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Phylogeography of the land snail genus *Circassina* (Gastropoda: Hygromiidae) implies multiple Pleistocene refugia in the western Caucasus region ☆

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ABSTRACT

The phylogeny and historical biogeography of the Caucasian land snail genus *Circassina* was reconstructed using multilocus amplified fragment length polymorphism (AFLP) data and mitochondrial DNA sequences. Diversification within the group started with a divergence of populations from the western Lesser Caucasus from those of the Greater Caucasus during the late Miocene. Distinct AFLP clusters and major mitochondrial clades separated by long internal branches lend evidence to the hypothesis of separate glacial refuges in the Lesser and Greater Caucasus during the Pleistocene. High genetic distances across low geographic distances and admixture analysis revealed a phylogeographic boundary running through the Colchis lowlands, which may have been established and maintained in part by repeated transgressions of the Black Sea during the Pleistocene and Holocene. Localities in Ciscaucasia were probably colonised through long-distance dispersal across the main ridge of the Greater Caucasus. The phylogeny implies multiple independent losses of accessory genital organs, i.e. dart sac and mucus glands, within *Circassina*. None of the anatomically defined (sub-) species distinguished so far is monophyletic and there is gene flow between the two main population groups across the Colchis lowlands. Thus, we propose to classify these population groups as subspecies of a single species.

1. Introduction

The Caucasus region is ranked among the 25 most important global biodiversity hotspots (Myers et al., 2000; Zazanashvili et al., 2004). The importance of the western Caucasus, especially the Colchis region, as a glacial refugium where, among others, Neogene relict species survived as well as a centre of ongoing radiation has increasingly been appreciated (Hewitt, 2000; Pokryszko et al., 2011; Tarkhnishvili et al., 2012; Nakhutsrishvili, 2013; Tarkhnishvili, 2014; Walther et al., 2014). There is dissent, however, on whether there existed a single continuous forest refugium at the eastern Black Sea coast as implied by some studies (van Andel and Tzedakis, 1996; Kikvidze and Ohsawa, 2001; Pokryszko et al., 2011; Tarkhnishvili et al., 2012; Volkova et al., 2013; Wielstra et al., 2013) or multiple forest refugia (Tarkhnishvili et al., 2000; Mumladze et al., 2013; Tarkhnishvili, 2014).

Circassina Hesse, 1921 (Gastropoda: Hygromiidae) is a group of land snails endemic to the western and central Caucasus region and the eastern Pontus. It includes only two species, *C. frutis* (Pfeiffer, 1859) and *C. lasistana* Hausdorf, 2001. *Abchasohela* Hudec & Lezhawa, 1971, which was formerly classified as a subgenus of *Circassina* (Hausdorf, 2001), proved to be more closely related to other hygromiid groups than to *Circassina* (Walther et al., in press). The *Circassina frutis* complex is exceptional among helicoid land snails because it is polymorphic with regard to the dart apparatus, i.e. the snails possess either a complete dart apparatus with a dart sac plus an accessory sac and mucus glands (*C. frutis circassica* (Mousson, 1863)), only mucus glands (*C. frutis frutis*) or none of these accessory genital organs (*C. frutis veselyi* (Frankenberger, 1919)). Schileyko (1978) classified these morphotypes as subspecies, whereas Giusti and Manganelli (1987) questioned whether they are related at all. Hausdorf (2001) followed Schileyko (1978), but questioned whether the morphotypes are actually genetic entities, because their ranges interdigitate in western Georgia. Furthermore, Hausdorf (2001) separated *C. lasistana* from *C. frutis* because of the much shorter spermatophore-forming flagellum.

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A dart apparatus with a calcareous dart and mucus glands is an apomorphy of a large group of helicoid land snails (Nordsieck, 1987; Hausdorf, 1998). The mucus glands produce an allohormone-like substance that is transmitted into the mating partner during courtship by the dart. This substance causes temporal changes in the female part of the genitalia of the mating partner, which delays the intake of the spermatophore into the bursa of the bursa copulatrix and allows more sperm to leave the spermatophore before it is digested in this organ, so that the fertilisation success of the dart shooter is increased (Koene and Chase, 1998; Chase and Blanchard, 2006; Chase, 2007). Since the fertilisation success of individuals that possess these organs is increased, sexual selection should favour the fixation of these traits. However, injuries or an increased infection risk of the recipient as well as a prolongation of the mating implied by the delayed intake of the spermatophore, during which the individuals are under risk of desiccation or predation, may have negative fitness effects. Thus, natural selection might counteract sexual selection. The population structure of land snails usually consists of local demes that often go extinct and may be founded again, potentially resulting in populations in which the lack of the dart sac or the complete dart apparatus is fixed.

The purpose of this study was twofold. First, we studied the phylogeography of *Circassina* based on mitochondrial sequences and amplified fragment length polymorphism (AFLP) markers to infer whether the Colchis region can be viewed as a homogeneous Pleistocene forest refugium or whether there were multiple separate refugia in this region. Second, we tested for associations between environmental variables and the structure of the dart apparatus to infer potential reasons for the unusual polymorphism of this accessory genital organ in *Circassina*. We also reassessed the taxonomy of *Circassina*, so far resting upon the anatomy of the genitalia, based on the results of our molecular phylogenetic and admixture analyses.

2. Material and methods

2.1. Sampling

Mitochondrial cytochrome c oxidase subunit I gene (*cox1*) and 16S rDNA fragments as well as amplified fragment length polymorphism (AFLP) data of *C. frutis frutis*, *C. frutis circassica*, *C. frutis veselyi* and *C. lasistana* covering most of the ranges of these taxa in Georgia, the Russian part of the Caucasus region and north-eastern Turkey were analysed (Fig. 1; Supplementary Table S1). Individuals of *Caucasigena eichwaldi* (Pfeiffer, 1846), *Fruticocampylaea narzanensis* (Krynicki, 1836), and *Kokotschashvilia holotricha* (Boettger, 1884) were included as outgroups in the analyses. *Circassina* specimens were identified according to the diagnoses given in Hausdorf (2001), i.e. dart sac, accessory sac and mucus glands missing (*C. frutis veselyi*), mucus glands present, but dart and accessory sacs missing (*C. frutis frutis*), dart sac plus accessory sacs as well as mucus glands present, but epiphallus shorter than flagellum (*C. frutis circassica*) or epiphallus longer than flagellum (*C. lasistana*) (Fig. 2). Data on sampling sites, voucher numbers and the classification of each specimen used in this study are compiled in Supplementary Table S1.

2.2. DNA amplification and sequencing

Samples of foot muscle tissue were stored in 100% isopropanol at −20 °C. DNA was extracted following a slightly modified version

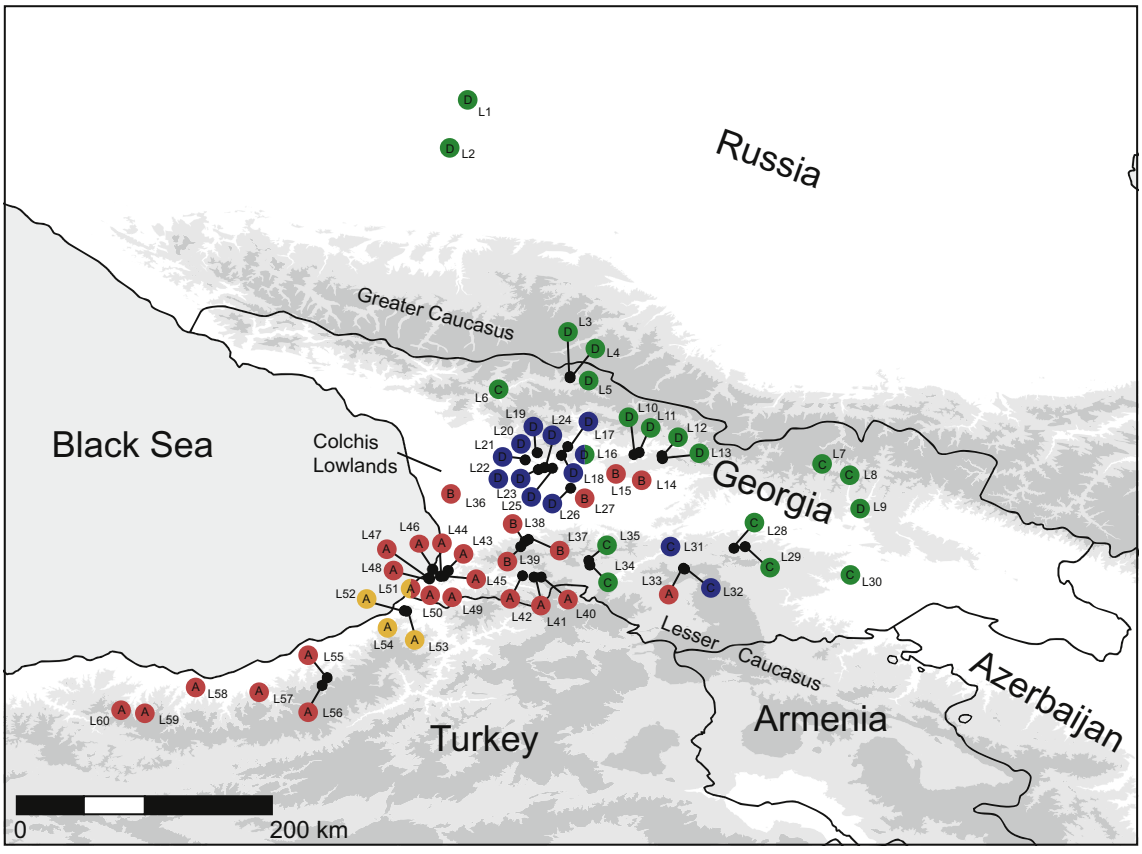

Fig. 1. Sampling sites (see Supplementary Table S1) and distribution of mitochondrial haplotype clades (for letters in the circles; see Fig. 2) and of morphotypes (red: *Circassina frutis fruits*; blue: *C. frutis circassica*; green: *C. frutis veselyi*; yellow: *C. lasistana*) in the Caucasus region and north-eastern Turkey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)