Hybridization and recurrent evolution of left–right reversal in the land snail genus *Schileykula* (Orculidae, Pulmonata)

Josef Harl1,2 | Elisabeth Haring2,3 | Barna Páll‐Gergely4

1 Institute of Pathology, University of Veterinary Medicine, Vienna, Austria
2 Central Research Laboratories, Museum of Natural History, Vienna, Austria
3 Department of Integrative Zoology, University of Vienna, Vienna, Austria
4 Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Correspondence
Josef Harl, Institute of Pathology, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria.
Email: josef.harl@vetmeduni.ac.at

Funding information
Austrian Science Fund, Grant/Award Number: 19592‐B17

Abstract
The land snail genus *Schileykula* Gittenberger, 1983 is distributed in arid limestone areas from western Turkey to north‐western Iran. It comprises eight species, which display high variation in shell size and morphology. The cylindrical shells are 5–12 mm in height and the last shell whorls bear several inner lamellae and plicae. Two taxa differ in their chirality having sinistral shells, while all the others are dextrals such as the vast majority of orculids. The aim of this study was to establish a molecular genetic phylogeny of *Schileykula* and to test whether it conforms to the current morphology‐based classification. Furthermore, we were interested in the phylogenetic position of the two sinistral forms in order to assess whether one or two reversals happened in the evolution of the genus. Nine out of ten species, including all four subspecies of *Schileykula trapezensis* and three of six subspecies of *Schileykula scyphus*, were investigated. A section of the mitochondrial *cytochrome c oxidase subunit I* gene was analyzed in 54 specimens of *Schileykula* and from a subsample, partial sequences of the mitochondrial genes for the 12S rRNA and the 16S rRNA, and a section of the nuclear *H4/H3* histone gene cluster were obtained. The phylogenetic trees based on the mitochondrial sequences feature high support values for most nodes, and the species appear well differentiated from each other. The two chiral forms evolved independently and are not sister lineages. However, some groupings disagree with the present morphology‐based classification and taxonomical conclusions are drawn. *Schileykula trapezensis* is polyphyletic in the molecular genetic trees; therefore, three of its subspecies are elevated to species level: *Schileykula acampsis* Hausdorf, 1996 comb. nov., *Schileykula neuberti* Hausdorf, 1996 comb. nov., and *Schileykula contraria* Neubert, 1993 comb. nov. Furthermore, *Schileykula sigma* is grouped within S. scyphus in the mitochondrial and nuclear trees and consequently treated as a subspecies of the latter (*Schileykula scyphus sigma* Hausdorf, 1996 comb. nov.). *Schileykula nordsiecki*, whose shell morphology is indistinguishable from that of the neighboring *Schileykula scyphus lycaonica*, but who differs in its genital anatomy, was confirmed to represent a distinct lineage. The phylogenies produced by the mitochondrial and nuclear data sets are to some extent conflicting. The patterns differ concerning the grouping of some specimens, suggesting at least two independent hybridization
events involving *S. contraria*, *S. scyphus* and *S. trapezensis*. The results exemplify the importance of integrating both mitochondrial and nuclear sequence data in order to complement morphology-based taxonomy, and they provide further evidence for hybridization across distantly related lineages in land snails.

**KEYWORDS**

12S, 16S, COI, histone genes, molecular phylogeny, taxonomy

1 | INTRODUCTION

The land snail genus *Schileykula* Gittenberger, 1983 is distributed in dry limestone areas of Asia Minor, from western Turkey to northwestern Iran (Hausdorf, 1996). Adults of *Schileykula* have cylindrical shells, which range from 5 to 12 mm in height. Shell shapes are less variable than the structure of inner lamellae and plicae in the adult shell. The morphology of the latter has thus been primarily used for taxonomy. Currently, ten species and 18 subspecies (including the nominate forms) are recognized in *Schileykula* as follows: *Schileykula aculeata* Gittenberger & Menkhorst, 1993, *Schileykula attilae* Páll-Gergely, 2010, *Schileykula batumensis* (Retowski, 1889), *Schileykula inversa* Schütt, 1993, *Schileykula maculata* Páll-Gergely & Asami, 2013, *Schileykula nordsiecki* Hausdorf, 1996, *Schileykula robusta* (Nägele, 1906), *Schileykula scyphus ciliicica* Hausdorf, 1996, *Schileykula scyphus crassa* (Pilsbry, 1922), *Schileykula scyphus enteropla* (Pilsbry, 1922), *Schileykula scyphus erecta* Hausdorf, 1996, *Schileykula scyphus lycaonica* Hausdorf, 1996, *Schileykula scyphus scyphus* (L. Pfeiffer, 1848), *Schileykula sigma* Hausdorf, 1996, *Schileykula trapezensis acampsis* Hausdorf, 1996, *Schileykula trapezensis contraria* Neubert, 1993, *Schileykula trapezensis neuberti* Hausdorf, 1996, and *Schileykula trapezensis trapezensis* (Stojsapal, 1981). The genital anatomy of almost all *Schileykula* species has been described and allows for distinguishing *Schileykula* from *Orcula* Steenberg, 1925 (Gittenberger, 1978; Hausdorf, 1996; Neubert, 1993; Páll-Gergely, 2010, 2011; Páll-Gergely & Asami, 2013; Páll-Gergely & DeMatta, 2016). Although the generic position of *S. robusta* has not been confirmed based on its genital anatomy (no living specimens have been found so far), similarities in shell traits suggest placing it within *Schileykula* (see Hausdorf, 1996).

Current records show that most *Schileykula* taxa are distributed in a patchy, parapatric pattern, and different species usually do not co-occur at the same localities but live in close vicinity. For example, *S. maculata* has only been recorded from a single site within the range of *Schileykula trapezensis* (Páll-Gergely & Asami, 2013). *Schileykula nordsiecki*, which was found at a single locality between two disjunct populations of *S. s. lycaonica*, differs from the latter in its genital anatomy, while it is indistinguishable based on shell morphology (Hausdorf, 1996). *Schileykula attilae* and *S. batumensis* are known to inhabit opposite sides of the same castle hill (Páll-Gergely, 2010). *Schileykula batumensis* and *S. t. acampsis* are the only *Schileykula* taxa known to occur in sympathy over a broad range, and the presence of populations with intermediate shell forms in the overlapping area was interpreted as an indication for hybridization between the two species (Neubert, 1993). Apart from the latter two taxa, potential hybrid populations were not reported for any other *Schileykula* taxa so far. However, the comparison of mt and nc sequence data in more recent studies showed that hybridization is not rare in land snails. There is evidence for hybridization between the orculid species *Orcula gularis* and *Orcula pseudodolium* (Harl, Páll-Gergely, et al., 2014), and the mixing of genetically and morphologically extremely distinct populations of *Orcula dolium* (Harl, Duda, Kruckenhaus, Sattmann, & Haring, 2014). Evidence for introgressive hybridization between morphologically divergent land snails was found in the family Bradybaenidae in the genus *Mandarina* (e.g., Chiba, 2005) and between the genera *Ainoheelix* and *Ezohelix* (Morii, Yokoyama, Kawata, Davison, & Chiba, 2015). RADseq data on the genus *Pyramidula* also provided evidence for minor ancestral gene flow between *Pyramidula pusilla* (Gittenberger & Bank, 1986) and *Pyramidula saxatilis* (Hartmann, 1842) (Razkin et al., 2016). Hybrid populations were investigated also in the genus *Albinaria* in Crete with SNP genotyping (Lammers et al., 2013). The presence of a hybrid population of the clausiliid species *Micropontica caucasica* (Schmidt, 1868) and *Micropontica circassica* (Boettger, 1888) in the Lagonaki plateau in the Caucasus, analyzed with AFLP markers, was interpreted as a case of hybrid speciation (Koch, Neiber, Walther, & Hausdorf, 2016).

A peculiarity of *Schileykula* is the presence of a sinistral (shell coiled counterclockwise) species and a sinistral subspecies, whereas all others are dextral (shell coiled clockwise). The sinistral subspecies (*S. t. contraria*) is known to occur between populations of the dextral *S. t. trapezensis*. *Schileykula inversa*, which is a sinistral species, lives close to populations of the dextral *S. s. erecta*, more than 200 km west of *S. t. contraria*. In gastropods reproducing by internal fertilization, the mating of snails with different chirality can cause genital mismatch (Asami, Cowie, & Ohbayashi, 1998; Gittenberger, 1988; Lipton & Murray, 1979). This mismatch is supposed to result in frequency-dependent selection against the chirality type with lower frequency (Johnson, 1982). Yet, there are examples of chirally dimorphic populations of snail species (subgenus *Amphidromus*) that exhibit no difficulty of interchiral mating (Nakadera et al., 2010; Schilthuizen et al., 2007; Sutcharih, Asami, & Panha, 2007; Sutcharih & Panha, 2006). The role of chirality reversal in speciation, that is, whether different chiral types cause sexual isolation (“single gene speciation”), has been a matter of debate (Hoso et al., 2010; Richards et al., 2017; Ueshima & Asami, 2003; Yamamichi & Sasaki, 2013). Van
Batenburg and Gittenberger (1996) investigated factors influencing the ease of fixation of a change in coiling direction. They found that the population size, number of invaders, and dominance of the mutant chirality gene are of higher importance than maternal effects and mobility. They also emphasize that the shift in coiling direction usually does not prevent mating in high-spired snails, but that it often leads to reproductive isolation in snails with globular shells (Batenburg & Gittenberger, 1996). While dextral species in temperate regions are by far more common (often exceeding 99%), in Turkey, sinistral land snail taxa reach 5.5% (excluding the Clausiliidae which are mostly sinistral; Gittenberger, Hamann, & Asami, 2012). Five out of the 45 orculid taxa are sinistral: Orculella heterostropha commagensis (Neubert, 1988), Orculella heterostropha heterostropha (O. Boettger, 1905) Orculella menkhorsti sinistrorsa Hausdorf, 1996, S. inversa, and S. t. contraria. So far, phylogenetic relationships of these taxa have not been investigated with molecular genetic methods. Schileykula t. contraria has been reported only from three close sites around which the dextral S. t. trapezensis occurs, raising the question of whether they are actually separate species.

In the present study, we tested the morphology-based systematics of Schileykula by molecular phylogenetic analyses using DNA sequences of three mitochondrial (mt) genes and one nuclear (nc) sequence region. We included almost all extant species (except for S. robusta) and most subspecies of Schileykula. We also included several specimens of Sphyridium doliolum (Bruguère, 1792) from the monotypic genus Sphyridium Charpenter, 1837, the sister group of Schileykula (Harl et al., 2017). Sphyridium doliolum has a wide distribution from the Pyrenees in the west to northern Iran in the east, partially overlapping with that of Schileykula (Hausdorf, 1996).

Besides the establishment of a DNA-based phylogeography of Schileykula, we addressed the following specific questions: (a) Is the polytypic S. trapezensis, with four morphologically distinct subspecies, monophyletic? (b) Is S. nordisheki a part of the S. scyphus group or is it a distinct species as the anatomy suggests? (b) Did hybridization occur between nearby occurring Schileykula taxa in north-eastern Turkey, the most speciose region within the range of the genus? (d) Do the two sinistral taxa represent independent lineages? That is, did a single reversal occur in the common ancestor of the two species or were there two independent reversals?

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

We performed DNA analyses on 56 specimens of 14 Schileykula taxa. Only S. robusta and three subspecies of S. scyphus, S. s. ciliacea Hausdorf, 1996, S. s. crassa (Pilsbry, 1922), and S. s. erecta, were not included. For out-group comparison, we included 31 individuals of Sp. doliolum from a variety of places in its wide distribution including Austria, Hungary, Greece, Croatia, Romania, Slovenia, and Turkey. Before they were broken for tissue preparation, shells of all specimens were pictured with a WILD MAKROSKOP M420 and a NIKON DS Camera Control Unit DS-L2 in frontal, lateral, apical, and umbilical view. Remaining parts of specimens are stored in the tissue collection of the Central Research Laboratories of the Natural History Museum Vienna (NHMW). Taxon names, individual identification IDs (IndIDs), and localities with GPS coordinates are listed in Table 1.

2.2 | Distribution maps

Distribution maps of all Schileykula taxa were prepared using ArcMap Desktop 10.0 and manually edited in Adobe Photoshop CC v.2015.01 (Adobe Systems). Distribution data originate from Hausdorf (1996), Neubert (1993), and the present authors. Figure 1 shows a rough overview on the known localities of Schileykula taxa as well as the sampling sites of specimens investigated in the present study. A detailed view of distribution ranges in north-eastern Turkey is provided in Figure 2.

2.3 | DNA extraction and markers

Molecular phylogenetic analyses were performed with two to five individual specimens per species and locality (87 individuals in total). DNA was extracted from small pieces of foot tissue (ca. 1–2 mm in diameter) using the DNeasy Blood and Tissue Kit (QIAGEN) following the standard protocol for DNA extraction from tissue. DNA is stored at ~80°C in the DNA collection of the Central Research Laboratories of the NHMW. A section of the mt cytochrome c oxidase subunit 1 (COI) was amplified from 86 specimens. For a selection of 35 Schileykula and nine Sphyridium specimens, we additionally obtained partial sequences of the mt genes for the 12S ribosomal RNA (12S) and the 16S ribosomal RNA (16S), and a fragment of the nc H4/H3 gene cluster including major parts of the histone H4 (H4) and histone H3 (H3) as well as the complete non-coding intergenic spacer region (IGS), which is positioned between H4 and H3. Information on the primers (names, sequences, references, amplicon sizes) and annealing temperatures is listed in Table 2.

2.4 | PCR and cloning

PCRs for the mt markers (COI, 12S, 16S) were performed with the Roche Taq Polymerase (Roche) in 25 µl volumes with 0.2 mM of each dNTP, 1 mM of each primer, 3 mM MgCl₂, 5 µl 10× PCR buffer, and Taq DNA Polymerase (1 U). PCRs started with 3 min at 94°C, followed by 35 cycles with 30 s at 94°C, 30 s at the respective annealing temperatures (Table 2), 1 min at 72°C, and a final extension for 7 min at 72°C. PCRs for the nc H4/H3 were performed with the proof-reading Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific) in 25 µl volumes with 0.2 mM of each dNTP, 1 mM of each primer, 5 μl 5x Phusion HF Buffer, and Phusion DNA Polymerase (0.4 U). Phusion PCRs started with 30 s at 98°C, followed by 35 cycles with 10 s at 98°C, 10 s at 71°C, 30 s at 72°C, and a final extension for 7 min at 72°C. All PCRs were run on a Master gradient thermocycler (Eppendorf). Purification and direct sequencing (in both directions, using the PCR primers) were performed at
| Species                      | Identification IDs | Locality [site no.] | WGS84 (N)  | WGS84 (E)  | m. asl |
|-----------------------------|--------------------|---------------------|------------|------------|--------|
| Schileykula aculeata        | 6256, 6257         | TR, Karadeniz, Bölgesi Artvin, Ardanuç, Ardanuç castle [21] | 41°08.4’   | 42°00.5’   | 530    |
| Schileykula attilae         | 6254               | TR, Karadeniz, Bölgesi Artvin, Şavşat, Şavşat, Kalesi [23] | 41°15.6’   | 42°19.6’   | 950    |
| Schileykula batumensis      | 6619, 6620         | TR, Artvin, Yusufeli, Boarder to Erzurum, 1km S of Kınalıçam [20] | 40°42.5’   | 41°40.7’   | 740    |
| Schileykula maculata        | 7116, 7117         | TR, Erzurum, Uzundere, Uzundere, 1km S of Uzundere [19] | 40°31.0’   | 41°31.7’   | 1,120  |
| Schileykula scyphus enteroplax | 6630, 6631     | TR, Gümüşhane, Gümüşhane, Gümsçe, Gümsçe, 1km NW of Mescitli [6] | 40°31.1’   | 39°24.9’   | 1,080  |
| Schileykula scyphus inversa | 6635, 6636, 6637  | TR, Amasya, Amasya, Amasya, Amasya [4] | 40°39.6’   | 35°50.9’   | 430    |
| Schileykula scyphus lycaonica | 6599, 6600, 6601  | TR, Kastamonu, Bozkır, Bozkır, 2,5km NE of Kızılık [3] | 37°12.9’   | 32°35.5’   | 1,360  |
| Schileykula scyphus scyphus | 5490, 5491         | TR, Bursa, Bursa, Iznik, Besevler [1] | 40°26.8’   | 30°02.8’   | 210    |
| Schileykula sigma           | 7108, 7109, 7110  | TR, Bayburt, Bayburt, Aşkale, 16km SW of Aşkale [16] | 39°50.3’   | 40°34.1’   | 1,836  |
| Schileykula sigma           | 6258, 6259         | TR, Erzinan, Tercan, Tercan, Tercan tunnel [15] | 39°50.4’   | 40°34.0’   | 1,800  |
| Schileykula trapezensis acampsis | 6616, 6617, 6618 | TR, Erzurum, Ispır, İspir, 3km SE of Çamlıkaya [17] | 40°37.5’   | 41°11.6’   | 930    |
| Schileykula trapezensis acampsis | 7114, 7115         | TR, Erzurum, Uzundere, Engüzekapı kalesi, 2km S of Uzundere [18] | 40°30.6’   | 41°31.6’   | 1,200  |
| Schileykula trapezensis contraria | 6608, 6609, 6610 | TR, Bayburt, Bayburt, Boarder to Erzurum, 12km S of Maden [11] | 40°05.3’   | 40°25.4’   | 1,800  |
| Schileykula trapezensis contraria | 7096, 7097         | TR, Bayburt, Bayburt, Bayburt, NW of Çalıdere [12] | 40°06.7’   | 40°25.4’   | 1,760  |
| Schileykula trapezensis contraria | 7098, 7099         | TR, Bayburt, Bayburt, Bayburt, 3km NW of Çalıdere [13] | 40°07.2’   | 40°25.5’   | 1,750  |
| Schileykula trapezensis neuberti | 6626, 6627, 6628 | TR, Artvin, Ardanuç, Ardanuç, 1km S of Ardanuç [22] | 41°07.7’   | 42°03.2’   | 530    |
| Schileykula trapezensis trapezensis | 6613, 6614, 6615 | TR, Bayburt, Bayburt, Boarder to Erzurum, 6km S of Maden [10] | 40°07.8’   | 40°25.1’   | 1,780  |
| Schileykula trapezensis trapezensis | 7091, 7092, 7093 | TR, Bayburt, Bayburt, Balkaynak, 2 km W of Balkaynak [8] | 40°21.7’   | 39°53.1’   | 1,750  |
| Schileykula trapezensis trapezensis | 7094, 7095         | TR, Bayburt, Bayburt, Balkaynak, 5 km N of Bayburt [9] | 40°18.1’   | 40°13.8’   | 1,525  |
| Schileykula trapezensis trapezensis | 7102, 7103, 7104 | TR, Bayburt, Bayburt, Maden, Kopca centre junction [14] | 40°03.2’   | 40°27.0’   | 2,000  |
| Sphyradium doliolum         | 833, 2803, 2804    | AT, Niederösterreich, Baden, Breitenstein, Adlitzgraben | 47°39.4’   | 15°50.2’   | 650    |
| Sphyradium doliolum         | 5443               | HR, Primorsko, goranska županja, Cres, Cres, Lubenice | 44°53.3’   | 14°20.6’   | 360    |
| Sphyradium doliolum         | 5451               | SI, Bovec, Bovec, Koritnica valley, Trdnjava Kluče | 46°21.6’   | 13°35.6’   | 534    |
| Sphyradium doliolum         | 2835, 2836, 2837   | HU, Baranya, Komlo, Manfa, Mecseki, erdő | 46°08.8’   | 18°14.7’   | 322    |
| Sphyradium doliolum         | 2839, 2840         | HU, Baranya, Komlo, Komlo city, Sikonda reservoir | 46°10.8’   | 18°13.1’   | 182    |
Nucleotide sequence analyses

2.5 | Nucleotide sequence analyses

The raw forward and reverse sequences were manually aligned in BioEdit v.7.1.3 (Hall, 1999) and checked for errors. Prior to the phylogenetic analyses, sequences of the separate data sets were aligned, which was straightforward for the COI because there were no indels. The other sequences of Schileykula and Sphyridium were aligned with MAFFT v.7 (Katoh & Standley, 2013) using the option “E-INS-i” for alignments with multiple conserved domains and long gaps (Katoh, Kuma, Toh, & Miyata, 2005) and refined in BioEdit v.7.1.3 (Hall, 1999). Subsequently, all positions in the 12S, 16S, and H4/H3 alignments containing gaps were removed with trim-al v1.2 (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009) using the “nogaps” option, followed by a second trimming step applying the “strictplus” algorithm (removal of highly saturated alignment regions). A second alignment including the H4/H3 sequences of Schileykula only was created for the calculation of median-joining networks, applying the same trimming options for the IGS region as for the latter data sets.

Phylogenetic trees were reconstructed with three different sequence data sets containing (a) all 86 COI sequences of Schileykula and Sphyridium; (b) the concatenated COI, 12S, and 16S alignment of 41 individuals (Schileykula: 33, Sphyridium: 8); and (c) the H4/H3 alignment with 53 sequences (including additional clones of some specimens) of 43 individuals (Schileykula: 35, Sphyridium: 8). The latter two data sets differ in individual numbers, because in two specimens either the mt COI (IndID: 6256) or 12S (7114) could not be amplified. Prior to the phylogenetic analyses, identical sequences in each of the three data sets were collapsed, resulting in 57 (a), 35 (b) and 42 (c) haplotypes. We did not calculate trees based on a concatenated data set combining mt and nc markers because the data sets partially give conflicting signals. We think this would only be justified if gene flow between lineages can be excluded.

Substitution models were selected separately for the alignments/partitions with JModelTest v.2.1.5 (Darriba, Taboada, Doallo, & Posada, 2012), based on the corrected Akaike information criterion (AICc). Information on sequence lengths, alignments, and the particular substitution models is provided in Table 3. Bayesian inference (BI) analyses were calculated with MrBayes v.3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), applying the substitution models best fit according to the AICc. Analyses for the three data sets were run for 107 generations each (two runs each with four chains, one of which was heated), sampling every hundredth tree. The first 25% of trees were discarded as burn-in and 50% majority rule consensus trees were calculated from the remaining 75,000 trees. Maximum likelihood (ML) analyses were performed with IQtree v.1.4.4 (Nguyen, Schmidt, Haeseler, & Minh, 2015) using the same substitution models and 1,000 bootstrap replicates each. In the BI and ML analyses, only the three sections of the combined mt data set were treated as separate partitions, whereas the H4/H3
alignment was not partitioned because of the low number of informative sites in the coding H4 and H3 regions. Mean Kimura 2-parameter (K2P) distances between and maximum K2P distances within species/subspecies clades were calculated for the 86 sequence COI data set using Mega v.6.6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

In order to visualize potential recombination between nc H4/H3 variants, median-joining networks were constructed for the three partitions (H4, IGS, and H3) with Network 5.0.0.1 (Fluxus Technology Ltd.) and post-processed using the Steiner (MP) algorithm (Polzin & Daneshmand, 2003). Haplotype networks were graphically arranged with Network Publisher (Fluxus Technology Ltd.) and post-edited in Adobe Photoshop CC v.2015.01 (Adobe Systems).

All sequences were uploaded to NCBI GenBank under the accession numbers MK332711–MK332746 (125), MK332747–MK332782.

**FIGURE 1** Distribution of *Schileykula* in Turkey and Iran. Numbers indicate sampling sites of the present study (see also Table 1).

**FIGURE 2** Distribution of *Schileykula* in eastern Turkey. Numbers indicate sampling sites of the present study (see also Table 1).
Table 2 Primers and annealing temperatures used in the present study

| Region   | Primer (5'–3')                      | Origin                   | PCR product length (sites) | Annealing T (°C) |
|----------|-------------------------------------|--------------------------|---------------------------|-----------------|
| COI      | LCO1490: GGTCAACAAATCATAAAGGATATTGG | Folmer, Black, Heah, Lutz, and Vrijenhoek (1994) | 706 | 50 (Taq) |
|          | HCO2198: TAAACTTCCAGGGTGACAAAAATCA  | Folmer et al. (1994)     |                           |                 |
| 12S      | 12SGastFw1: AGTACGGGCGATTGT         | Cadahia et al. (2014)    | 706–746                   | 50–54 (Taq)     |
|          | 12SGastRv4: TAAGCTTGGGGCTATAAC      | Cadahia et al. (2014)    |                           |                 |
| 16S      | 16SLorc1_fwd: TTACCTTCTGATATAAGGTAAACTA | Harl, Duda, et al. (2014) | 882–912                   | 50–54 (Taq)     |
|          | 16SLorc_rev: CGGTCTGAACCTAGATCATG    | Harl, Duda, et al. (2014) |                           |                 |
| H4/IGS/H3| Orch4_left1: GTTGGTCTTCTGCGCTTCTA    | Harl, Duda, et al. (2014) | 1.125–1,186               | 55–57 (Taq)     |
|          | Orch3_right1: TGGGCGATGATGTCACAGCCT  | Harl, Duda, et al. (2014) |                           | 71 (Phusion)    |

IGS, intergeneric spacer.

Table 4. The mean K2P distance between the *Schileykula* taxa and *Sp. doliolum* was 22.5%. The maximum intraspecific K2P distances were highest at 12.9% in *S. t. acampsis*, 7.1% in *S. t. trapezensis*, 6.5% in *S. batimensis*, and 2.5% in *S. s. enteroplax*. No genetic variability in the COI was found in *S. s. lycaonica*, *S. nordsiecki*, *S. inversa*, *S. attilae*, and *S. aculeata*. However, there is a sampling bias because of the latter taxa only a few specimens from single localities were investigated. In addition, we also provide the uncorrected p-distances in Table S1.

3 | RESULTS

3.1 | Mitochondrial gene trees

In the BI and ML trees based on the concatenated mt nucleotide sequences (COI, 12S, 16S), most relationships between the clades were well supported, although deeper nodes of the trees did not obtain maximum support (Figure 3a). In the following, we indicate BI posterior probability values (in decimals) and ML bootstrap support values (in %) in brackets when referring to the clades. The combined mt tree shows two main clades, one of which (1.0/93) bifurcates into the *S. nordsiecki* lineage and a subclade comprising *S. scyphus* and *S. sigma*. The second main clade (1.0/100) contains all three subspecies of *S. scyphus*, namely *S. s. scyphus*, *S. s. enteroplax*, and *S. s. lycaonica* as well as *S. sigma*. The second main clade (1.0/96) comprises the remaining *Schileykula* taxa included in the study. *Schileykula aculeata* is the sister lineage of *S. t. neuberti* (1.0/100) and their sister clade contains *S. attilae* and *S. batimensis* (1.0/87). The sister group relationship between *S. maculata* and *S. inversa* is highly supported (1.0/100). Together with *S. t. acampsis*, they form a clade with high support (1.0/93). *Schileykula t. trapezensis* forms the sister lineage of the latter clade with moderate support (0.99/88) and *S. t. contraria* branches off from a more basal node with low support (0.89/82). Thus, in the mt gene trees, the four subspecies of *S. trapezensis* represent distinct, polyphyletic lineages. Moreover, the two sinistral taxa *S. inversa* and *S. t. contraria* are located at distinct positions in the tree, strongly supporting independent evolution of right-left reversal.

In the BI and ML phylograms based on the COI nucleotide sequences only (including all samples of *Schileykula* and *Sphyradium*), deeper splits within *Schileykula* are not resolved (Figure S1). Similarly, as in the combined mt tree, the position of *S. sigma* within the clade containing the three subspecies of *S. scyphus* is not essentially supported (0.76/74). The K2P distances in the COI sequences between *Schileykula* taxa and the out-group *Sp. doliolum* are provided in (16S), MK332783–MK332862 (COI), and MK332863–MK332910 (H4/H3). Alignments are available as Alignments S1–S4.

3.2 | Nuclear gene trees

Concerning the H3/H4 region, several individuals provided more than a single variant of the H4/H3 sequence section, which can be explained by the presence of distinct alleles at the two homologous chromosomes or the presence of distinct variants in the multi-copy histone cluster. Of the 35 *Schileykula* individuals analyzed, 16 were homozygous in the H4/H3 region, ten each had two variants differing only in a single nucleotide position in the IGS, six showed two variants differing at two to three sites, and three had two variants differing at multiple sites.

The BI and ML trees of the H4/IGS/H3 data set (Figure 3b) exhibit complex patterns, which substantially differ in topology from the combined mt gene tree (Figure 3a). Similarly, as in the mt tree, there are two main clades, however, with different taxon compositions. One main clade (1.0/100) contains sequences of all *S. scyphus* and *S. sigma* specimens analyzed and a subclade with sequences of most *S. t. acampsis* (6613, 6614, 7102) and *S. t. contraria* (6608, 6610, 7096, 7099) specimens (1.0/100). Besides that, one specimen of *S. s. scyphus* (5491) and two specimens of *S. s. lycaonica* (6599, 6601) yielded in addition very different H4/H3 variants forming a short-branched clade (S4/−) at the base of the in-group clade. All other sequences cluster in one weakly supported (0.84/76) clade. Within the latter, a well-supported subclade (1.0/100) contains all sequences of *S. t. acampsis*, *S. inversa*, and *S. maculata* as well as the sequences of two specimens of *S. t. trapezensis* (7092, 7094). This subclade corresponds in taxon composition with one of the clades in the combined mt tree. *Schileykula t. neuberti*, *S. aculeata*, *S. attilae*, and *S. batimensis*
TABLE 3 Sequence data sets and alignments

| Partition | Sequence length (bp) | Alignm. method (MAFFT) | Alignment length (bp) | Nogaps (trim-al) (bp) | Strictplus (trim-al) (bp) | Align. final (bp) | Subst. model (Akaike information criterion) |
|-----------|----------------------|-------------------------|-----------------------|-----------------------|--------------------------|------------------|---------------------------------|
| H4/IGS/H3 | 271/435–537/346 | E-INS-i | 271/672/346 | 271/379/346 | 271/353/346 | 970 | SYM+G |

Alignments for phylogenetic tree inference

| COI | 655 | manual | 655 | — | — | 655 | HKY+H+G |
| 12S | 669–709 | E-INS-i | 763 | 563 | 500 | 500 | GTR+H+G |
| 16S | 836–866 | E-INS-i | 915 | 747 | 628 | 628 | GTR+H+G |

Alignments for H4/IGS/H3 network calculations

| H4/IGS/H3 | 271/435–537/346 | E-INS-i | 271/647/346 | 271/389/346 | 271/364/346 | — | — |

are grouped in a subclade (1.0/88) together with one specimen of S. t. contraria (7097). Schileykula nordsiecki forms the sister lineage to the latter clade (0.84/88).

3.3 Median-joining networks of H4, IGS, and H3

While the BI and ML trees were generated with the complete H4/H3 region, median-joining haplotype networks were calculated separately for the three sections (H4, IGS, H3) in order to visualize potential recombination products between different variants (Figure 4). In particular, the placement of three clones of S. s. scyphus (5491 c1) and S. s. lycaonica (6599 c2, 6600 c1) in the short-branched clade at the base of the H4/H3 tree (Figure 3b) suggested that recombination might have happened. In the network, the H3 sections of these cloned sequences cluster with the sequences from the other S. scyphus individuals. However, their IGS regions had similar sequences to those of S. batumensis, S. attilae, and S. t. neuberti, whereas the H4 sections were similar to those of S. t. acampsis, S. maculata, S. inversa, and two S. t. trapezensis specimens (7092, 7094). The three haplotypes from S. s. scyphus (5491 c1) and S. s. lycaonica (6599 c2, 6600 c1) seem to be recombination products between the alleles in the two main clades. These three cloned sequences also differ in a unique 30 bp insertion at the 5′-end of the IGS region (not visible in the networks). Moreover, in S. nordsiecki (6594, 6595), the placements of the three sections are not concordant. The H3 sections are identical to the haplotypes from S. maculata (7089, 7090) and two S. t. trapezensis specimens (7090, 7092), whereas the H4 sections are similar to those from S. batumensis (7118) and related species, and the IGS region is positioned between the two main haplotype clades.

4 DISCUSSION

We performed phylogenetic analysis on 18 species and subspecies of the land snail genus Schileykula using nc (H4/H3) and mt (COI, 12S, 16S) data sets, with several samples of the monotypic sister genus Sphyridium as out-group. It is worth mentioning that the mt phylogeny roughly reflects anatomical characters: The taxa grouped in the two main clades in the mt tree each share similar anatomical traits with respect to the size and shape of the penial cecum (Figure S2). Schileykula scyphus and S. nordsiecki are characterized by a rather vestigial, slender penial cecum, whereas all other species possess a large cecum with widened basis (Hausdorf, 1996; Páll-Gergely, 2011; Páll-Gergely & Asami, 2013; Páll-Gergely & DeMattia, 2016). On the other hand, the trees partly disagree with the morphology-based classification asking for some taxonomic changes. Furthermore, the mt and nc trees show different patterns implying several hybridization events.

4.1 Hybridization

The nc phylogeny of Schileykula reveals complex patterns, which indicate at least two hybridization events between distinct taxa. The dextral S. t. trapezensis and the sinistral S. t. contraria are separated by a genetic K2P distance of 13.6% in the COI and do not form sister lineages in the combined mt tree (Figure 3a). In the nc H4/H3 tree (Figure 3b) most, but not all specimens of S. t. contraria and S. t. trapezensis form a highly supported subclade within the S. scyphus clade. The most parsimonious explanation for this pattern is that two specimens of S. t. trapezensis (7092, 7094) and one specimen of S. t. contraria (7097) carry original variants, whereas the H4/H3 variants in the other specimens originate from two hybridization events. In a first event, H4/H3 variants may have introgressed from S. scyphus into S. t. trapezensis. Their distribution ranges are adjacent in three regions (Figure 2). In a second hybridization event, the newly acquired H4/H3 variants were probably passed from S. t. trapezensis to the narrow ranged S. t. contraria. The mixed sequence pattern of the recombinant sequences as visible in the nc H4/H3 data set implies that hybridization and recombination might have occurred several times. The genuine and the recombinant H4/H3 alleles of three specimens (IndID 5491, 6599, 6600) were probably maintained in populations of S. scyphus over a long time because they are present in two geographically distinct subspecies (>400 km geographic distance) and differ in several nucleotide positions. The aberrant H4/H3 variants theoretically might represent a paralogous histone cluster, but this would be in conflict with its absence in other Schileykula taxa (if it was present, we would have expected to co-amplify it, as it was the case with the three specimens). Moreover, the separate analyses of the three sections clearly indicate that these sequences
are recombinants. Generally, multigene families such as those encoding histones and rRNA are subject to so-called concerted evolution (Liao, 1999). The primary driving force for concerted evolution in tandemly repeated multigene families is probably intra-chromosomal homogenization, whereas inter-chromosomal genetic exchange is much rarer (Liao, 1999; Liao, Pavelitz, Kidd, Kidd, & Weiner, 1997; Schlötterer & Tautz, 1994). In two studies on the genus Orcula Held, 1837, the H4/H3 sequence section was analyzed in over a hundred specimens. Approximately 90% of the Orcula specimens were homozygous regarding the H4/H3 loci and only three provided more than two distinct variants (Harl, Duda, et al., 2014; Harl, Páll-Gergely, et al., 2014), which were consequently interpreted as alleles of one histone cluster and not as paralogues.

Another possible explanation for some sequence patterns would be ancestral polymorphism in the H4/H3 genes. However, the nc haplotypes of S. t. contraria and S. t. trapezensis, which cluster with S. scyphus, are very similar to each other and also to S. scyphus (Figure 3b).

Considering that these haplotypes were affected throughout time in the same way by mutations as those found in the other specimens, they should also be more distinct (have longer branches in the tree), which is not the case. The morphological similarity of S. t. trapezensis and S. t. contraria and the close vicinity of populations further support the assumption of introgression after secondary contact. However, genome-wide nuclear sequence data would be required to confirm our assumption and to clarify the true extent of admixture between populations.

Geographically, hybridization between S. trapezensis and S. scyphus might have occurred particularly in the eastern Black Sea Region (Gümüşhane, Bayburt, Trabzon, and parts of Erzurum province), where their distribution ranges partly overlap. Hybridization might have occurred also in the north-easternmost part of Turkey (Erzurum and Artvin provinces), where five Schileykula taxa are distributed in a relatively narrow range (see Figure 2). Neubert (1993) reported hybrid specimens in populations of S. batumensis and...
S. t. acampsis, which are the only Schileykula taxa known to occur in sympatry over a broad range. We only sampled a few specimens and localities each. Therefore, although the sequence data did not indicate mixing between these two taxa, analyzing a larger sample covering a wider area could reveal hybridization between them and/or other taxa.

### 4.1.1 Schileykula trapezensis is polyphyletic

Following the current classification, S. trapezensis comprises four subspecies, all of which were included in the present study. They all inhabit north-eastern Turkey, whereby S. t. trapezensis and S. t. acampsis each occupy relatively large areas spanning a distance of about 100 km. The other two subspecies have extremely narrow ranges and occur in parapatry with the latter two. The sinistral S. t. contraria was so far found only at three sites between populations of the dextral S. t. trapezensis, separated by a distance of 1 km only. Schileykula t. neuberti is known from a valley about 2 km east of the north-eastern margin of the distribution area of S. t. acampsis. The mt and nc trees show that S. trapezensis is actually polyphyletic and the subspecies rather should be considered as distinct species. Schileykula t. neuberti is closest related to S. aculeata in both the mt and nc phylogenies (Figure 3), separated by a genetic K2P distance at 6.5% in the COI only; their sister group relationship is highly supported. Schileykula t. acampsis represents the sister group of S. maculata + S. inversa in the combined mt tree. Therefore, S. t. acampsis should be considered as a separate species independent from S. t. trapezensis. Schileykula t. contraria forms a quite distinct lineage in the combined mt tree. Despite some indications of past hybridization between S. t. contraria and S. t. trapezensis (the latter issue is discussed below), we suggest treating S. t. contraria as independent species.
4.1.2 | Schileykula scyphus, S. nordsiecki, and S. sigma

Schileykula scyphus shows the widest distribution of all Schileykula species. Only S. nordsiecki occurs within the western range of S. scyphus. It is only known from one single locality in south-east Turkey, geographically located between populations of S. s. lycaonica. The shells of S. nordsiecki are indistinguishable from those of S. s. lycaonica, but the morphology of the genital anatomy is strikingly different, which resulted in its differentiation on species level (Hausdorf, 1996). Both the mt and nc phylogenies (Figure 3) provide clear evidence that S. nordsiecki is genetically distinct from S. scyphus. Although S. scyphus and S. nordsiecki are closest related to each other in the combined mt tree, they are separated by relatively high genetic K2P distances at 16.5%–19.0% in the COI, which is in accord with their proposed species status.

Schileykula sigma has been considered as an independent species because of its peculiar shell characters, in particular, the sigmoid formation of the columellar lamella (Hausdorf, 1996), the genital anatomy, however, is similar to that of S. scyphus (Pál-Gergely, 2011). In the mt and nc phylogenies, S. sigma is nested within S. scyphus. Genetic K2P distances between the three subspecies of S. scyphus (5.1%–5.5%) are in a similar range as that between the latter and S. sigma (6.4%–7.5%). In order to clarify the status of S. sigma, that is, to test whether it is reproductively isolated from S. scyphus or not, hybridization experiments would be necessary. However, controlled laboratory experiments have not been performed with any Schileykula species so far. Given the similarities in the mt and nc genes analyzed as well as in the genital anatomy, we consider S. scyphus and S. sigma as conspecific.
4.2 | Origin of sinistral taxa

The two sinistral species do not form sister groups in the nc and mt trees, suggesting that the change of coiling direction from dextral to sinistral happened twice independently within the genus. However, since our data indicate that hybridization affected several taxa (including S. t. contraria), it is possible that there was a single origin of sinistrality in this group, which has subsequently been obscured by hybridization events. Generally, Turkish Orculidae exhibit a comparably high proportion of sinistral taxa (Gittenberger et al., 2012). Sinistral snails are more frequent in high-spired taxa, and opposite-coiled specimens have less difficulties in mating than enantiomorphic pairs possessing flat or globular shells (Asami et al., 1998). Whether the changes in chirality might have contributed to the speciation of both S. inversa and S. contraria remains speculative.

4.3 | Classical taxonomy versus molecular phylogeny

The present study provides a phylogenetic basis for the systematics of Schileykula for the first time. Our DNA-based analysis allowed us to re-evaluate some pre-existing hypotheses on the systematics within Schileykula. However, this revised systematics should be further tested using a higher sample size and optimally analyzing genome-wide nuclear sequence data. Figure 5 shows selected specimens of the taxa studied.

Schileykula aculeata Gittenberger & Menkhorst, 1993
Schileykula acampsis Hausdorf, 1996
Schileykula attilae Päll-Gergely, 2010
Schileykula batumensis (Retowski, 1889)
Schileykula contraria Neubert, 1993
Schileykula inversa Śchütz, 1993
Schileykula maculata Päll-Gergely & Asami, 2013
Schileykula neuberti Hausdorf, 1996
Schileykula nordsiecki Hausdorf, 1996
Schileykula (?) robusta (Nägele, 1906) (not included in study)
Schileykula scyphus ciliica Hausdorf, 1996 (not included in study)
Schileykula scyphus crassa (Pilsbry, 1922) (not included in study)
Schileykula scyphus enteropla (Pilsbry, 1922)
Schileykula scyphus erecta Hausdorf, 1996 (not included in study)
Schileykula scyphus lycaonica Hausdorf, 1996
Schileykula scyphus scyphus (L. Pfeiffer, 1848)
Schileykula scyphus sigma Hausdorf, 1996
Schileykula trapezensis (Stojsapal, 1981).

5 | CONCLUSION

The molecular genetic data presented here provide a first phylogenetic hypothesis based on a combination on mt and nc DNA sequences. Our results suggest some taxonomic revisions. Since S. trapezensis is polyphyletic, we propose treating the four subspecies as independent species. Schileykula nordsiecki was confirmed to represent an independent lineage distinct from S. scyphus. Schileykula sigma was shown to be closely related to S. scyphus and is therefore treated as a subspecies of the latter. The sinistral taxa S. contraria and S. inversa did not emerge as sister lineages in the mt and nc trees, implying that the change of the coiling direction might have happened two times independently. The incongruences between mt and nc trees suggest at least two independent hybridization events involving S. contraria, S. scyphus, and S. trapezensis. However, in order to shed more light on the complex patterns, future studies should include a larger number of samples from more localities as well as they would benefit from the analysis of additional nc markers.

ACKNOWLEDGEMENTS

We are very grateful to Zoltán Fehér, Takashi Ishibe, Kenji Ohara, Kanji Okubo, and Jame Uiriamu Otani who provided specimens to the NHMW collection.

ORCID

Josef Harl https://orcid.org/0000-0002-4915-3943
Elisabeth Haring https://orcid.org/0000-0002-5411-1879
Barna Päll-Gergely https://orcid.org/0000-0002-6167-7221

REFERENCES

Asami, T., Cowie, R. H., & Ohbayashi, K. (1998). Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. American Naturalist, 152(2), 225–236. https://doi.org/10.1086/286163
Cadahía, L., Hari, J., Duda, M., Sattmann, H., Kruckenhaus, L., Fehér, Z., ... Haring, E. (2014). New data on the phylogeny of Arioitinae (Pulmonata, Helicidae) and the systematic position of Cylindrus obtusus based on nuclear and mitochondrial DNA marker sequences. Journal of Zoological Systematics and Evolutionary Research, 52(2), 163–169.
Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics, 25(15), 1972-1973. https://doi.org/10.1093/bioinformatics/btp348
Chiba, S. (2005). Appearance of morphological novelty in a hybrid zone between two species of land snail. Evolution (N.Y.), 59(8), 1712–1720.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. Nature Methods, 9(8), 772. https://doi.org/10.1038/nmeth.2109

Folmer, O., Black, M., Heah, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3, 294–299.

Gittenberger, E. (1978). Beiträge zur Kenntnis der Pupillacea. VIII. Einiges Über Orculidae. Zoológische Verhandelingen, 163(19), 1–44.

Gittenberger, E. (1983). Beiträge zur Kenntnis der Pupillacea. IX. Nachbemerkungen über Orculidae. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen C, 86(3), 325–342.

Gittenberger, E. (1988). Sympatric speciation in snails; a largely neglected model. Evolution (N.Y.), 42(4), 826–828.

Gittenberger, E., Hamann, T. D., & Asami, T. (2012). Chiral speciation in terrestrial pulmonate snails. PLoS ONE, 7(4), e34005. https://doi.org/10.1371/journal.pone.0034005

Hall, T. A. (1999). BioEdit: A user-friendly biological sequences alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.

Harl, J., Duda, M., Krucken hauser, L., Sattmann, H., & Haring, E. (2014). In search of glacial refuges of the land snail Or cula dolium (Pulmonata, Orculidae) - an integrative approach using DNA sequence and fossil data. PLoS ONE, 9(5), e96012. https://doi.org/10.1371/journal.pone.0096012

Harl, J., Haring, E., Asami, T., Sittenthaler, M., Sattmann, H., & Päll- Gergely, B. (2017). Molecular systematics of the land snail family Orculidae reveal polyphyly and deep splits within the clade Orthurethra (Gastropoda: Pulmonata). Zoological Journal of the Linnean Society, 181(4), 778–794. https://doi.org/10.1093/zoolinnean/zlx022

Harl, J., Päll-Gergely, B., Kirchner, S., Sattmann, H., Duda, M., Krucken hauser, L., & Haring, E. (2014). Phylogeography of the land snail genus Or cula (Orculidae, Stylommatophora) with emphasis on the Alpine xena: Speciation, hybridization and morpho logical variation. BMC Evolutionary Biology, 14(1), 223. https://doi.org/10.1186/s12862-014-0223-y

Hausdorf, B. (1996). Die Orculidea Asiens (Gastropoda: Stylommatophora). Archiv Für Molluskenkunde, 125, 1–86.

Hoso, M., Kameda, Y., Wu, S.-P., Asami, T., Kato, M., & Hori, M. (2010). A speculation gene for left-right reversal in snails results in anti-predator adaptation. Nature Communications, 1, 133. doi:10.1038/ncomms1133

Huelsbeck, J. P., & Ronquist, F. (2001). MrBAYES: Bayesian inference for phylogeny. Bioinformatics, 17, 754–755.

Johnsson, M. (1982). Polymorphism for direction of coil in Partula su ‐ turalis: Behavioural isolation and positive frequency dependent selection. Heredity (Edinb), 49(2), 145. doi:10.1038/hdy.1982.80

Katoh, K., Kuma, K., Toh, H., & Miyata, T. (2005). MAFFT version 5: Improvement in accuracy of multiple sequence alignment. Nucleic Acids Research, 33(2), 511–518. doi:10.1093/nar/gki198

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30(4), 772–780. doi:10.1093/molbev/msu010

Koch, E. L., Neiber, M. T., Walther, F., & Hausdorf, B. (2016). Presumable incipient hybrid speciation of door snails in previously glaciated areas in the Caucusus. Molecular Phylogenetics and Evolution, 97, 120–128. https://doi.org/10.1016/j.ympev.2015.12.016

Lammers, Y., Kremer, D., Brakefield, P. M., Groenenberg, D. S. J., Pirovano, W., & Schilthuizen, M. (2013). SNP genotyping for detecting the ‘rare allele phenomenon’ in hybrid zones. Molecular Ecology Resources, 13(2), 237–242. https://doi.org/10.1111/1755-0998.12044

Liao, D. (1999). Concerted evolution: Molecular mechanism and biological implications. American Journal of Human Genetics, 64(1), 24–30. https://doi.org/10.1086/302221

Liao, D., Pavelitz, T., Kidd, J. R., Kidd, K. K., & Weiner, A. M. (1997). Concerted evolution of the tandemly repeated genes encoding human U2 snRNA (the RNU2 locus) involves rapid intrachromosomal homogenization and rare interchromosomal gene conversion. EMBO Journal, 16(3), 588–599. https://doi.org/10.1093/embj/16.3.588

Lipton, C. S., & Murray, J. (1979). Courtship of land snails of the genus Partula. Malacologia, 19, 129–146.

Morii, Y., Yokoyama, J., Kawata, M., Davison, A., & Chiba, S. (2015). Evidence of introgressive hybridization between the morphologically divergent land snails Ainohelix and Ezohelix. Biological Journal of the Linnean Society, 115(1), 77–95.

Nakadera, Y., Sutchitarian, C., Ubakata, T., Seki, K., Utsuno, H., Panha, S., & Asami, T. (2010). Enantiomorphs differ in shape in opposite directions between populations. Journal of Evolutionary Biology, 23(11), 2377–2384. https://doi.org/10.1111/j.1420-9101.2010.02099.x

Neubert, E. (1993). Contribution to the knowledge of the Orculinae from Northeastern Turkey (Mollusca: Stylommatophora: Orculidae). Zoology in the Middle East, 8(1), 53–68. https://doi.org/10.1080/09397 140.1993.10637637

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQTREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution, 32(1), 268–274. https://doi.org/10.1093/molbev/msu300

Päll-Gergely, B. (2010). New and little-known land snails from Turkey (Gastropoda: Pulmonata). Zoology in the Middle East, 50, 89–94. https://doi.org/10.1080/09397140.2010.10638416

Päll-Gergely, B. (2011). Description of the genital structure of four Turkish orculids (Gastropoda: Pulmonata: Orculidae). Journal of Conchology, 40(4), 471–476.

Päll-Gergely, B., & Asami, T. (2013). A new, ribbed Schleykula species from north-eastern Turkey (Gastropoda: Pulmonata: Orculidae). North-Western Journal of Zoology, 9(1), 214–216.

Päll-Gergely, B., & DeMattia, W. (2016). Notes on the taxonomy of Turkish Orculidae (Gastropoda: Pulmonata: Orthurethra). Soosiana, 33, 5–10.

Polzin, T., & Daneshmand, S. V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters, 31(1), 12–20. https://doi.org/10.1016/S0167-6377(02)00185-2

Razkin, O., Sonet, G., Breugelmans, M., Madeira, M. J., Gómez-Moliner, I., & Daneshmand, S. V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters, 31(1), 12–20. https://doi.org/10.1016/S0167-6377(02)00185-2

Raykova, M., & Tautz, D. (1994). Chromosomal homogeneity of Drosophila ribosomal DNA arrays suggests intrachromosomal exchanges drive concerted evolution. Current Biology, 4(9), 777–783. https://doi.org/10.1016/S0960-9822(00)00175-5

Sutchitarian, C., Asami, T., & Panha, S. (2007). Evolution of whole body enantiomorph in the tree snail genus Amphilorus.
Sutcharit, C., & Panha, S. (2006). Taxonomic review of the tree snail Amphidromus Albers, 1850 (Pulmonata: Camaenidae) in Thailand and adjacent areas: Subgenus Amphidromus. *Journal of Molluscan Studies*, 72(1), 1-30. https://doi.org/10.1093/moluscy/044

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729. https://doi.org/10.1093/molbev/mst197

Ueshima, R., & Asami, T. (2003). Evolution: Single-gene speciation by left-right reversal. *Nature*, 425(6959), 679. https://doi.org/10.1038/nature02172

Van Batenburg, F. H. D., & Gittenberger, E. (1996). Ease of fixation of a change in coiling: Computer experiments on chirality in snails. *Heredity (Edinb)*, 76(3), 278-286. https://doi.org/10.1038/hdy.1996.41

Yamamichi, M., & Sasaki, A. (2013). Single-gene speciation with pleiotropy: Effects of allele dominance, population size, and delayed inheritance. *Evolution (N. Y)*, 67(7), 2011-2023. https://doi.org/10.1111/evo.12068

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at end of the article.

**Figure S1.** Phylogenetic tree of the mt COI sequences obtained from *Schileykula* and *Sphyradium*. Black and grey dots indicate nodes with high BI posterior probabilities and ML bootstrap values (see figure). The scale bars indicate the expected number of substitutions per site according to the model of sequence evolution applied.

**Figure S2.** Reproductive anatomy of *Schileykula attilae* Páll-Gergely, 2010 (left) and male part of the reproductive anatomy of *S. scyphus* sigma Hausdorf, 1996 (right) to highlight the differences of relative penial caecum sizes. Abbreviations: e: epiphallus, p: penis, pa: penial appendix, pc: penial caecum, rm: retractor muscle. Not to scale.

**Table S1.** Uncorrected p-distances of the COI sequences between taxa and maximum p-distances between taxa.

**Alignment S1.** Alignment of all COI sequences included in the study.

**Alignment S2.** Alignment of 12S rRNA sequences included in the concatenated mt tree.

**Alignment S3.** Alignment of 16S rRNA sequences included in the concatenated mt tree.

**Alignment S4.** Alignment of H4/H3 sequences included in the nc tree.

**How to cite this article:** Harl, J., Haring, E., Páll-Gergely, B. Hybridization and recurrent evolution of left–right reversal in the land snail genus *Schileykula* (Orculidae, Pulmonata). *J Zool Syst Evol Res*. 2019;00:1-15. https://doi.org/10.1111/jzs.12353