B. & Brendonck, L. Anostracan monopolisation of early successional phases in temporary waters?. *Fundam. Appl. Limnol.* **176**, 127–132 (2010).
38. Lukić, D., Horváth, Z., Vad, C. F. & Ptacnik, R. Food spectrum of *Branchinecta orientalis*—Are anostracans omnivorous top consumers of plankton in temporary waters?. *J. Plankton Res.* **40**, 436–445 (2018).
39. Lukić, D., Ptacnik, R., Vad, C. F., Póda, C. & Horváth, Z. Environmental constraint of intraguild predation: Inorganic turbidity modulates omnivory in fairy shrimps. *Freshw. Biol.* **65**, 226–239 (2020).
40. Waterkeyn, A., Grillas, P., Anton-Pardo, M., Vanschoenwinkel, B. & Brendonck, L. Can large branchiopods shape microcrustacean communities in Mediterranean temporary wetlands?. *Mar. Freshw. Res.* **62**, 46–53 (2011).
41. Brendonck, L. & De Meester, L. Egg banks in freshwater zooplankton: Evolutionary and ecological archives in the sediment. *Hydrobiologia* **491**, 65–84 (2003).
42. Hairston, N. G., Brunt, R. A. V., Kearns, C. M. & Engstrom, D. R. Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology* **76**, 1706–1711 (1995).
43. Lukić, D. *et al.* High genetic variation and phylogeographic relations among Palearctic fairy shrimp populations reflect persistence in multiple southern refugia during Pleistocene ice ages and postglacial colonisation. *Freshw. Biol.* **64**, 1896–1907 (2019).
44. Marrone, F., Alfonso, G., Naselli-Flores, L. & Stoch, F. Diversity patterns and biogeography of Diaptomidae (Copepoda, Calanoida) in the Western Palearctic. *Hydrobiologia* **800**, 45–60 (2017).
45. Vanschoenwinkel, B. *et al.* Toward a global phylogeny of the “living fossil” crustacean order of the Notostraca. *PLoS ONE* **7**, e34998 (2012).
46. Boileau, M. & Hebert, P. Genetic consequences of passive dispersal in pond-dwelling Copepods. *Evolution* **45**, 721–733 (1991).
47. Deng, Z., Chen, Y., Ma, X., Hu, W. & Yin, M. Dancing on the top: Phylogeography and genetic diversity of high-altitude freshwater fairy shrimps (Branchiopoda, Anostraca) with a focus on the Tibetan Plateau. *Hydrobiologia* **848**, 2611–2626 (2021).
48. Ketmaier, V. *et al.* Mitochondrial DNA regionalism and historical demography in the extant populations of *Chirocephalus kerkyrensis* (Branchiopoda: Anostraca). *PLoS ONE* **7**, e30082 (2012).
49. Korn, M. *et al.* Phylogeny, molecular ecology and taxonomy of southern Iberian lineages of *Triops mauritanicus* (Crustacea: Notostraca). *Org. Divers. Evol.* **10**, 409–440 (2010).
50. Stoch, F., Korn, M., Turki, S., Naselli-Flores, L. & Marrone, F. The role of spatial environmental factors as determinants of large branchiopod distribution in Tunisian temporary ponds. *Hydrobiologia* **782**, 37–51 (2016).
51. Lindholm, M., d'Auriac, M. A., Thaulow, J. & Hobaek, A. Dancing around the pole: Holarctic phylogeography of the Arctic fairy shrimp *Branchinecta paludosa* (Anostraca, Branchiopoda). *Hydrobiologia* **772**, 189–205 (2016).
52. Vörös, J., Alcobendas, M., Martínez-Solano, I. & García-París, M. Evolution of *Bombina bombina* and *Bombina variegata* (Anura: Discoglossidae) in the Carpathian Basin: A history of repeated mt-DNA introgression across species. *Mol. Phylogenet. Evol.* **38**, 705–718 (2006).
53. Zharov, A. A. *et al.* Pleistocene branchiopods (Cladocera, Anostraca) from Transbaikalian Siberia demonstrate morphological and ecological stasis. *Water* **12**, 3063 (2020).
54. Velonà, A., Luchetti, A., Scanabissi, F. & Mantovani, B. Genetic variability and reproductive modalities in European populations of *Triops cancrivormis* (Crustacea, Branchiopoda, Notostraca). *Ital. J. Zool.* **76**, 366–375 (2009).
55. Vanschoenwinkel, B., Gielen, S., Vandewaerde, H., Seaman, M. & Brendonck, L. Relative importance of different dispersal vectors for small aquatic invertebrates in a rock pool metacommunity. *Ecography* **31**, 567–577 (2008).
56. Hulsmans, A., Moreau, K., Meester, L. D., Riddoch, B. J. & Brendonck, L. Direct and indirect measures of dispersal in the fairy shrimp *Branchipodopsis wolffi* indicate a small scale isolation-by-distance pattern. *Limnol. Oceanogr.* **52**, 676–684 (2007).
57. Vanschoenwinkel, B., Vries, C. D., Seaman, M. & Brendonck, L. The role of metacommunity processes in shaping invertebrate rock pool communities along a dispersal gradient. *Oikos* **116**, 1255–1266 (2007).
58. Sánchez, M. I., Green, A. J., Amat, F. & Castellanos, E. M. Transport of brine shrimps via the digestive system of migratory waders: Dispersal probabilities depend on diet and season. *Mar. Biol.* **151**, 1407–1415 (2007).
59. Horváth, Z. *et al.* Eastern spread of the invasive *Artemia franciscana* in the Mediterranean Basin, with the first record from the Balkan Peninsula. *Hydrobiologia* **822**, 229–235 (2018).
60. Muñoz, J., Amat, F., Green, A. J., Figuerola, J. & Gómez, A. Bird migratory flyways influence the phylogeography of the invasive brine shrimp *Artemia franciscana* in its native American range. *PeerJ* **1**, e200 (2013).
61. Muñoz, J. *et al.* Phylogeography and local endemism of the native Mediterranean brine shrimp *Artemia salina* (Branchiopoda: Anostraca). *Mol. Ecol.* **17**, 3160–3177 (2008).
62. Sánchez, M. I., Hortas, F., Figuerola, J. & Green, A. J. Comparing the potential for dispersal via waterbirds of a native and an invasive brine shrimp. *Freshw. Biol.* **57**, 1896–1903 (2012).
63. Viana, D. S., Santamaria, L., Michot, T. C. & Figuerola, J. Migratory strategies of waterbirds shape the continental-scale dispersal of aquatic organisms. *Ecography* **36**, 430–438 (2013).

64. Green, A. J. *et al.* Dispersal of invasive and native brine shrimps *Artemia* (Anostraca) via waterbirds. *Limnol. Oceanogr.* **50**, 737–742 (2005).
65. Kappas, I. *et al.* Molecular and morphological data suggest weak phylogeographic structure in the fairy shrimp *Streptocephalus torvicornis* (Branchiopoda, Anostraca). *Hydrobiologia* **801**, 21–32 (2017).
66. Rogers, D. C. Larger hatching fractions in avian dispersed anostracan eggs (Branchiopoda). *J. Crustac. Biol.* **34**, 135–143 (2014).
67. Angeler, D. G., Viedma, O., Sánchez-Carrillo, S. & Alvarez-Cobelas, M. Conservation issues of temporary wetland Branchiopoda (Anostraca, Notostraca: Crustacea) in a semiarid agricultural landscape: What spatial scales are relevant?. *Biol. Cons.* **141**, 1224–1234 (2008).
68. Horváth, Z., Vad, C. F., Vörös, L. & Boros, E. Distribution and conservation status of fairy shrimps (Crustacea: Anostraca) in the astatic soda pans of the Carpathian basin: the role of local and spatial factors. *J. Limnol.* **72**, 103–116 (2013).
69. Svensson, L., Mullarney, K. & Zetterström, D. *Collins Bird Guide* 2nd edn. (HarperCollins Publishers Ltd., 2009).
70. Horváth, Z., Vad, C. F., Vörös, L. & Boros, E. The keystone role of anostracans and copepods in European soda pans during the spring migration of waterbirds. *Freshw. Biol.* **58**, 430–440 (2013).
71. Gill, J. L. Ecological impacts of the late Quaternary megaherbivore extinctions. *New Phytol.* **201**, 1163–1169 (2014).
72. Neretina, A. N. *et al.* Crustacean remains from the Yuka mammoth raise questions about non-analogue freshwater communities in the Beringian region during the Pleistocene. *Sci. Rep.* **10**, 859 (2020).
73. Chang, D. *et al.* The evolutionary and phylogeographic history of woolly mammoths: A comprehensive mitogenomic analysis. *Sci. Rep.* **7**, 44585 (2017).
74. Lister, A. M., Sher, A. V., van Essen, H. & Wei, G. The pattern and process of mammoth evolution in Eurasia. *Quatern. Int.* **126–128**, 49–64 (2005).
75. Vanschoenwinkel, B. *et al.* Passive external transport of freshwater invertebrates by elephant and other mud-wallowing mammals in an African savannah habitat. *Freshw. Biol.* **56**, 1606–1619 (2011).
76. Waterkeyn, A., Pineau, O., Grillas, P. & Brendonck, L. Invertebrate dispersal by aquatic mammals: A case study with nutria *Myocastor coypus* (Rodentia, Mammalia) in Southern France. *Hydrobiologia* **654**, 267–271 (2010).
77. Belk, D. & Brtek, J. Checklist of the Anostraca. *Hydrobiologia* **298**, 315–353 (1995).
78. Marrone, F., Korn, M., Stoch, F., Naselli Flores, L. & Turki, S. Updated checklist and distribution of large branchiopods (Branchiopoda: Anostraca, Notostraca, Spinicaudata) in Tunisia. *Biogeogr. J. Integr. Biogeogr.* **31**, 27–53 (2016).
79. Mura, G. & Brtek, J. Revised key to families and genera of the Anostraca with notes on their geographical distribution. *Crustaceana* **73**, 1037–1088 (2000).
80. Atashbar, B., Agh, N., Van Stappen, G., Mertens, J. & Beladjal, L. Combined effect of temperature and salinity on hatching characteristics of three fairy shrimp species (Crustacea: Anostraca). *J. Limnol.* **73**, 574–583 (2014).
81. Eder, E., Hödl, W. & Gottwald, R. Distribution and phenology of large branchiopods in Austria. *Hydrobiologia* **359**, 13–22 (1997).
82. Šćiban, M., Marković, A., Lukić, D. & Miličić, D. Autumn populations of *Branchinecta orientalis* G. O. Sars, 1903 and *Chirocephalus diaphanus* Prevost, 1803 (Crustacea, Branchiopoda) in the Central European Lowlands (Pannonian Plain, Serbia). *North-West. J. Zool.* **10**, 435–437 (2014).
83. Alonso, M. A survey of the Spanish Euphyllipoda. *Miscelania Zool.* **9**, 179–208 (1985).
84. Petkovski, S. On the presence of the genus *Branchinecta* Verrill, 1869 (Crustacea, Anostraca) in Yugoslavia. *Hydrobiologia* **226**, 17–27 (1991).
85. Dimentman, C. The rainpool ecosystems of Israel: Geographical distribution of freshwater Anostraca (Crustacea). *Israel J. Ecol. Evol.* **30**, 1–15 (1981).
86. Eid, E. K. New records of large branchiopods from northern Jordan (Crustacea: Branchiopoda). *Zool. Middle East* **46**, 116–117 (2009).
87. Mura, G., Ozkutuk, S. R., Aygen, C. & Cottarelli, V. New data on the taxonomy and distribution of anostracan fauna from Turkey. *J. Biol. Res.* **15**, 17–23 (2011).
88. Rogers, D. C., Quinney, D. L., Weaver, J. & Olesen, J. A new giant species of predatory fairy shrimp from Idaho, USA (Branchiopoda: Anostraca). *J. Crustac. Biol.* **26**, 1–12 (2006).
89. Rodríguez-Flores, P. C., Jiménez-Ruiz, Y., Forró, L., Vörös, J. & García-París, M. Non-congruent geographic patterns of genetic divergence across European species of *Branchinecta* (Anostraca: Branchinectidae). *Hydrobiologia* **801**, 47–57 (2017).
90. Atashbar, B., Agh, N., Van Stappen, G. & Beladjal, L. Diversity and distribution patterns of large branchiopods (Crustacea: Branchiopoda) in temporary pools (Iran). *J. Arid. Environ.* **111**, 27–34 (2014).
91. Belk, D. & Esparza, C. E. Anostraca of the Indian Subcontinent. *Hydrobiologia* **298**, 287–293 (1995).
92. Brtek, J. & Thiéry, A. The geographic distribution of the European Branchiopods (Anostraca, Notostraca, Spinicaudata, Laevicaudata). *Hydrobiologia* **298**, 263–280 (1995).
93. Horn, W. & Paul, M. Occurrence and distribution of the Eurasian *Branchinecta orientalis* (Anostraca) in Central Asia (Northwest Mongolia, Uvs Nuur Basin) and in other holarctic areas. *Lauterbornia* **49**, 81–91 (2004).
94. Marrone, F., Alonso, M., Pieri, V., Augugliaro, C. & Stoch, F. The crustacean fauna of Bayan Onjuul area (Tov Province, Mongolia) (Crustacea: Branchiopoda, Copepoda, Ostracoda). *North West. J. Zool.* **11**, 288–295 (2015).
95. Mura, G. & Takami, G. A. A contribution to the knowledge of the anostracan fauna of Iran. *Hydrobiologia* **441**, 117–121 (2000).
96. Naganawa, H. *et al.* Does the dispersal of fairy shrimps (Branchiopoda, Anostraca) reflect the shifting geographical distribution of freshwaters since the late Mesozoic?. *Limnology* <https://doi.org/10.1007/s10201-019-00589-9> (2019).
97. Padhye, S. M., Kulkarni, M. R. & Dumont, H. J. Diversity and zoogeography of the fairy shrimps (Branchiopoda: Anostraca) on the Indian subcontinent. *Hydrobiologia* **801**, 117–128 (2017).
98. Petkovski, S. *Taksonomsko-morfološka i zoogeografsko-ekološka studija Anostraca (Crustacea: Branchiopoda) jugoslovenskih zemalja*. (Prirodno-matematički fakultet, Novi Sad, 1993).
99. Pretus, J. L. A commented check-list of the Balearic Branchiopoda (Crustacea). *Limnetica* **6**, 157–164 (1990).
100. van den Broeck, M., Waterkeyn, A., Rhazi, L. & Brendonck, L. Distribution, coexistence, and decline of Moroccan large branchiopods. *J. Crustacean Biol.* **35**, 355–365 (2015).
101. Hijmans, R. J., Phillips, S., Leathwick, J. & Elith, J. Package 'dismo'. **9**, 1–68 (2017).
102. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>. (2014).
103. Hijmans, R. J., Cameron, S. E. & Parra, J. L. *Climate Data from Worldclim* (2004).
104. Alfonso, G. & Marrone, F. Branchiopoda Anostraca, Notostraca, Spinicaudata. In *Checklist of the Italian fauna* (in press).
105. Defaye, D., Rabet, N. & Thiéry, A. Atlas et bibliographie des crustacés branchiopodes (Anostraca, Notostraca, Spinicaudata) de France métropolitaine. *Collection patrimoines naturels* (1998).
106. Song, H., Buhay, J. E., Whiting, M. F. & Crandall, K. A. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**, 13486–13491 (2008).
107. Hall, T. A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* **41**, 95–98 (1999).
108. Aguilar, A. *et al.* High intraspecific genetic divergence in the versatile fairy shrimp *Branchinecta lindahli* with a comment on cryptic species in the genus *Branchinecta* (Crustacea: Anostraca). *Hydrobiologia* **801**, 59–69 (2017).

109. Jeffery, N. W., Elías-Gutiérrez, M. & Adamowicz, S. J. Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. *PLoS ONE* **6**, e18364 (2011).
110. Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **34**, 772–773 (2017).
111. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).
112. Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
113. Huelsenbeck, J. P. & Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755 (2001).
114. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574 (2003).
115. Ronquist, F. *et al.* MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
116. Bandelt, H. J., Forster, P. & Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48 (1999).
117. Leigh, J. W. & Bryant, D. popart: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116 (2015).
118. Xia, X. & Kumar, S. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* **35**, 1550–1552 (2018).
119. Xia, X. & Lemey, P. Assessing substitution saturation with DAMBE. In *The phylogenetic Handbook* 615–630 (Cambridge University Press, 2009).
120. Xia, X., Xie, Z., Salemi, M., Chen, L. & Wang, Y. An index of substitution saturation and its application. *Mol. Phylogenet. Evol.* **26**, 1–7 (2003).
121. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120 (1980).
122. Rozas, J. *et al.* DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* **34**, 3299–3302 (2017).
123. Nychka, D. *et al.* *fields: Tools for Spatial Data* (2020).
124. Oksanen, J. *et al.* *vegan: Community ecology package*. – R package ver. 2.0-4. <http://CRAN.R-project.org/package=vegan>. (2012).

Acknowledgements

The work was supported by the Interreg V-A Austria-Hungary program of the European Regional Development Fund (“Vogelwarte Madárvárta 2”) and ÖAD (Erasmus+ internship mobility grant). D.L. was a recipient of the DOC fellowship of the Austrian Academy of Sciences and Nationalparks Austria research scholarship at the WasserCluster Lunz and is currently employed on an FWF project—FWF P 32714. Z. H. acknowledges support by the NKFIH OTKA FK-132095, NKFIH-471-3/2021, and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The work of M. M. was supported by grant no. 2017/27/B/NZ8/01056 from the National Science Centre, Poland. Authors thank Lake Neusiedl Biological Station, Thomas Zechmeister, Richard Haider, Jordi Sala, Raquel Ortells, Priscila Pons, Bart Hellemans, Csenge Póda, Adam Petrusek and Luca Vecchioni for their help in field work, sample collection, work in the molecular lab or performing part of the analyses.

Author contributions

D.L., Zs.H., Cs.V. and R.P. conceived this study. D.L. and M.M. performed the lab work with help from T.P. and L.B. D.L. analysed the data with the help of Zs.H., T.P., M.M. and F.M. D.L. and Zs.H. wrote the first version of the manuscript, after which all authors contributed to improving the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-02164-8>.

Correspondence and requests for materials should be addressed to D.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021