INTRODUCTION

Dispersal ability influences how much gene flow will occur between populations, how quickly organisms can colonise new habitats, and how isolated habitat patches they can reach. Lower dispersal ability leads to more isolation and differentiation between parts of species ranges (e.g. Papadopoulou et al., 2009; Riginos et al., 2014), and, intuitively, species with low dispersal ability may be expected to have smaller distribution ranges because they face difficulties expanding their range or more easily split into allopatric species. The dispersal limitation could then lead to high spatial turnover of species and intraspecific lineages and to spatial grouping of related lineages. In a
similar vein, older species may have larger ranges as they had more time for dispersal. However, this relationship between dispersal ability and range size often does not hold up (e.g. Lester et al., 2007; Sheth et al., 2020). A relation between range size and species age has been found in some groups but not others, with the theoretical expectations and details of the observed relationships varying (e.g. Abellán & Ribera, 2011; Sheth et al., 2020; Webb & Gaston, 2000). Other important determinants of range size are clearly the ecological niche of the species, its breadth (Slattery et al., 2013) and the distribution of suitable habitat patches. The latter may change quickly during the existence of a species leading to large changes in range size. Even taxa with relatively low powers of dispersal like land snails are able to extend their range limits for hundreds of kilometres within a couple of millennia, as is evidenced by expansions after the Last Glacial (e.g. Korábek et al., 2018). Similarly, the distribution of a higher clade may be uncoupled from its age or dispersal ability of its constituent species as the number of species and species ranges are influenced by changes of the environment, which may be at some point exceptionally favourable for their expansion but may also lead to range contractions and species extinctions.

Dispersal of large-sized rock-dwelling land snails is particularly limited. Rock outcrops are in many cases an island-like habitat and the life of rock-dwelling snails is closely tied to the rock crevices in which they hide, which both reduces dispersal rates and gene flow (Criscione & Köhler, 2016; Köhler & Burghardt, 2016; Neiber et al., 2019; Ursenbacher et al., 2010). It is among rock-dwelling snails where narrowly distributed species and localised conchological forms appear most commonly. In the Western Palaearctic, well-studied examples of spatial differentiation on a small scale are found especially in the families Clausiliidae and Helicidae (e.g. Bamberger et al., 2021; Elejalde, Madeira, Arrébola et al., 2008; Elejalde, Madeira, Muñoz et al., 2008; Päll-Gergely et al., 2019; Rensch, 1937; Sacchi, 1955; Schilthuizen, 2018; Walther et al., 2016), and limited dispersal is among the explanations invoked for the high spatial turnover.

The helicid genus Levantina Kobelt, 1871, is an example of a rock-dwelling taxon with a broad distribution. Most of its constituent species live in south-eastern Turkey, western Iran and Iraqi Kurdistan (Schütt & Subai, 1996). Others are found in the Levant up to its south (Germain, 1921, 1922; Heller, 1979, 1984; Neubert et al., 2015; Schütt, 2005), along the western Arabian Peninsula (Neubert, 1998) and on Cyprus (Glaubrecht, 1993a; Schütt & Subai, 1996). The eastern range limits are unclear, as Levantina species are known to occur approximately up to the region near Tehran, but a single report exists for an isolated location also in south-east Samarqand in eastern Uzbekistan (Dohrn, 1882). There are also occurrences in south-western Anatolia and the south-eastern Aegean region (Glaubrecht, 1993a, 1994; Pfeiffer, 1949), but these include one or two taxa that have never been collected alive (see below) and the origin of the remaining two (possibly conspecific) taxa remains controversial (Ketmaier & Glaubrecht, 2015; Neubert et al., 2015). The range of the genus, thus, extends c. 2000 km along its longest dimension, but may even be significantly larger.

The phylogenetic relationships of Levantina to the other Eastern Mediterranean rock-dwelling Helicini, namely Neocrassa Subai, 2005, Codringtonia Kobelt, 1898, Isaurica Kobelt, 1901, and Amanica Nordsieck, 2017, remain unresolved (Neiber et al., 2021). Neocrassa and Amanica are narrowly distributed (Subai, 1994, 2005) and isolated (Neiber et al., 2021) monotypic genera. Isaurica has a limited range in the south-west of Anatolia (Subai, 1994). Codringtonia includes several narrowly distributed species on the Peloponnese and the adjacent Greek mainland (Kotsakiozi et al., 2012; Subai, 2005). If dispersal limitation due to the rock-dwelling lifestyle is similar in Codringtonia and Levantina, we may expect a considerable diversity within the latter given its much larger distribution range.

Three subgenera were recognised within Levantina: Levantina s. str., Laevihelix Neubert, 1998, and Gyrostomella Hesse, 1911 (Hesse, 1909, 1918; Neubert, 1998; Subai, 1994). Most of the species currently included in the genus were placed into a separate genus Assyrilla Hesse, 1908 (Hesse, 1918; Schütt & Subai, 1996; Subai, 1994). This arrangement has been overturned by Neiber et al. (2021), who demonstrated that Gyrostomella actually belongs to a different tribe of Helicinae and both the subgenera Levantina and Laevihelix are nested among species assigned to Assyrilla. Laevihelix and Assyrilla are, therefore, now both considered junior synonyms of Levantina.

The former subgenus Levantina s. str. has been comprehensively revised by Pfeiffer (1949), who recognised several taxa classified within the “Rassenkreis” Levantina spiriplana (Olivier, 1801; see also Zilch, 1952). The classification that prevailed distinguished fewer valid taxa than he did, though the exact number was not agreed upon (Avnimelech, 1933; Glaubrecht, 1993a; Heller, 1979, 2009; Hesse, 1918; Ketmaier & Glaubrecht, 2015; Neubert et al., 2015; Schütt, 2005). Up to four taxa were classified as species: L. spiriplana and Levantina malziana (Pfeiffer, 1861) in the south-eastern Aegean and adjacent south-western Anatolia, and Levantina lithophaga (Conrad, 1852) and Levantina caesarea (Mousson, 1854) in the Levant (Neubert et al., 2015). Levantina spiriplana and L. lithophaga are characterised by an open umbilicus, while L. malziana and L. caesarea have a closed umbilicus, so in both the Levant and
the south-eastern Aegean there are two conchological forms differing by the state of the umbilicus. The first phylogeny of the species included in *Levantina* s. str., published by Ketmaier and Glaubrecht (2015), uncovered a recent connection between the two areas in the form of a shared haplotype, supporting the hypotheses of a human-mediated dispersal from the Levant into the Aegean region as first suggested by Glaubrecht (1993a, 1993b, 1994). However, the study concluded that *Levantina* originally colonised the Aegean probably at the latest in the Pliocene based on the presence of multiple haplotypes and a molecular clock dating. This conclusion has been criticised on grounds of insufficient sampling in the Levant by Neubert et al. (2015), and it remains possible that there are only two (sub)species (one umbilicated, the other with a closed umbilicus; Neubert et al., 2015) and that both were introduced to the Aegean by humans. That would not be surprising as there are edible helicids other than *Levantina*, where anthropogenic introductions from the Levant to south-western Anatolia and the eastern Aegean were suspected (Korábek et al., 2015, 2021).

Neubert (1998) described four species from the western Arabian Peninsula, but only three of them have been collected alive so far. *Levantina semitecata* Neubert, 1998, from an unknown locality near the northern Red Sea is known only from its two type specimens collected in the 19th century. The other three species were all found east or south-east of Makkah. Neubert (1998) established a subgenus *Levantina* (*Laevihelix*) for these Arabian species.

Schütt and Subai (1996) revised the taxonomy of species formerly placed in *Assyriella* based on morphological characters and accepted 17 species, two of which were newly described. The majority of them are distributed in south-eastern Turkey, western Iran and Iraqi Kurdistan. In the west, the range extends to the Central Taurus Mountains near Mersin and to Cyprus. There are records of *Assyriella* further west, but they are somewhat problematic. *Levantina rechingeri* Fuchs & Käufel, 1936, is known only from empty shells found at a few sites in the centre of Karpathos Island in the south-eastern Aegean (Glaubrecht, 1994; Grano & Cattaneo, 2021). Nordsieck (2017) suggested that *Isaurica callirhoe* (Rolle, 1894), known only from shells of the type series collected at an imprecisely known location in the far south-west of Anatolia, actually belongs to *Levantina*. The eastern and south-eastern distribution limits are also not well documented. The eastern-most species, *Levantina longinqua* (Schütt & Subai, 1996), which was described based on material allegedly from south-east of Samarqand, Uzbekistan (Dohrn, 1882), is known only from shells. Reliable records of *Levantina* extend, however, to the central Alborz Mountains in Iran.

The current taxonomy of *Levantina* is based solely on morphological characters, the usefulness of which for species delimitation in helicids varies from taxon to taxon and which may evolve rapidly, even in a single species (Fiorentino et al., 2008; Walther et al., 2016). The relationship between morphological variation, phylogenetic relatedness and species limits would remain unknown without genetic data. An important issue is that the actual extent of intraspecific variation may not be fully appreciated because several species are known only from a limited number of sites. Additional forms are likely to be uncovered with more complete sampling (see Gümüş & Neubert, 2012, for the most recently described taxon), including intermediates between seemingly distinct groups of populations.

The study of *Levantina* has been always plagued by a scarcity of material from most of its large range, which to this day prevents a thorough phylogenetic and taxonomic analysis of the genus. However, we were recently able to revise the delimitation of the genus by excluding the North African *Gyrostromella* and synonymising *Assyriella* and *Laevihelix* with *Levantina*, thanks to the utilisation of museum specimens (Neiber et al., 2021). Here, we follow up by a first comprehensive phylogenetic analysis of the diversity of *Levantina* in its revised sense covering the full extent of its confirmed distribution range. We show that the present large range of *Levantina* is not due to the age of the genus and that its geographic expansion was a fast process indicating the potential of these snails for long-distance dispersal. We also re-examine the origin of the populations of *Levantina* in the Aegean and conclude that human-mediated dispersal is the most likely explanation for the colonisation of that region by *Levantina*.

### 2 | MATERIAL AND METHODS

#### 2.1 | Material

We obtained samples of 20 of the 26 currently accepted species (four previously classified in *Levantina* s. str., four in *Levantina* (*Laevihelix*) and 18 in *Assyriella*). This includes material from the type localities of *Levantina bellardi* (Mousson, 1854), *Levantina mardinensis* Kobelt, 1900, *L. malziana*, *Levantina mazenderanensis* (Kobelt, 1883), *Levantina naegelei* Kobelt, 1901, *Levantina vanensis* (Schütt & Subai, 1996), *Levantina symensis* Neubert, 1998, *Levantina asagittata* Neubert, 1998, and *Levantina asira* Neubert, 1998. The type localities of *Levantina ciliacea* (Kobelt, 1895) and *Levantina ghilanica* (Mousson, 1876) are not identifiable, but our samples originate from their proximity. The analysed
samples include paratypes of *L. symensi*, *L. asira* and *L. asagittata*, and probably also of *Levantina thospitis* (Schütt & Subai, 1996).

No samples were available from *L. rechingeri*, *L. (?) calirhoe*, *L. longinqua* and *L. semitecta*, four taxa known only from shell material. Also missing are *Levantina mahanica* Kobelt, 1910, and *Levantina ninivita* (Galland, 1885). *Levantina kurdistanica* (Pfeiffer, 1862) is represented only by its subspecies *L. kurdistanica baschkira* (Pfeiffer, 1862).

We sequenced c. 810 bp of the 16S rRNA (16S) gene and 655 bp of cytchrome c oxidase subunit I (cox1) gene, the two mitochondrial loci most used in land snail systematics. The laboratory methods used for DNA extraction, PCR and sequencing corresponded to those given in Neiber et al. (2021). The primer pairs 16Scs1 + 16Scs2 (Chiba, 1999) and LCO1490 + HC02198 (Folmer et al., 1994) were used for amplification of 16S and cox1 fragments, respectively. Internal primers 16S-F: 5′-CGG CGC CCT GTT TAT CAA AAA CAT-3′ (Misof et al., 2000), 16S_MN3R: 5′-GCT ACC TTT GCA CTA GGA CWA GWR-3′ (Neiber et al., 2017), COI_OK1F: 5′-TTG TTA CTG CYC AYG CRT TTG-3′ and COI_OK3R: 5′-AAA GGT GGR TAA ACA GTY CAN CC-3′ were used for amplification of museum samples with degraded DNA, and the targeted segment was then amplified in two overlapping fragments.

The data we used also include sequences of 22 of the *Levantina* s. str. individuals analysed by Ketmaier and Glaubrecht (2015). Although they used in their analysis only very short sequence fragments, in some individuals they successfully sequenced longer segments. We inspected the original chromatogram files and used those reads from which we could extract longer sequences corresponding to the full cox1 fragment targeted here and roughly half of the 16S fragment.

The material and sequences (with GenBank accession numbers) used are listed in Table S1.

### 2.2 | Phylogenetic analyses

The 16S sequences were aligned with MAFFT 7.471 (Katoh & Standley, 2013) using the Q-INS-i algorithm (Katoh & Toh, 2008) with default settings except for a scoring matrix set to 20 PAM (option—kimura 20). The mitochondrial cox1 sequences were aligned using Muscle (Edgar, 2004) in MEGA 7 (Kumar et al., 2016) using default settings. For the analyses, we collapsed identical sequences per locality. The partitioning scheme and substitution models were selected with ModelFinder in IQ-TREE 1.6.12 (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015). We initially partitioned the data into three codon positions of cox1 and separate partitions for the 16S rRNA gene, which was supported by ModelFinder. The following substitution models were selected for the three codon positions of cox1 and for 16S, respectively: TN + G, F81 + I, GTR + G, HKY + I + G.

Maximum likelihood analyses were then run with IQ-TREE in ten replicate runs; branch support was assessed with standard bootstrap (1000 pseudoreplicates) and mapped onto the tree with the highest likelihood. Bayesian analysis was run in MrBayes 3.2.7 (Ronquist et al., 2012) using the default scheme of two runs with four chains each. The analysis was run for 40,000,000 generations. Diagnostics calculated by MrBayes and Tracer 1.7.1 (Rambaut et al., 2018) were used to check stationarity and convergence of the runs. The first 10,000,000 generations were discarded as burn-in. The root was assumed to be between a distinctively divergent *Levantina* lineage from near Lake Van and the rest of the samples as suggested by the analysis of Neiber et al. (2021).

### 2.3 | Biogeographic reconstruction

To investigate the geographic origins of the diversity of *Levantina*, we conducted a continuous biogeographic analysis with a random walk model in BEAST 1.10.4 (Suchard et al., 2018). We performed the analysis with three versions of the dataset: the full one as above, one with the Aegean samples removed and the full dataset with the addition of two additional samples of Aegean *Levantina* from Ketmaier and Glaubrecht (2015). These represent a third mitochondrial lineage of *L. spiriplana* in the Aegean, and their inclusion provides a dataset most favourable to diversification in the Aegean, but only the published short sequences were available and, therefore, they were not used for the phylogenetic analyses otherwise. We specified a lognormal uncorrelated relaxed clock model, a lognormal relaxed random walk spatial diffusion model and Bayesian skyline as a flexible tree prior. Our data combine intraspecific and interspecific divergences and, therefore, combine speciation and the coalescent. This may potentially bias the estimated ages, and it has been suggested that the skyline prior should be robust against these effects (Ritchie et al., 2017). To enable direct comparison of clade ages within Helicidae, we used the absolute ages inferred by Neiber et al. (2021) to calibrate the molecular clock, despite the substantial uncertainty concerning the original fossil calibration and the estimated ages. We specified lognormal priors for the ages of the root and for the most recent common ancestor (MRCA) of *Levantina guttata* (Oliver, 1804), *L. mazenderanensis*, *L. spiriplana*, *Levantina escheriana* (Bourguignon, 1864) and *L. symensi*. The mean and standard deviation of the prior distributions were set to correspond to the node ages and 95% highest posterior density intervals inferred by
A random jitter of 0.001° was added to duplicate geographic locations. The analyses were run for 100,000,000 generations in two replicate runs, sampling each 10,000th generation. Convergence of the runs was checked with Tracer, and the results were summarized with TreeAnnotator after excluding the first 25% of generations.

3 | RESULTS

3.1 | Phylogeny

Most of the analysed *Levantina* samples group in a large and widely distributed clade (Figure 1; the “core clade” hereafter) comprising the taxa previously included in *Levantina* s. str., and some of the taxa included in *Assyriella*, namely *L. bellardi* from Cyprus, *L. cilicica* and *L. naegelei* from central southern Turkey, and five species from south-eastern Turkey: *L. kurdistana*, *L. guttata*, *L. vanensis*, *L. thospitis* and *L. mardinensis*. This clade was recovered in all analyses, but received statistical support only in the Bayesian analysis. Basal relationships within this clade were largely unresolved.

*Levantina* s. str. is probably monophyletic, but lacks statistical support. We found three well-supported clades (Figures 1 and 2) that were already represented in the data of Ketmaier and Glaubrecht (2015). All were recorded in both the Aegean region and the Levant. In addition, we uncovered a fourth one represented by an individual corresponding to a form described as *Levantina werneri* (Kobelt, 1889) from Israel. This nominal taxon has...
been considered a form of *L. caesareana* (Heller, 1979, 2009; Neubert et al., 2015). The Aegean *L. spiriplana* and *L. malziana* do not form separate branches, but are nested among samples from the Levant. Furthermore, the umbilicated forms are nested among those without an umbilicus. Similarly, the umbilicated *L. cilicica* is nested within a group of non-umbilicated *L. naegelei*. *Levantina guttata* groups with *L. vanensis* and *L. mardinensis* with
L. *thospitis*; in both cases, the p-distances are small (for combined 16S and *cox1* 4.7% and 3.3%, respectively) and the species have adjacent ranges (Schütt & Subai, 1996).

Three additional clades were recovered besides the core one that included fewer taxa. Their mutual relationships were also unresolved, but the monophyly of each was well supported. One consists only of *Levantina ceratomma* (Pfeiffer, 1856) from near Lake Van. The second one unites three species from the north-east of the range of the genus: *L. djulfensis*, *L. mazenderanensis* and *L. ghilanica*. In the third one, *L. escheriana* is found to be the sister lineage to a clade uniting the three Arabian species, *L. symensi*, *L. asagittata* and *L. asira*.

Finally, there is the lineage which was used to root the tree, which comprises one population from south-eastern Turkey near the western end of Lake Van. Its basal position is supported also by the molecular clock analysis. However, note that the large divergence between this population and the other *Levantina* taxa is not observed in the 5.8S rRNA-ITS2-28S rRNA data of Neiber et al. (2021).

### 3.2 Biogeographic analysis

The results of the biogeographic analysis in BEAST were very similar irrespective of the inclusion of *Levantina* samples from the Aegean region. The initial diversification of *Levantina* took place in the south-east of the present-day territory of Turkey, and this area was inferred to be from where the spatial expansion took place (Figure 2). The MRCA of the clade comprising *L. escheriana* and the Arabian *Levantina*, of the eastern clade uniting *L. djulfensis*, *L. ghilanica* and *L. mazenderanensis*, as well as of the broadly distributed core clade including *L. spiriplana* were all placed in this region. It is possible, but not strongly supported, that the expansion from this area to the east, south and west was broadly contemporaneous in these three clades. According to the calibration used, the mitochondrial tree of *Levantina* spans over 15 My. The expansion to the Central Taurus Mountains, Levant and Arabia has apparently happened within ca 2.2 My or less (possibly well under 1 My) during the Pliocene. All three lineages of *L. spiriplana* reported from the Aegean region by Ktmaier and Glaubrecht (2015) were found to have started diversification in the Levant, regardless whether the Aegean populations were included in the analysis or not. Although an individual of *L. spiriplana* from Rhodes which shared its haplotype with a sample from Jerusalem in the data of Ktmaier and Glaubrecht (2015) was not included in our dataset, there was in one case only one nucleotide difference between a sample from Jerusalem and one from the western Turkish coast over the 1064 sites of the two concatenated loci.

### 4 DISCUSSION

#### 4.1 Origin and dispersal of *Levantina*

Our new dataset including the majority of the currently accepted species of *Levantina* confirmed that *Levantina* s. str. and *Levantina* (*Laevihelix*) are nested among *Assyriella* taxa (Neiber et al., 2021). Therefore, *Laevihelix* and *Assyriella* have to be considered junior synonyms of *Levantina*. The crown group of the genus originated in what is currently south-eastern Turkey. *Levantina* s. str., as recognised for the last century, represents one branch of this radiation that diversified in the Levant. Unexpectedly, the Arabian *Levantina* (*Laevihelix*) is not the sister group of the neighbouring *Levantina* s. str., but of *L. escheriana*, a species distributed in south-eastern Turkey and extending to Armenia and marginally to northern Syria (Schütt & Subai, 1996). The mitochondrial phylogeny clearly suggests that the Levantine and Arabian *Levantina* species are the result of two independent expansions from the diversification centre to the south-west, which is surprising given that colonisation from or through the Levant seemed to be the most likely explanation for the presence of *Levantina* in the western Arabian Peninsula (Neubert, 1998). However, there exists a similar case, as the closest relatives of *Lejeania Ancey*, 1887 (*Hygromiidae*) from the south-western Arabian Peninsula and Eastern Africa were found to be taxa from the Caucasus region (Neiber et al., 2017).
Besides the sister-group relationship between the Arabian *Levantina* and *L. escheriana*, the Bayesian analysis supported also close relationships among a group of species distributed broadly from Cilicia and Cyprus (*L. ciliica* and *L. bellardii*) to the very south-east of Turkey (*L. kurdistana*) and to the southern Levant (*L. spiriplana*). Within both of these groupings, species from distant parts of the distribution range of *Levantina* are closely related. Given the rock-dwelling lifestyle of *Levantina*, we expected the phylogeny to more clearly reflect geographic relationships between species. In contrast, the results indicate that the core clade of *Levantina* quickly expanded, creating a crescent-shaped range extending at least 1400 km (the south-eastern extent is uncertain because data are missing for *L. mahanaica*). Even more surprisingly, a clade was found containing *L. escheriana* and the Arabian species which has a disjunctive range with a gap in distribution of c. 1000 km (1600 km if *L. semitecta* is not related to *L. asira*, *L. asagittata* and *L. symensi*). The molecular clock analysis of Neiber et al. (2021, fig. 4) combined with our results suggests that the core clade of *Levantina* is approximately as old as the radiation of *Codringtonia* on the Peloponnese (longest range dimension >290 km). The mitochondrial diversity of *Levantina* except the basal-most lineage is then approximately as old as *Codringtonia* as a whole (maximum range extent also ~290 km). The rock-dwelling lifestyle does not seem to be, at least in *Levantina*, associated with limited dispersal in terms of the ability to extend range across more than a thousand kilometres over the timescales involved. However, range expansions involving long-distance dispersal are unpredictable chance events like in the recently uncovered dispersal of *Cornu cretense* Hausdorf, Bamberger & Walther, 2020, from Sicily into the Eastern Mediterranean region (Hausdorf et al., 2021), and as such are not directly informative about dispersal ability differences.

4.2 *Levantina spiriplana* and its dispersal into the Aegean region

A special problem in the biogeography of *Levantina* is the occurrence of the genus in the south-eastern Aegean region, viz. on the Dodecanese islands Karpathos, Rhodes, Symi and Kalymnos along with some smaller islands and the adjacent Anatolian coast (Glaubrecht, 1993a). Because of the accessibility of this area for European malacologists, this is where *Levantina* s. str. was found and described for the first time and where it has been studied in most detail (Glaubrecht, 1993a, 1993b; Ketmaier & Glaubrecht, 2015; Pfeiffer, 1949). However, similar to identical forms of *Levantina* s. str. are found also in the Levant and on Cyprus (see Heller, 1979; Neubert et al., 2015; Pfeiffer, 1949, for figures). This distribution pattern was always difficult to explain (Kobelt, 1898; Pfeiffer, 1949), and human-mediated transport between the regions has been eventually considered as an explanation (Glaubrecht, 1993a). In the most recent study, which was the first one employing molecular genetic data, Ketmaier and Glaubrecht (2015) proposed a complex biogeographic scenario involving both natural and anthropogenic colonization of the Aegean region by *Levantina* s. str. from Anatolia and the Levant, respectively. Our results based on a broader sampling in Anatolia and the Levant allow for a revised interpretation of their findings and show that, regardless whether naturally or with human aid, *Levantina* s. str. colonised the Aegean region from the Levant.

Ketmaier and Glaubrecht (2015) uncovered in the Aegean two principal mitochondrial clades corresponding to the non-umbilicated form *L. malziana*. Most samples belonged to a clade found by them only on the Aegean islands Karpathos, Rhodes, Symi and Nimos. In our phylogeny (Figure 1), specimens from these islands are nested within *L. caesarea* samples from the Levant, namely Israel/Palestine and the Turkish province of Hatay. A minority of the non-umbilicated samples of Ketmaier and Glaubrecht (2015) belonged to a second lineage, which they found on the Turkish coast (near Bodrum), on the Aegean island Symi and near Lake Tiberias (Kinneret) in the Levant. This latter clade is represented in our dataset by the sample from near Lake Tiberias and the two fragmentary sequences added for the biogeographic analysis. It is found to be sister to a third clade consisting mostly of samples of the umbilicated morphotype (*L. spiriplana* and *L. lithophaga*), which was found by Ketmaier and Glaubrecht (2015) in the Levant, on Rhodes, Karpathos and Symi, as well as on the Turkish coast. Within this clade, Ketmaier and Glaubrecht (2015) found one haplotype to be shared between the Levant and the supposed type locality of *L. spiriplana* (Glaubrecht, 1993a, 1993b) on Rhodes; this haplotype was found in the Levant at multiple sites. Our data also confirm the high similarity of haplotypes between Jerusalem and the Aegean, although this particular sample was not included. The association between an umbilicated shell and this third clade of Ketmaier and Glaubrecht (2015) is not absolute, as the authors reported haplotypes of this group also from the samples collected near Lake Tiberias, which was a population without umbilicus.

The non-umbilicated forms from the Aegean and the Levant belong to the same taxon, that is, *L. malziana* is a junior synonym of *L. caesarea*. Analogously, the umbilicated *L. lithophaga* (=*Levantina hierosolyma* (Mousson, 1854)) is a junior synonym of *L. spiriplana*. Our phylogenetic analysis (Figure 1) suggests that the umbilicated form is derived from the non-umbilicated one, which is
more broadly distributed in the Levant and is much more diverse. Heller (1979) analysed the hybrid zone at the contact between the umbilicated and non-umbilicated *Levantina* in the Levant (which runs westwards from the Dead Sea and close to Jerusalem) and found it to be only a couple of kilometres broad despite contact lasting probably at least 9000 years. This strongly suggests selection against hybrids, which was supported by lower fecundity and higher juvenile mortality in pairings of presumed *L. spiriplana x L. caesareana* hybrids. However, no similar clear-cut border between the range of the umbilicated and non-umbilicated form exists east of the Dead Sea in Jordan (Neubert et al., 2015). In the Aegean, hybrid populations have been found on Symi and Rhodes (Glaubrecht, 1993a). Therefore, we suggest treating the *Levantina spiriplana* complex as a single species with subspecies *L. spiriplana spiriplana* with umbilicus and *L. spiriplana caesareana* without umbilicus. The status of *L. werneri* needs further evaluation.

The presence of three divergent mitochondrial lineages in the Aegean (Ketmaier & Glaubrecht, 2015) implicates multiple colonisations of the region. In the case of *L. s. spiriplana*, the close link to the south of Israel/Palestine clearly indicates recent spread to the Aegean suggestive of anthropogenic dispersal. A natural origin of some of the Aegean populations of *L. s. caesareana* cannot be formally ruled out based on the currently available genetic data. Most of its Aegean haplotypes form a monophyletic group where there is a haplotype diversity that, if evolved in situ, would mean colonisation probably too early to be explained by human transport, although our dating gives a substantially more recent MRCA than that of Ketmaier and Glaubrecht (2015). However, *L. s. caesar- eana* is represented in the region by two divergent lineages, which according to our analysis both originated in the Levant. The total of three different lineages in the region means that the Aegean has been colonised multiple times or at least by a greater number of individuals. In our view, this is most easily explained by intentional transport, considering that *Levantina* is an edible snail (e.g. Bar, 1977). The presence of multiple haplotypes implies colonisation by a greater number of individuals, which is less likely to be accidental. *Levantina* has been transported even within the Aegean region several times in any case (Glaubrecht, 1993a; Ketmaier & Glaubrecht, 2015; Orstan, 2004). Furthermore, besides the Aegean and Levant, *L. spiriplana* occurs also on Cyprus, where both the umbilicated and non-umbilicated forms occur, but each one lives on an opposite end of the island and both in a restricted area (Glaubrecht, 1993a; Pfeiffer, 1949). Such a distribution pattern is also suggestive of anthropogenic introductions. Nevertheless, only thorough sampling of *L. s. caesareana* in the Levant could resolve this issue if haplotypes more similar or identical to those in the Aegean would be found.

### 4.3 Species-level taxonomy

Our results revealed also little differentiation between some of the other currently accepted *Levantina* species (Figure 1), which suggests that some of them may be synonyms. The individuals with an umbilicus classified as *L. cilicica* by Schütt and Subai (1996) are nested in the phylogenetic tree within those without an umbilicus classified as *L. naegelei* (Figure 1). The *L. cilicica* populations are found at the margin of the range of *L. naegelei*. We consider *L. naegelei* a synonym of *L. cilicica*, because these two nominal taxa do not differ in characteristics other than the state of the umbilicus (similar to *L. s. spiriplana* and *L. spiriplana caesareana*).

The divergence between the samples of *L. mardinen-sis* and *L. thospitis* (p-distance between *cox1* sequences: 0.035) is within a range that does not exceed intraspecific variation in most other stylommatophoran land snails (e.g. Koch et al., 2020; Korábek et al., 2015; Table S3). The two nominal taxa have adjacent ranges (Schütt & Subai, 1996) and might perhaps be classified as subspecies. A similar case is the widely distributed *L. guttata* and the conchologically very similar *L. vanensis* (p-distance between *cox1* sequences: 0.061). However, we refrain from formally synonymising the species because we lack topotypic samples of *L. thospitis* and *L. guttata*. Species-level taxonomy of the genus is in need of an integrative revision including genetic analyses based on a more comprehensive sampling.

### ACKNOWLEDGEMENTS

We thank Ronald Janssen (Senckenberg Museum, Frankfurt, Germany), Joseph Heller (Hebrew University of Jerusalem, Jerusalem, Israel), Inga Gryn (University of Duisburg–Essen, Essen, Germany), Eike Neubert (Natural History Museum, Bern, Switzerland), Zoltán Fehér (Hungarian Natural History Museum, Budapest), Frank Walther (Essen, Germany) and Jasna Simonová (Charles University, Praha) for collecting, loaning and/or donating material. Sigrid Hof and Julia Sigwart provided information on the *Levantina* material in SMF. The first author thanks Zoltán Fehér, Eike Neubert, Beat Pfarrer and Estée Bochud for great time in Budapest and Bern. We thank the Volkswagen Foundation for funding within the project ‘Biogeography of the land molluscs of the Caucasus region’. The first author’s work at LIB is funded by the Alexander von Humboldt Foundation. Open access funding enabled and organized by ProjektDEAL.
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How to cite this article: Korábek, O., Glaubrecht, M., Hausdorf, B., & Neiber, M. T. (2022). Phylogeny of the land snail Levantina reveals long-distance dispersal in the Middle East. Zoologica Scripta, 51, 161–172. https://doi.org/10.1111/zsc.12526