INTRODUCTION

Radiations display a wide array of different stages of speciation and offer an opportunity to study factors affecting the differentiation of populations and the segregation of incipient species. The intermediate stages of speciation that help the evolutionary biologist to understand the process are a challenge for the taxonomist. Adaptive radiations, which are the...
result of ecological speciation by divergent selection and a rapid adaptation of incipient species to different niches, have been studied in detail, for example the Darwin's finches in the Galápagos Islands (Grant & Grant, 2008), Anolis lizards of the Caribbean (Losos, 2009) or cichlid fish in Africa (Seeheisen, 2006). In contrast, non-adaptive radiations, in which diversification is not accompanied by adaptation into different niches, but results in a group of usually allopatric species occupying similar niches (Dominey, 1984; Fehér et al., 2018; Gittenberger, 1991; Rundell & Price, 2009) have been neglected for a long time. However, the importance of non-adaptive radiations may be underestimated. It is likely that in some apparently adaptive radiations non-adaptive speciation triggered by geographical isolation preceded the later ecological differentiation of the species (Rundell & Price, 2009). Thus, a better insight into non-adaptive radiations is essential for our understanding of the origin of biodiversity.

A radiation can be defined as the evolution of a relatively large monophyletic group of species (Gittenberger, 1991). Thus, we also have to define what a species is. We do not use the restrictive biological species concept (Mayr, 1942, 1963) that requires reproductive isolation. Many species in recent radiations like the ground finches (Grant & Grant, 2008; Lamichhaney et al., 2015) do not fulfil this criterion and, thus, would not be considered distinct species under this concept. Instead, we use the differential fitness species concept (Hausdorf, 2011) that defines species as groups of individuals that are reciprocally characterized by features that would have negative fitness effects in other groups and that cannot be regularly exchanged between groups upon contact. In an adaptive radiation, niche divergence is connected to speciation. Thus, the features that would have negative fitness effects in other groups may be adaptations to different environments or different functions in an environment. In a non-adaptive radiation, the niches of the emerging species do not differ distinctly. Thus, the speciation process is not driven by niche-related factors and depends only on features affecting reproduction or the intrinsic fitness of the offspring.

Non-adaptive radiations are frequent in groups with limited dispersal capability. The classical example of a non-adaptive radiation is a radiation of the door-snail (Clausiliidae) genus Albinaria inhabiting limestone rocks on Crete (Gittenberger, 1991). Many other snail taxa are restricted to limestone rocks, where they feed mainly on lichens. Limestone rock outcrops are often isolated habitat islands that are separated by areas without rocks. The geographical isolation of limestone outcrops facilitates the differentiation and radiation of rock-dwelling snail species. For example, there are several species-rich radiations of door-snail genera in southern Europe (Nordsieck, 2007).

In this study, we investigate the door-snail genus Alopia in the Bucegi Mountains in the southern Carpathians in Romania. Although this mountain massif covers less than 10 km × 20 km, up to ten endemic Alopia (sub-)species were recorded from that area (Fehér, Németh, Nicoara, & Szekeres, 2013; Grossu, 1981; Nordsieck, 2008). The mitochondrial gene tree of Alopia (Fehér et al., 2013) showed that the taxa inhabiting the Bucegi Mountains belong to three different clades, each of which has further representatives in other parts of the Carpathian Mountains and each of which is more closely related to clades from other parts of the Carpathian Mountains than to the geographically adjacent clades in the Bucegi Mountains. This can be explained by two hypotheses. Either the Bucegi Mountains were colonized three times by different Alopia clades or the Bucegi Mountains were part of the ancestral area of Alopia, where the different clades evolved and from where other regions of the Carpathian Mountains were colonized. The latter hypothesis had already been proposed by Kimakowicz (1894). In any case, the Alopia taxa from the Bucegi Mountains do not represent a separate radiation but are only a part of the Alopia radiation in the Carpathian Mountains. We focus here on the Bucegi Mountains because several taxa occur there in close proximity so that we can study potential gene flow between taxa, whereas in many other areas, different taxa are separated by wide geographical barriers so that migration between ranges of different taxa is very low.

There are taxa with differing coiling direction in Alopia. Most taxa are coiled counterclockwise (sinistral) as it is usual in the family Clausiliidae, but several unrelated taxa are coiled clockwise (dextral) (Fehér et al., 2013). Reversals of the primary asymmetry in spiral snails may affect gene flow because the position of the genital orifice on different body sides in dextral and sinistral snails can impede mating of differently coiled individuals (Asami, Cowie, & Ohbayashi, 1998; Johnson, 1982; Ueshima & Asami, 2003). It has been assumed that mutations of the gene determining the coiling direction might result even in single-gene speciation (Gittenberger, 1988; Gittenberger, Hamann, & Asami, 2012; Orr, 1991; Ueshima & Asami, 2003; but see Johnson, Clarke, & Murray, 1990; Davison, Chiba, Barton, & Clarke, 2005; Koch, Neiber, Walther, & Hausdorf, 2017). Thus, reversals of the coiling direction may have contributed to the Alopia radiation. However, there is morphological (Nordsieck, 2007, 2008, 2016; Szekeres, 1976) as well as genetic (Koch et al., 2017) evidence for hybridization and introgression between differently coiled Alopia taxa in the Bucegi Mountains.

We investigated the stages of the non-adaptive radiation of the Alopia taxa in the Bucegi Mountains and the processes that drive or counteract the differentiation process using mitochondrial DNA sequences as well as nuclear AFLP markers. We also propose a new classification of the Alopia taxa from the Bucegi Mountains considering their genetic differentiation and the admixture between population groups.
2 | MATERIAL AND METHODS

2.1 | Sampling and classification

Alopia populations from 24 locations (Figure S1) were sampled in the Bucegi Mountains in the southern Carpathian Mountains in Romania in June 2013. Herilla ziegleri dacica, and Albinaria p. puella were used as outgroups for the phylogenetic analysis of coxl sequences. The classification, locality data and voucher numbers of the specimens used in this study are compiled in Table S1. The samples were stored in 100% isopropanol at −20°C.

The samples were identified morphologically to the species and subspecies distinguished by Nordsieck (2008) and Fehér et al. (2013) (Table 1). In the Results section, we used the nomenclature proposed by Nordsieck (2008) with exception of the subspecies of A. livida (in the traditional sense). Both, Nordsieck (2008, 2015, 2016) and Fehér et al. (2013) separated the populations of A. livida from higher altitudes as a separate subspecies A. livida bipalatalis (Kimakowicz, 1883). Nordsieck (2008) replaced this name by A. livida sororcula Soós, 1928 because it is preoccupied by Clausilia bipalatalis Martens (in Boettger, 1878), but Nordsieck (2016) argued that A. livida sororcula is a synonym of Alopia livida hypula Soós, 1928 and used A. livida bipalatalis again. Alopia livida bipalatalis is allegedly characterized by the frequent possession of palatal folds. However, the percentage of specimens with palatal folds varies between 55% and 100% in the populations at higher altitudes and in populations at lower altitudes between Ciubotea and Grohotiș up to 35% of the specimens also possess palatal folds (Nordsieck, 2015). A. livida cannot be classified into subspecies based on morphological characters because of the lack of consistent differences between population groups. In the Results section, we preliminarily subdivide A. livida according to the network based on nuclear AFLP marker. We assigned available names to these population clusters based on their proximity to the type localities compiled in Table S2. We provisionally follow Nordsieck (2015, 2016) and Fehér et al. (2013) in using the name A. livida bipalatalis for the populations of A. livida from higher altitudes. Nordsieck (2015) argued that the type of A. livida was from the north-western foothills of the Bucegi Mountains between Ciubotea and Grohotiș, but included also the populations from the southern and south-eastern parts of the Bucegi Mountains in A. l. livida. However, these populations probably do not form a coherent evolutionary unit with those from the north-western foothills, because they are separated by populations at higher altitude along the ridge of the mountains that Nordsieck (2008, 2015) classified as A. livida bipalatalis. Thus, we use the oldest name A. livida nubila (Kimakowicz, 1894) for the populations from the catchment area of the upper Ialomița River. For descriptive purposes, we use the name A. livida kimakowiczi Kimakowicz, 1933 for the populations of A. livida from the south-eastern ridge of the Bucegi Mountains, which differ genetically from those from lower altitudes of the Ialomița catchment area. However, see the discussion for the validity of A. livida kimakowiczi and of A. livida bipalatalis. Finally, we follow Nordsieck (2016) in using the name A. livida hypula Soós, 1928 for populations from the Velican and Țigănești valleys instead of the younger name A. livida velicana Kimakowicz, 1933 used by Nordsieck (2008).

2.2 | DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissue samples of the foot following the protocol proposed by Sokolov (2000) with slight modifications as detailed in Scheel and Hausdorf (2012).

A fragment of the mitochondrial cytochrome c oxidase subunit 1 (coxl) gene was amplified by polymerase chain reaction (PCR), using the primer pair L1490-Alb 5′-ACT CAA GAT GAA GGA TCA AAA AAT CA-3′ (Gittenberger, Piel, & Groenenberg, 2004). Amplifications were performed in 25 μl volumes containing 2 μl 10x amplification buffer B (biolabproducts), 4 μl MgCl2 (25 mM, biolabproducts), 1 μl dNTP mix (5 mM each, biolabproducts), 1 μL of each primer (10 μM), 0.2 μl Crystal Taq DNA polymerase (5 U/µl; biolabproducts), 1 μl 1:30 diluted template DNA and 14.8 μl ddH2O under the following reaction conditions: an initial denaturation step at 94°C for 2 min, 35 PCR cycles (94°C for 30 s, 50°C for 35 s, 72°C for 30 s) and a final extension step at 72°C for 5 min. PCR products were enzymatically cleaned up by adding 0.65 μl FastAP thermosensitive alkaline phosphatase (1 U/μl; Thermo Fisher Scientific) and 0.35 μl exonuclease I (20 U/μl; Thermo Fisher Scientific) to a 5 μl aliquot of the PCR mixture followed by an incubation step at 37°C for 15 min. The enzymes were inactivated by heating the mixture to 85°C for 15 min. Both strands of the amplified products were sequenced at Macrogen Europe Laboratory.

2.3 | Alignment and analyses of DNA sequences

Forward and reverse sequence reads were assembled using CHROMASPRO version 1.7.1 (Technelysium). The sequences were aligned with MUSCLE (Edgar, 2004) as implemented in MEGA version 7 (Kumar, Stecher, & Tamura, 2016) using the default settings. MEGA 7 was also used to calculate p-distances.
The coxI alignment was divided into three partitions corresponding to the three codon positions. The HKY + G model was selected for all three partitions using the Akaike information criterion (AIC) implemented in Treefinder (Jobb, 2011; Jobb, von Haeseler, & Strimmer, 2004). Heuristic maximum-likelihood analyses were performed in Treefinder setting the search depth to 2 and allowing for independent estimation of parameters for individual partitions. Confidence values were computed by bootstrapping (1,000 replications).

Alignment files (including GENBANK (www.ncbi.nlm.nih.gov/genbank) accession numbers) and phylogenetic trees are available from TREEBASE (www.treebase.org; study accession number 25308).

### 2.4 AFLP

AFLP were generated following the protocol proposed by Vos et al. (1995) with modifications as detailed in Scheel and Hausdorf (2012). Five primers with two or three additional bases at the 3’ end (Nägele & Hausdorf, 2015) were used for selective amplifications. Six primer combinations (SMsI/SEcoRI/PYE) were run: AGC/CA**FAM**, AGC/CG**NED**, AGC/ **GH**,**HEX**, TGC/CA**FAM**, TGC/CG**NED** and TGC/GG**HEX**.

Electropherograms were analysed with PEAK SCANNER version 1.0 (Applied Biosystems) to detect AFLP bands and calculate their size using default settings except for a light peak smoothing, sizing quality with a pass range of 0.1–1 and a low-quality range of 0.0. Fluorescent threshold was set to 50 relative fluorescence units. Binning and scoring were performed using RAWGENO version 2.0 (Arrigo, Tuszynski, Ehrich, Gerdes, & Alvarez, 2009), an add-on package for the statistical software suite R (R Core Team, 2012), with the following settings: scoring range = 50–500 bp, minimum intensity = 100 rfu, minimum bin width = 1.5 bp and maximum bin width = 2.0 bp. Bins with reproducibility lower than 80% (the default value of the reproducibility filter) were eliminated. A replicate

### Table 1

Comparison of the classification and nomenclature of the discussed Alopia taxa of the Bucegi Mountains proposed in this study with previous classifications

| Proposed in this study | Nordsieck (2008) | Fehér et al. (2013) |
|------------------------|-----------------|---------------------|
| A. pomatias albicostata (M. Kimakowicz, 1894) | A. pomatias albicostata (M. Kimakowicz, 1894) | A. pomatias (L. Pfeiffer, 1865) part |
| A. livida straminicollis (Charpentier, 1852) | A. straminicollis straminicollis (Charpentier, 1852) | A. straminicollis (Charpentier, 1852) |
| A. livida monacha (M. Kimakowicz, 1894) | A. straminicollis monacha (M. Kimakowicz, 1894) | A. monacha (M. Kimakowicz, 1894) |
| A. livida nixa (M. Kimakowicz, 1894) | A. nixa (M. Kimakowicz, 1894) | A. nixa nixa (M. Kimakowicz, 1894) |
| A. livida fassi (M. Kimakowicz, 1894) | A. fassi (M. Kimakowicz, 1894) | A. nixa fassi (M. Kimakowicz, 1894) |
| (A. livida bipalatalis (M. Kimakowicz, 1883)) | A. livida sororcula Soős, 1928 | A. livida bipalatalis (M. Kimakowicz, 1883) |
| (A. livida kimakowiczi R. Kimakowicz, 1933) | A. livida livida (Menke, 1828) part | A. livida livida (Menke, 1828) part |
| A. livida nubila (M. Kimakowicz, 1894) | A. livida livida (Menke, 1828) part | A. livida livida (Menke, 1828) part |
| A. livida hypula Soős, 1928 | A. livida velicana R. Kimakowicz, 1933c | A. livida livida (Menke, 1828) part |

*aThe names A. livida bipalatalis and A. livida kimakowiczi are used in this study for descriptive purposes, but are not considered valid (see discussion). |

*bNordsieck (2016) regarded A. livida sororcula Soős, 1928 as a synonym of A. livida hypula Soős, 1928 and used the preoccupied name A. livida bipalatalis (M. Kimakowicz, 1883) (non-Martens in Boettger, 1878) again.

*cReplaced by A. livida hypula Soős, 1928 by Nordsieck (2016).*
reproducibility rate of 91.4% \((n = 13)\) was calculated as the mean percentage of matching character states between replicates of the same individual (Pompanon, Bonin, Bellemain, & Taberlet, 2005).

A neighbour-net (Bryant & Moulton, 2004) was constructed based on Jaccard distances calculated with the AFLP data using SPLITSTREE4 version 4.14.3 (Huson & Bryant, 2006).

Individual-based clustering and admixture analyses of the AFLP data were performed with STRUCTURE version 2.3.4 (Falush, Stephens, & Pritchard, 2007; Pritchard, Stephens, & Donnelly, 2000) as well as BAPS 6.0 (Corander & Marttinen, 2006; Corander, Marttinen, Sirén, & Tang, 2008). We carried out ten runs with 800,000 iterations after a burn-in of 200,000 iterations for each cluster number \(K\) from 1 to 12 with STRUCTURE. We used the mean estimates of the posterior probabilities of the data for a given cluster number \(L(K)\) and the ad hoc quantity \(\Delta K\) proposed by Evanno, Regnaut, and Goudet, (2005) computed with STRUCTURE HARVESTER (Earl & vonHoldt, 2012) to estimate the number of clusters. DISTRUCT version 1.1 (Rosenberg, 2004) was used for visualizing the admixture calculated in the STRUCTURE run with the highest posterior probability for a given \(K\). With BAPS, 10 repetitions for each \(K = 10, 20\) and 30 as maximum bounds of the numbers of clusters were carried out. The results of the mixture analysis served as input for BAPS admixture analysis based on 500 simulations from posterior allele frequencies. A network of clusters where gene flow is indicated by weighted arrows, such that the weights equal relative average amounts of ancestry in the source cluster among the individuals assigned to the target cluster was estimated as described by Tang, Hanage, Fraser, and Corander (2009) using the function Plot Gene Flow in BAPS.

We used analysis of molecular variance (Excoffier, Smouse, & Quattro, 1992) as implemented in GenAlEx version 6.501 (Peakall & Smouse, 2006, 2012) to estimate the partitioning of genetic variance among taxa, among populations and within populations. We determined significance with 9,999 permutations.

3 | RESULTS

3.1 | Mitochondrial gene tree and sequence diversity

Maximum-likelihood analyses of \(\text{cox}1\) sequences (655 positions) of the Alopia taxa and one Albinaria puella puella and one Herilla ziegleri dacica as outgroups showed that the Alopia specimens from the Bucegi Mountains form three strongly supported clades, representing \(A.\) pomatias, \(A.\) straminicollis and the \(A.\) livida group including \(A.\) fussi and \(A.\) nixa (Figure 1). Within \(A.\) pomatias \(p\)-distances reached 0.6%, within the \(A.\) straminicollis clade 5.3% and within the \(A.\) livida clade 4.2%; between the three clades \(p\)-distances reached 10.1% (Table S3).

The relationships between the three clades were not robustly resolved. Within the \(A.\) livida group, only the \(A.\) nixa specimens formed a well-supported monophylum, whereas individuals identified as \(A.\) livida nubila, \(A.\) livida bipalatalis, \(A.\) livida hypula and \(A.\) fussi intermingle. In the \(A.\) straminicollis clade, the individuals from the northern slope of the Bucegi Mountains representing \(A.\) s. straminicollis formed the sister group of the individuals from the southern part of the mountains representing \(A.\) straminicollis monacha. Among \(A.\) straminicollis monacha, there was also a dextral specimen from the hybrid zone with \(A.\) livida, identified as \(A.\) livida because of its dextral coiling. Likewise, there was a sinistral specimen from the hybrid zone between \(A.\) s. straminicollis and \(A.\) livida in the \(A.\) livida group that was identified as \(A.\) s. straminicollis because of its sinistral coiling.

3.2 | Network based on AFLP data

Using six primer combinations, we scored 2,215 AFLP fragments of 50–500 bp length in 250 Alopia specimens (Table S4). In contrast to the mitochondrial gene tree, the individuals belonging to one population usually form a cluster in the neighbour-net based on the nuclear AFLP markers (Figure 2). The only species that is separated from the other species by a long branch is \(A.\) pomatias. The other taxa form an almost star-like radiation. The arrangement of the other populations in the network reflects their geographical relationships. At the one side of the network, the array starts with the \(A.\) straminicollis monacha populations from the southern part of the Bucegi Mountains (see Figure S1). Then, the hybrid population between \(A.\) straminicollis monacha and \(A.\) livida nubila (population 2) and the populations of \(A.\) livida nubila follow. The next branches are populations from higher altitudes classified as \(A.\) livida kimakowicz and \(A.\) fussi. Among the \(A.\) fussi populations, the populations of \(A.\) nixa form a distinct cluster. Only one individual of \(A.\) nixa is separated from this cluster. The populations of \(A.\) livida bipalatalis, which are geographically placed between \(A.\) fussi and \(A.\) livida hypula, are also placed between these taxa in the network. Finally, the hybrid population between \(A.\) livida hypula and \(A.\) s. straminicollis (population 20) forms the transition to \(A.\) s. straminicollis.

3.3 | Population genetic structure

The ad hoc quantity \(\Delta K\), proposed by Evanno et al. (2005) to estimate the number of clusters, shows a maximum for
$K = 2$ (Figure S2B). However, a plot of the likelihood of $K$ for $K = 1–12$ showed that $L(K)$ does not reach a plateau in this range, but that it further increases with higher values (Figure S2A). Thus, we show the STRUCTURE results for $K = 2–8$ that give additional insights into the genetic structuring of the *Alopia* populations in the Bucegi Mountains and the gene flow between population groups (Figure 3).

With $K = 2$, the two subspecies of *A. straminicollis* and *A. livida hypula* are separated from the other taxa of the *A. livida* complex. With $K = 3$, *A. straminicollis monacha* is separated from *A. s. straminicollis* + *A. livida hypula*. With $K = 4$, *A. livida nubila* and *A. nixa* + *A. fussi* are separated. With $K = 5$, *A. pomatias albicostata*, which is shown as an admixed population with lower $K$ values, is separated as a distinct cluster. With $K = 6$, *A. s. straminicollis* and *A. livida hypula* are separated. With $K = 7$, populations 3 and 8 of *A. livida nubila* are separated. With $K = 8$, STRUCTURE runs show different groupings. In the run with the highest likelihood, *A. nixa* and *A. fussi* are separated and the division of the *A. livida nubila* populations disappears. Alternative
classifications found in other runs show the former separation of populations 3 and 8 of *A. livida nubila*, but combine *A. s. straminicollis* and *A. livida hypula* in one cluster. In all results with $K = 8$, an additional cluster is found that never reaches a proportion above 75% and does not represent a specific taxon or population group. When $K$ is further increased, the additional clusters do not represent distinct population groups.

The STRUCTURE analysis provides evidence for extensive gene flow between *A. straminicollis* and *A. livida*. In the contact zones (2 and 20), all individuals have a mixed ancestry and also in the neighbouring populations several individuals show high genetic proportions of the other species. There is also admixture between the other enantiomorph pair, *A. nixa* and *A. fussi* when they form separate clusters with $K = 8$ (Figure 3). Individuals of populations 6 and 7

**FIGURE 2** Neighbour-net based on Jaccard distances between AFLP data of 250 *Alopia* individuals from the Bucegi Mountains. Encircled numbers refer to sampling localities and colours refer to *Alopia* taxa (see Figure S1 and Table S1). Coiling direction is indicated by D = dextral or S = sinistral after the taxon names [Colour figure can be viewed at wileyonlinelibrary.com]
of A. nixa show approximately 30% genetic proportion of A. fussi and one individual of A. fussi has an inferred ancestry of 50% from the A. nixa cluster. There is also evidence for gene flow between A. livida and A. fussi (Figure 3). In population 11 of A. fussi some of the individuals show up to 50% inferred ancestry to A. livida nubila and the individuals of A. livida kimakowiczi have an inferred ancestry of 30% up to 50% from the A. nixa + A. fussi cluster. In contrast, there is no admixture between A. pomatias and A. nixa, despite they occur sympatrically.

BAPS recognized 6 clusters that correspond to A. pomatias albicostata, A. straminicollis monacha + A. livida nubila from the hybrid population (population 2), A. livida nubila (populations 3, 8) + 2 specimens of A. fussi from population 11, A. nixa + A. fussi + A. livida bipalatalis + A. livida kimakowiczi, A. livida hypula + A. s. straminicollis from the hybrid population (population 20) and A. s. straminicollis. The BAPS admixture solution showed less admixture than the STRUCTURE analysis with K = 6 (Figure 3).

An analysis of gene flow between predefined population groups corresponding to A. pomatias albicostata, A. straminicollis monacha, A. livida nubila, A. livida kimakowiczi, A. nixa, A. fussi, A. livida bipalatalis, A. livida hypula and A. s. straminicollis using a gene flow plot calculated with BAPS (Figure S3) indicated that gene flow downhill is distinctly higher than uphill in four of five cases in which gene flow exceeds 1% at least in one direction (Table S5). The exception is a higher amount of gene flow from A. livida nubila to A. fussi at higher altitudes.

An analysis of molecular variance (Table S6) attributed only a small part of the genetic variation (13%) to the division into five species proposed by Nordsieck (2008, 2007, 2016).
The variation between populations accounted for 26% and the variation within populations for 61% of the total variation.

4 | DISCUSSION

4.1 | Stages of the Alopia radiation

Alopia in the Bucegi Mountains offers the opportunity to study all stages of a radiation from slight differentiation of populations to complete speciation within a few kilometres. We will discuss the different stages beginning from the case of complete speciation. Alopia pomatias is monophyletic in the mitochondrial gene tree (Figure 1), is distinctly separated from all other taxa in the neighbour-net based on nuclear markers (Figure 2) and shows little admixture with other taxa (Figure 3). It is the only taxon in the Bucegi Mountains belonging to clade F in the mitochondrial gene tree of Koch et al. (2013). This clade corresponds to the subgenus Kimakowiczia that differs from the other Alopia in elongated male organs and an elongated diverticulum of the bursa copulatrix (Grossu, 1981; Nordsieck, 2008). Nordsieck (2016) reported a possible hybrid between A. pomatias and A. fussi and raised the question whether the elongation of the male organs of A. nixa and of A. fussi (Grossu, 1981; Szekeres, 1976) might be the result of introgression. In any case, A. pomatias is the only taxon in the Bucegi Mountains that co-occurs with other taxa without fusing with them. At each of its localities, it is associated with a species of the A. livida complex (Figure S3). There is also frequent introgression of mitochondrial haplotypes (Figure 1; Koch et al., 2017). The hybrid zones between A. livida and A. straminicollis in the Tatar Gorge and the Velican Valley were known based on morphological evidence (Nordsieck, 2007; Szekeres, 1976, 2008, 2016). However, Nordsieck (2008, 2007) supposed that the two taxa hybridize only to a limited extent. In contrast, the admixture analyses of the nuclear markers (Figure 3; see Koch et al., 2017 for additional analyses) showed that most specimens in the hybrid zones have a combination of similarly large components from the genomes of the parental taxa irrespective of their classification (Figure 3; Koch et al., 2017). The introgression between the two taxa in the hybrid zones resulted in a fusion of their populations. This is also visible in the neighbour-net in which the individuals of the hybrid populations are not separated according to the classification (Figure 2). The introgression is not limited to the hybrid zones. Apparently "pure" A. s. straminicollis and A. straminicollis monacha populations showed some introgression with genomic components of A. livida as well, albeit the exact pattern depends on the number of assumed clusters (Figure 3). Genes for the opposite coiling direction are probably erased from these populations by frequency-dependent selection as long as there are only a few immigrants with
the opposite coiling direction. This results in a mosaic-like geographical pattern of populations of A. livida nubila and A. straminicollis monacha in the southern part of the Bucegi Mountains (Figure S1; Szekeres, 1976: Figure 2). However, if the number of immigrants increases, for example, because they are regularly washed down from an upstream population, frequency-dependent selection breaks down as observed in the hybrid zones between A. livida and A. straminicollis in the Tatar Gorge and the Velican Valley. It had already been observed by Nordsieck (2016) that the percentage of the hybrid phenotypes depends on the frequencies of the enantiomorph taxa. Nordsieck (2008) argued that dextral and sinistral taxa should be classified as distinct species because of their mosaic-like distribution. This conclusion was also based on the assumption that there is only limited hybridization between these taxa. In contrast, our data showed that the A. straminicollis populations fuse with neighbouring populations of A. livida when in contact and that even apparently "pure" populations showed some introgression (Figures 2 and 3). Thus, A. s. straminicollis and A. straminicollis monacha cannot be separated as a distinct species from A. livida. We suggest classifying them as subspecies of A. livida as it has been proposed by Szekeres (1976).

The dextral taxa of the A. livida complex represent the least differentiated stage of the radiation. The A. livida complex forms a shallow clade in the mitochondrial gene tree in which only the sinistral A. nixa is monophyletic (Figure 1). Alopia livida nubila from the southern part of the Bucegi Mountains and A. livida hypula from the northern slope are placed at different ends of the neighbour-net (Figure 2). In the admixture analyses with BAPS and corresponding STRUCTURE analyses (Figure 3), A. livida nubila and A. livida hypula form distinct clusters. Thus, we suggest classifying these population groups as subspecies despite the lack of morphological differentiation. They are not directly in contact but are connected across the main ridge of the mountains by populations classified as A. fussi and A. livida bipalatalis. The populations usually classified as A. livida bipalatalis include genomic components of the A. nixa + A. fussi cluster and of A. livida hypula (Figure 3). Likewise, the populations from the south-eastern range called here A. livida kimakowiczii for descriptive purposes include genomic components of the A. nixa + A. fussi cluster and of A. livida nubila (Figure 3). Because A. livida bipalatalis and A. livida kimakowiczii do not form separate clusters, we do not consider these transitional groups as distinct subspecies. However, the delimitation of population clusters may depend on the sampling. A representative sampling across the whole range of A. livida will be necessary to achieve a final classification. The transitions between the A. nixa + A. fussi cluster and the neighbouring A. livida subspecies show that A. nixa and A. fussi cannot be separated as distinct species and we suggest classifying them also as subspecies of A. livida. Consequently, the Alopia species of the Bucegi massif are classified as two species, A. pomatias (with the subspecies albicostata and pomatias) and A. livida (with the subspecies fussi, hypula, livida, monacha, nixa, nubila and straminicollis). In the following, we will use this classification (see also Table 1).

A comparison of the p-distances between coxl sequences within and between Alopia clades with corresponding values of other stylommatophoran radiations (Table S3) confirms that Alopia represents an early stage of radiation (see also Fehér et al., 2013). Although species status should be assessed based on the criteria of the applied species concept and not on distances between marker sequences (see also Davison, Blackie, & Scothern, 2009; Sauer & Hausdorf, 2012), the low distances within and between Alopia clades show that our suggestion to classify most of the taxa in the Bucegi Mountains as subspecies of A. livida is not indiscriminate lumping, but is also justified in comparison with the classification of other stylommatophoran groups.

4.2 Dispersal processes and their effects on diversification

If we want to understand non-adaptive radiations, we must also understand the processes that provide opportunities for speciation and those counteracting differentiation and speciation. Beside differentiation, dispersal processes are most important. The distribution patterns of Alopia species and the admixture between Alopia populations in the Bucegi Mountains indicate that dispersal of Alopia specimens occurs more frequently than expected before. Dispersal processes in land snails can be classified into three categories: active dispersal, passive short-distance dispersal and passive long-distance dispersal. In land snails, active dispersal is generally slow and limited to short distances. In habitat specialists like the rock-dwelling Alopia, it is further limited by the restriction that unsuitable habitat has to be crossed to reach the next suitable habitat island. There are no data about active dispersal in Alopia, but we can assume that Alopia behaves similar as the rock-dwelling Albinaria. Albinaria individuals move actively at most a few metres in their whole life (Schilthuizen & Lombaerts, 1994). Thus, active dispersal might contribute to the coherence of demes of neighbouring rock outcrops, but it is unlikely to act as a homogenizing force at spatial scales larger than a few tens of metres (Schilthuizen & Lombaerts, 1994).

Short-distance passive dispersal is more important from an evolutionary perspective. The main agents of passive dispersal of snails in the mountains are probably streams and heavy rains as well as falling rocks that transport snails downhill. Thus, we expect that the direction of short-distance passive dispersal is biased. The higher gene flow estimates from Alopia populations groups at higher altitudes...
to neighbouring groups at lower altitudes than vice versa (Figure S3, Table 1) support the importance of short-distance passive dispersal. There is also evidence for the importance of downhill passive dispersal by streams in other land snails (Arter, 1990). Although it has been shown for Arianta arbustorum that gene flow by passive short-distance dispersal is low and allows the differentiation of local populations (Arter, 1990), it might counteract the speciation process between populations that adapt to different altitudes (e.g. A. livida fussi vs. A. livida hypula and A. livida nubila) in an evolutionary perspective.

The distribution patterns within Alopia provide also evidence for the third dispersal category, long-distance passive dispersal. The phylogenetic analysis of Fehér et al. (2013) revealed or confirmed at least three disjunctions over more than 100 km that cannot be explained as remnants of formerly continuous ranges; for example, the colonization of central Transylvania by A. livida from the Bucegi Mountains. Many disjunctions over shorter distances probably also belong in this category. For example, the populations of A. livida straminicollis at the northern slopes of the Bucegi Mountains and of A. livida monacha in the southern part (Figure S1), which are sister groups according to the mitochondrial gene tree (Figure 1) were probably not directly connected, but originated also by a long-distance dispersal event. Long-distance dispersal requires other dispersal agents than short-distance passive dispersal. The most likely vehicles for long-distance transport of snails are birds (Gittenberger, Groenenberg, Kokshoorn, & Preece, 2006; Rees, 1965; Simonová, Simon, Kapic, Nehasil, & Horsák, 2016; Wada, Kawakami, & Chiba, 2012). In contrast to the discussed short-distance passive dispersal, successful transport of living snails by birds is usually undirected (though major migration routes of birds may influence its likelihood) and much rarer. Whereas short-distance passive dispersal may counteract differentiation of neighbouring populations, long-distance dispersal provides new opportunities for speciation because it results in geographically isolated populations that can differentiate without gene flow. Moreover, only few individuals are involved in long-distance dispersal events resulting in founder events that further promote speciation (Gavrillets & Hastings, 1996; Mayr, 1963).

The opposite effects of the different modes of dispersal on the generation of biodiversity are nicely shown by the taxa of the clade C2 in the mitochondrial gene tree of Fehér et al. (2013). Whereas short-distance dispersal resulted in the fusion of A. livida straminicollis and A. livida monacha with neighbouring populations of clade D1, namely A. livida hypula and A. livida nubila, long-distance dispersal of a lineage closely related to A. livida monacha from the Bucegi Mountains to Cetăţeni and the Făgăraş Mountains allowed the differentiation of A. maffeiana and its splitting into A. m. maffeiana and A. maffeiana valeriae (Fehér et al., 2013).

4.3 Non-adaptive radiations versus polytypic species

Species may be kept separate by reproductive barriers and/or by disruptive natural selection resulting from differential adaptation to different environments. Disruptive natural selection often maintains the distinctness of species long before reproductive isolation is complete (Wu, 2001). However, in a non-adaptive radiation, disruptive natural selection is lacking. Thus, reproductive barriers may evolve only by sexual selection or by random genetic drift in isolated populations. Genetic drift is expected to be slow so that the waiting time to speciation may last much longer if no selection is involved (Gavrillets, 2003). In the case of the Alopia taxa in the Bucegi Mountains, this is demonstrated by the fusion of populations belonging to the distantly related clades C2 (including A. livida straminicollis and A. livida monacha) and D1 (including A. livida sensu stricto) of the mitochondrial gene tree of Fehér et al. (2013) in the southern part as well as on the northern slopes of the mountains. Despite the long separation of these lineages (Figure 1; Fehér et al., 2013), no sufficient barriers evolved so that extensive gene flow between the northern populations of the two lineages, A. livida straminicollis and A. livida hypula, as well as between the southern lineages, A. livida monacha and A. livida nubila, initiated the fusion of these taxa. The geographical adjacent representatives of the two lineages became more similar to each other on the genetic level than they are to the populations of the same clade inhabiting the opposite region of the Bucegi Mountains (Figures 2 and 5).

Morphological disparity between populations may increase even if they are connected by limited gene flow. However, increasing morphological disparity is not equivalent to speciation. Even if we do not require reproductive isolation as in the biological species concept (Mayr, 1942, 1963), but accept the differential fitness species concept (Hausdorf, 2011), species have to be characterized by features that would have negative fitness effects in other groups. In a non-adaptive radiation, these features cannot be adaptations to different ecological niches, but can only be features affecting reproduction itself or the intrinsic fitness of the offspring. Groups of populations which are neither adapted to different environmental conditions nor have characteristics that restrict gene flow to a level that prevents the fusion of the populations upon contact, cannot be considered distinct species irrespective of the morphological or genetic disparity they display and irrespective of the time they were separated. Rather, they have to be classified as polytypic species. The definition of radiation usually includes "the evolution of a relatively large, monophyletic group of species" (Gittenberger, 1991), the divergence of "a single ancestor … into a host of species" (Schluter, 2000) or "a pattern of species diversification" (Rundell & Price,
The differentiation of subspecies may be the first step in a radiation process. However, as such it is not yet radiation. Particularly in taxa like land snails in which there is little gene flow between populations because of their low dispersal capability, disparity between populations can increase considerably despite a lack of intrinsic characteristics that restrict gene flow. The disparity between populations may exceed that between distinct species, which evolved more quickly as a result of disruptive selection. Other examples of such polytypic species that were classified into multiple species because of their morphological disparity, but did not reach the level of non-adaptive radiations are the Sicilian Murella (Fiorentino, Manganelli, & Giusti, 2008a; Fiorentino, Salomone, Manganelli, & Giusti, 2008b) or the Moroccan Rossmaessleria (Walther, Neiber, & Hausdorf, 2016).

The fusions of A. livida straminicollis with A. livida nubila and of A. livida monacha with A. livida hypula show that the time since isolation or the position in a gene tree is irrelevant for the decision whether a group of populations represents a subspecies of a polytypic species or a distinct species. Decisive is only whether the taxon is characterized by features that would have negative fitness effects in other groups. The taxa that are located in the mitochondrial gene tree between the clades C2 (including A. livida straminicollis and A. livida monacha) and D1 (including A. livida sensu stricto) (Fehler et al., 2013) are not necessarily all subspecies of a single polytypic species. If it can be shown that they have characteristics that restrict gene flow with other population groups to a level that prevents the fusion of these groups upon contact, they should be classified as distinct species. Our results challenge the uncritical classification of taxa that have low dispersal abilities but show high disparity between populations as non-adaptive radiations. We need to prove that such taxa are not just polytypic species and we need to investigate the conditions under which polytypic species evolve into radiations despite niche conservatism and how frequently they become extinct and fuse with each other before they reach species level (as in the cases of A. livida straminicollis and A. livida nubila or A. livida monacha and A. livida hypula).

The radiation of the land snail genus Xerocrassa in Crete illustrated how non-adaptive radiation can originate despite of the lack of disruptive natural selection and the long waiting time for speciation based on genetic drift alone by sexual selection (Sauer & Hausdorf, 2009). Sexual selection may increase the rate of reproductive divergence between populations and thereby drive speciation (Dominy, 1984; Gavrilets, 2000, 2014; Panhuis, Butlin, Zuk, & Tregenza, 2001; Ritchie, 2007). In adaptive radiations, it may act in concert with divergent natural selection. In contrast, it is the only mechanism that can speed up the diversification process beyond the slow pace possible with genetic drift alone that will rarely result in radiation, the rapid diversification of a lineage into multiple species. The fact that the Alopia taxa that reached the most advanced stage of speciation, that is A. pomatias, shows the most distinct differences in the genitalia indicates that sexual selection has also facilitated the radiation of Alopia. In A. pomatias and, to a lesser degree, in A. livida nixa and A. livida fussi, the epiphallus that produces the spermatophore is elongated compared to A. livida straminicollis and A. l. livida (Grossu, 1981; Szekeres, 1976). In stylommatophoran snails, the spermatophore is transferred into the bursa copulatrix complex during the copulation, where the spermatophore with most of the sperm is digested (Lind, 1973; Rogers & Chase, 2001). An elongation of the spermatophore-producing organs, the epiphallus and the flagellum (if present) results in longer spermatophores that increase the number of sperm cells that can leave the spermatophore before it is introduced into the bursa copulatrix complex and digested. Thus, there is a sexual conflict between the male partner that wants to fertilize as many eggs as possible and the female partner that wants to control the fertilization of the eggs. This conflict can result in a co-evolutionary arms race between spermatophore-producing and spermatophore-receiving organs (Koene & Schulenburg, 2005; Sauer & Hausdorf, 2009). The elongation of the spermatophore-producing epiphallus and of the diverticulum of the bursa copulatrix, the spermatophore-receiving-organ, in the Alopia taxa that represent the most advanced stages of speciation in the radiation, A. pomatias and A. livida nixa, indicates that also the Alopia radiation was driven by sexual selection. This further supports the general importance of sexual selection in non-adaptive radiations.

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