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RESEARCH NOTES

Ecological observations on *Arianta aethiops aethiops* (Helicidae), a land snail endemic to the South Carpathian mountains, Romania

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The evolutionary history and isolation of the Carpathian mountains are reflected in a high number of endemic plants and invertebrates including several terrestrial gastropods[1–3]. *Arianta aethiops* (Bielz, 1851) is a helicid land snail that has been described as an endemic species at altitudes between 1900 and 2370 in two restricted areas in the Carpathians.[1–6] Nineteenth century authors reported the subspecies *A. aethiops aethiops* from about 10 localities in the South Carpathians in Romania,[4–6] whereas the subspecies *A. aethiops petrii* has been found in the East Carpathian mountains in North Romania and Western Ukraine.[1,2,3,7] Nothing is known about the reproductive biology and ecology of *A. aethiops*. However, such knowledge is a prerequisite for the assessment of the species' extinction threats and the development of conservation action.[8,9]

Here we report on shell and reproductive characteristics, population density and activity and resting sites of *A. a. aethiops* living sympatrically with *Arianta arbustorum* (L., 1758) in an alpine meadow in the South Carpathians. *A. arbustorum* has—in contrast to *A. a. aethiops*—a much wider distribution. Its range extends from the Alps to North Scandinavia and from Romania to the British Isles.[10]

Field work was conducted on a N- to NW-exposed slope (inclination: 20–45°) in the nature reserve at the cirque Lake Bilea at an elevation of 2100–2250 m in the Fagarasi mountains, South Carpathians, Romania (45°36′ N, 24°30′ E). The alpine meadow is partly covered with rocks and rock debris. The underlying bedrock is dominated by slate. To estimate the density of *A. a. aethiops*, 10 plots measuring 2 m × 2 m were randomly chosen. The plots were carefully searched for *A. a. aethiops* and *A. arbustorum*. For each snail found we recorded the species, age class (individuals with a reflected peristome at the shell aperture were considered as adults, those with a shell breadth > 12 mm but without flanged peristome as subadults, and smaller ones as juveniles) and the type of substrate on which the snail was moving or resting. In adult snails we measured shell breadth and height to the nearest 0.1 mm using vernier callipers. To obtain more data on shell size and activity and resting sites, a larger area of the slope was searched for individuals of both species, between 9 and 12 August 1997. During this period it was rainy and the air temperature varied between 2 and 10°C. The snails were released where they had been found. As an exception, we collected one individual from both species for dissection and a few individuals for breeding experiments (see below).

A. a. aethiops has a depressed globular shell with 5–6 slightly convex whorls (Fig. 1A). The shell is dark brown to black and thin-walled, the lip is thin and white. The umbilicus is narrow and semicovered by the reflected part of the columellar edge. The soft body is black. In the Lake Bilea population the shell breadth of adult *A. a. aethiops* averaged 19.6 mm, that of the co-existing *A. arbustorum* 20.5 mm (Table 1). The shell size of *A. arbustorum* in the Lake Bilea population is similar to that of conspecifics living in alpine

Table 1. Shell and reproductive characteristics of *A. a. aethiops* and *A. arbustorum* from an alpine meadow near Lake Bilea, Romania. Sample size is indicated in parentheses. t and P-values result from unpaired t-tests.

	A. a. aethiops Mean ± SD	*A. arbustorum* Mean ± SD	t	P
Shell breadth (mm)	19.6 ± 1.0 (30)	20.5 ± 1.2 (11)	2.38	0.0225
Shell height (mm)	13.2 ± 0.9 (30)	16.7 ± 1.3 (11)	9.45	<0.0001
Shell shape (shell height/shell breadth)	0.68 ± 0.03 (30)	0.82 ± 0.03 (11)	12.78	<0.0001
Clutch size	38.3 ± 14.7 (6)	38.7 ± 16.0 (11)	0.05	0.96
Egg length (mm)	3.54 ± 0.09 (6)	3.11 ± 0.33 (11)	3.11	0.0072
Egg breadth (mm)	3.27 ± 0.06 (6)	2.94 ± 0.27 (11)	2.88	0.0113
Egg shape (egg length/egg breadth)	1.081 ± 0.026 (6)	1.056 ± 0.023 (11)	2.03	0.0609

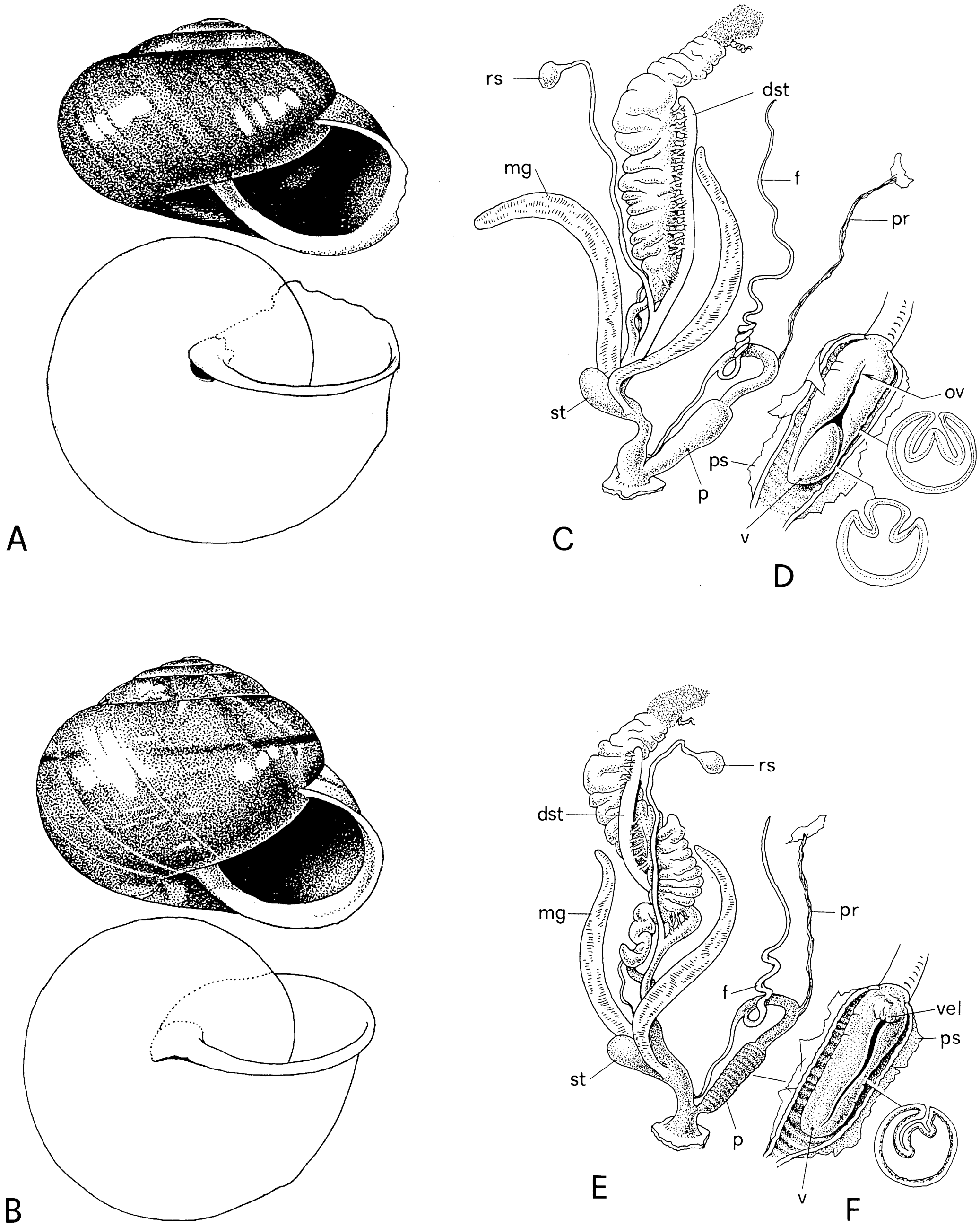

Figure 1. Shell and genitalia of *A. a. aethiops* and *A. arbustorum* from an alpine meadow near Lake Bilea, Romania. **A.** *A. a. aethiops* (shell breadth 19.6 mm). **B.** *A. arbustorum* (shell breadth 20.5 mm). **C.** genitalia of *A. a. aethiops*. **D.** detail of the penis, opened to show the penial papilla and orifice of verge. **E.** genitalia of *A. arbustorum*. **F.** detail of the penis. Abbreviations: dst—diverticle of spermatheca, f—flagellum, mg—mucus gland, ov—orifice of verge, p—penis, pr—penis retractor, ps—penial sheat, rs—reservoir of spermatheca, st—stylophore, v—verge (penial papilla), vel—velum.

habitats of central Europe.[11,12] *A. arbustorum* has a globular shell with 5–6 slightly convex whorls (Fig. 1B). The shell is dark yellow or light brown with an extensive paler flecking and a dark brown spiral band at the periphery. As in *A. a. aethiops*, the shell of *A. arbustorum* is thin-walled. The soft body colour is variable ranging from yellow to dark grey. The two species differed in shell size and shape (ratio shell height to shell breadth; Table 1). No intermediate forms between *A. a. aethiops* and *A. arbustorum* were found.

The genitalia of both species are presented in Fig. 1C–F. The only difference between the two species is the structure of the penial papilla (verge). In *A. arbustorum* the verge is represented by a grooved process with the orifice of the epiphallus situated in the depth of the groove and covered by a sort of corrugated velum. In *A. a. aethiops* the velum is absent, but the superficial groove on the verge is forked at the anterior end and embraces a swollen portion of the verge. However, similar variation in penis morphology has been found in a single *A. arbustorum* population in the Austrian Alps.[13] This suggests that the interspecific difference in genital anatomy observed in the present study might be an artefact of the small sample size.

The density of *A. a. aethiops* averaged 0.35 individuals per m^2 (SD = 0.41, range 0–1 individual/m^2). Considering different age classes there were 0.15 juveniles/m^2, 0.05 subadults/m^2 and 0.15 adults/m^2. The density of *A. arbustorum* was less than 0.02 individuals/m^2.

A total of 44 *A. a. aethiops* and 9 *A. arbustorum* were observed in the Lake Bilea population. Active snails were most frequently found on the sparse vegetation (*A. a. aethiops*: 64.5%; *A. arbustorum*: 83.3%) and on horizontal rock surfaces (*A. a. aethiops*: 25.8%; *A. arbustorum*: 16.7%). Individuals of *A. aethiops* were observed to feed on *Saxifraga rotundifolia*, other vascular plants, several lichen species and on sheep faeces. Resting snails were most frequently found attached to the lower side of pieces of stone (*A. a. aethiops*: 92.3%; *A. arbustorum*: 100.0%). Some individuals of *A. a. aethiops* rested attached to vertical rock surfaces (7.7%). Resting attached to the lower side of pieces of stone includes a high risk of mortality to the snails (see below).

To examine reproductive traits, 14 adult individuals of *A. a. aethiops* and six of *A. arbustorum* were collected and transported to the laboratory. The snails were kept singly in transparent beakers (8 cm deep, 6.5 cm in diameter) with moist soil (approximately 3 cm deep) in the bottom at 16°C and on a cycle of 18 h light: 6 h dark. The beakers were cleaned and checked for eggs twice per week, and fresh lettuce was provided *ad libitum* as food. The eggs of each batch were collected, cleaned on moist paper towel, counted, and kept separately in a plastic dish (6.5 cm in diameter) lined with moist paper towelling for at least 24 h before measuring. In both species newly laid eggs absorb water from the environment to reach their normal turgid state. The length and breadth of 10 randomly chosen eggs from each batch were measured to the nearest 1/16 mm using a binocular microscope with a stage micrometer. To determine hatching success the eggs were kept at 16°C.

The two species did not differ in clutch size (Table 1). However, *A. a. aethiops* produced larger eggs than *A. arbustorum*. Furthermore, the eggs differed slightly in shape (ratio egg length to egg breadth): eggs of *A. a. aethiops* tended to be elongate, whereas those of *A. arbustorum* were more globular (Table 1). The structure of the eggshell was very similar in both species. The eggshell was partly calcified containing discrete crystals of calcium carbonate in a jelly matrix. In *A. arbustorum* egg size was negatively correlated with clutch size ($r = -0.83$, $n = 11$, $P = 0.0017$). In *A. a. aethiops* there was no correlation between the two traits ($r = -0.25$, $n = 6$, $P = 0.63$). Hatching success of eggs did not differ between the species (*A. a. aethiops*: mean 58.6%, range 30.6–95.2%; *A. arbustorum*: mean 78.7%, range 56.0–95.7%; $t = 1.21$, d.f. = 6, $P = 0.27$). In both species, hatching snails ate their own eggshell and tried to consume unhatched sibling eggs. This type of egg cannibalism has been found in hatchlings of several pulmonate land snails.[14]

After hibernating at 4°C and darkness for 6 months the snails of both species were allowed to mate with conspecifics in transparent plastic containers. Mating trials (*A. a. aethiops* 14 trials, *A. arbustorum* 6 trials) consisted of pairs of snails of the same species and were performed outdoors under the more benign conditions of the Swiss lowlands near Basel. However, in no trial any snail showed courtship and copulation behaviour. The reason for the absence of mating behaviour under the experimental conditions is unknown. Most probably, the conditions offered lacked some essential stimuli to elicit mating behaviour in *A. a. aethiops* and *A. arbustorum* from the Carpathians (individuals of *A. arbustorum* from the Swiss Alps kept under identical conditions mated in simultaneously conducted trials). In the field, we observed one copulating pair of *A. a. aethiops* in the Lake Bilea population in the evening of 9 August 1997.

We searched for *A. a. aethiops* at two localities described in the literature.[2,3] At Lake Bilea there was still an abundant population, whereas at Lake Doamnei no *A. a. aethiops* could be found, but a few individuals of *A. arbustorum*. Most probably the *A. a. aethiops* population at Lake Doamnei had become extinct. A major threat to any *A. a. aethiops* population might be large flocks of sheep. In the Carpathians a flock of sheep is allowed to graze in a given valley during the whole vegetation period. However, traditional shepherding has become increasingly uneconomical. Since 1993, the owners have increased the size of sheep flocks fivefold,[15] resulting in a dramatic increase in the grazing pressure to alpine plants within a short period. As a consequence, the composition of plant species is changing; grasses are becoming more dominant and a variety of herb species disappear, which in turn leads to a decrease in invertebrate diversity. Furthermore, to protect sheep against large predators such as wolves and bears, the sheep are driven to safe fences at lower altitudes every evening. This means that sheep are travelling large distances every day. Moving sheep dislocate pieces of stone which can be fatal to snails resting attached to their lower sides. We found numerous individuals of *A. a. aethiops* with crushed shells after a flock of 700 sheep had passed the nature reserve adjacent to Lake Bilea. Our observations indicated that the new form of shepherding is a series threat to *A. a. aethiops* and *A. arbustorum* living in South Carpathian mountains.

A. a. aethiops is not listed in the IUCN Red List of Threatened Animals. However, its restricted distribution and the fact that most populations of the species are isolated and probably decline due to alterations in shepherding suggest that *A. a. aethiops* is threatened. Unfortunately, the information available does not allow an accurate assessment of the status of the species. Nonetheless, we hope that this report might be a first step towards any conservation action for molluscs, other invertebrates and plants in the Carpathians.

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