

Adventures in the field and in the lab – investigation of an enigmatic door-snail species, *Montenegrina apfelbecki* (Sturany, 1907)

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Introduction

In the frame of the Conchologists of America 2014 Academic Grant, the first author had the possibility to organise a field trip to Albania and neighbouring Kosovo, in order to collect *Montenegrina* taxa/populations needed for the systematic revision and molecular phylogenetic reconstruction of this genus. Aside from some other localities, our primary target was the Shënt Mountain in Northern Albania, where we expected to find the species most in demand, *Montenegrina apfelbecki* (Fig. 1).

The need for a systematic revision of *Montenegrina*

Montenegrina is a genus of terrestrial, obligate rock-dwelling gastropods, distributed in the western part of the Balkan Peninsula (SE Europe). Due to the patchy distribution of their special habitat type (they live on bare limestone cliffs), the genus comprises several small range endemic taxa. According to the current system (Bank 2012), there are 88 known subspecies, which are classified into 22 species. Moreover, due to the intensive fieldwork activity of the researchers of the Hungarian Natural History Museum (HNHM) (Fehér et al. 2004, Fehér & Erőss 2009, Murányi et al. 2011) there are several recently discovered populations, some of which are presumed to be new taxa, which remain to be described. The systematics of the door-snails in the Mediterranean region were almost exclusively set-up on a conchological basis (Nordsieck 2007), however, our preliminary molecular studies revealed that most of the conchological traits, which were once considered taxonomically important, might have arisen several times independently and many 'species' in the current system might be paraphyletic. Thus for the taxonomic revision of this group, a sound molecular phylogenetic footing seems necessary.



Figure 1. *Montenegrina apfelbecki* (Sturany, 1907), syntype specimen collected by Buljubašić in 1905 (NHMW 41166). Note the mummified body showing through the shell.

Following the footsteps of our forefathers

In 1905, Dr. Rudolf Sturany, curator of the mollusk collection of the Natural History Museum, Vienna (NHMW) and Viktor Apfelbeck, curator of the entomological collection of the museum in Sarajevo, performed a fieldtrip to Albania (Sturany 1905). They were accompanied by Latif Buljubašić, 'museum servant' from the Sarajevo museum. It took them several days to reach Albania, where they spent most of their time on mule-back. At that time, Albania was still under the Ottoman Turkish rule, however, their force barely intruded into the mountain settlements, which were dominated by Albanian tribes in constant conflict with each other. Wading across several rivers, the 50 km ride from Shkodra to Oroshi – center of 'darkest Albania' – lasted two days. While exploring the nearby mountains and their fauna, they were hosted by the local bishop (Fig. 2). Shortly afterwards the two curators left, but Buljubašić stayed in Oroshi for further, more extensive sampling of that area. During his two-months-long, hazardous stay (e.g. he survived an earthquake, in which the bishop's residency perished), he managed to collect a huge amount of invertebrate samples from the Shënt (Fig. 3), Munela, Zebë, and Koritnik mountains. A large part of this material was deposited and can still be found in the scientific collections at the NHMW. In his first travel report, Sturany (1905) mentioned a strange-looking and most probably new door-snail species, which was formally described just two years later, as *Clausilia apfelbecki*. Unfortunately, Buljubašić provided rather incomplete locality data for the collected material, namely, "Mal i Shëit bei Oroshi, Merdita, in einer Höhe von zirka 1500 m."

The progress of the zoological exploration of the northern Albanian territories did not become easier after this beginning. Due to the 1st World War, the Italian invasion, the 2nd World War, and finally the communist regime, it was very difficult for naturalists to reach that region until the 1990s (Dhora & Welter-Schultes 1996). Thus, hardly any zoologists have collected there in the past 110 years and, to our knowledge, nobody has ever managed to collect this species since it was originally collected. A few taxa have been described from northern Albania as subspecies of *M. apfelbecki*. Preliminary molecular analyses, however, indicate that these taxa are paraphyletic. Their systematic positions (and their correct taxonomic names) could only be defined after clarifying the phylogenetic relationship of the nominate taxon first.

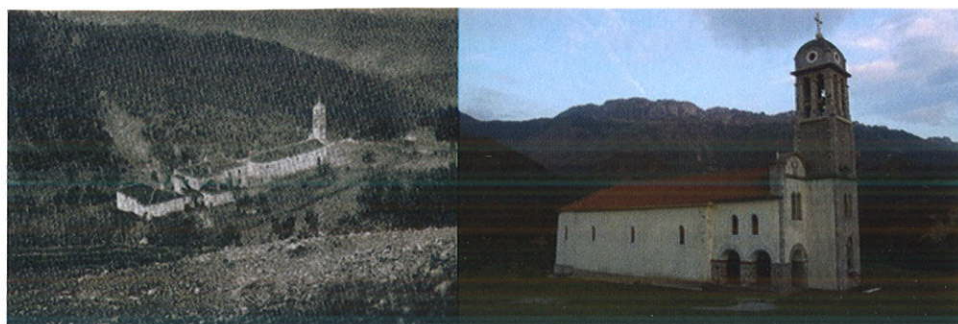


Figure 2. The abbey of Oroshi in Sturany's time and today. Black and white photo is an illustration from Sturany's travelogue from 1905.



Figure 3. The Shënt Mts. 110 years ago and now. Black and white photo is an illustration from Sturany's travelogue from 1905.

Our expedition in 2014

Before starting this project the majority of the known *Montenegrina* taxa were already available for DNA analysis (as recently collected and alcohol-stored museum lots). Thus, during the project's field campaign we aimed to obtain only the few missing taxa, among others, the enigmatic *M. apfelbecki*.

Our 10-day-long field trip started from Budapest. Participants in the expedition were Zoltán Erőss (HNHM) and Zoltán Fehér (NHMW), malacologists; Jozef Grego, malacologist and speleologist; his son, Maroš Grego, responsible for photo documentation during the trip; and Dorottya Angyal (HNHM), speleobiologist (Fig. 4). As the type locality of *M. apfelbecki*, as well as most of the other planned collecting sites, could be reached only on rugged backcountry roads, the 4WD Toyota Hilux of the HNHM was our indispensable companion (Fig. 5).



Figure 4. Zoologists of the expedition, (Tërthorë pass).

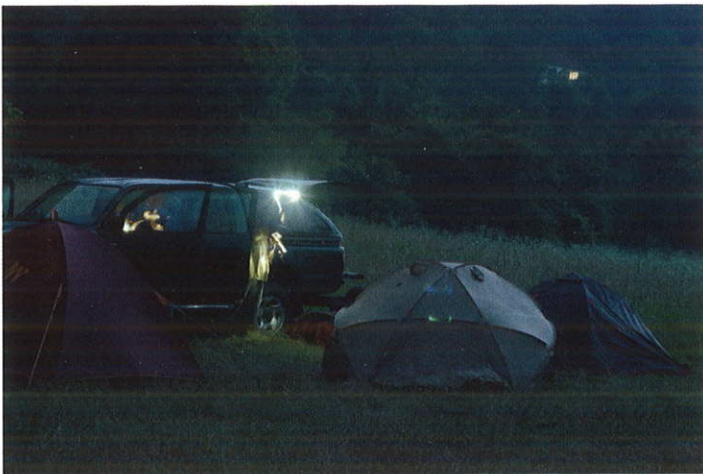


Figure 5. Night shelter in Montenegro and the Hilux, our 'best friend' during the trip.



Figure 6. Prokletije Mts., Tërthorë pass: one of the most beautiful places in Albania.



Figure 7. Entrance to the newly discovered 'Strawberry Cave' in Shënt Mts.



Figure 8. Double curse – double tire puncture far away from civilization.



Figure 9. First *Montenegrina* specimen collected by the expedition in the Tropojë Gorge, north of Tropojë village (northern Albania).

We reached Albania from the north, through Montenegro. After a short stop at the stunning Thertorë pass (Prokletije Mts., Fig. 6), we proceeded to the plateau of the Shënt Mts., where we stayed for three days and checked several sites that seemed suitable habitats for rock-dwelling snails (Fig. 3). We found rare and endemic gastropod species, like *Napaeopsis merditanus* (Sturany, 1907) and *Cochlostoma georgi* (A. J. Wagner, 1906), but despite our efforts, we could not manage to find any *Montenegrina* specimens. We discovered a previously unknown vertical cave on the plateau and collected some noteworthy cavernicolous macroinvertebrates from its walls and wooden debris (Fig. 7). Continuing our misadventure, we suffered a double tire puncture in the mountains, a couple of hours walk from any inhabited settlement (Fig. 8). Due to a very helpful local family we finally managed to fix the car and get down from the mountain. The main goal of the expedition – to find *M. apfelbecki* – seemed to be a failure and we already had more than a half-day delay. Therefore we had to strictly adhere to the schedule for the rest of the trip. We tried our luck in Kosovo, in the Bistrica valley, the type locality of *Montenegrina janinensis sporadica* H. Nordsieck, 1974, near a monastery closely guarded by the KFOR military legion. We were unsuccessful again, the desired subspecies remained elusive. Our luck finally turned only on the fifth day of the trip, when we discovered a new *Montenegrina* species in the vicinity of Tropojë in the gorge of the Tropojë Creek (Figs. 9,



Figure 10. Enroute through the Tropojë Gorge (northern Albania), the site of the new *Montenegrina* species.



Figure 11. Despite great poverty, people in small villages of the Albanian mountains seem to live in harmony and satisfaction. This old man picked oregano for us in Fushë Bardlë village.

10). We then headed south for some interesting sites in the Shpat, Kurveleshi, Kendrevicë, Lunxherisë, Nemerçkë, and Vallamarë Mountains. Our last campsite was on the shore of Lake Ohrid (Fig. 17), which is the oldest and one of the most spectacular lakes in Europe, hosting a large number of endemic freshwater molluscs.

Albania is a biodiversity hotspot for not only gastropods, but other groups as well (Fig. 16). We collected numerous millipedes, chilopods [centipedes], crustaceans, freshwater insects, harvestmen, beetles, and other invertebrates, which now enrich the collections of the NHMW and the HNHM.

The duality of the crowded cities with all of their technical achievements and the untouched nature of the mountain areas with their modest, near-natural inhabitants assigns a unique atmosphere to Albania (Figs. 13, 14, 15), making it a great adventure and chance to meet local people with their unusual customs and history (Figs. 11, 12).



Figure 12. According to Albanian superstition, hanging puppets on windows or doors helps to keep away wicked ghosts, called 'vojtans'.



Figure 13. Civilization has yet to touch some of the mountain ranges of Albania, preserving them in near natural condition.



Figure 14. Living as they have for centuries, in many Albanian villages breeding of goats for milk and meat is still practised.



Figure 15. Mules are extensively used in everyday life in Albania.



Figure 16. Remarkable creatures of our trip. Scorpion (*Euscorpium* sp.), Hermann's tortoise (*Testudo hermanni*), long-nosed, horned, or sand viper (*Vipera ammodytes*) and bush crickets (*Poecilimon jonicus*). The venomous but not overly aggressive long-nosed viper is protected.

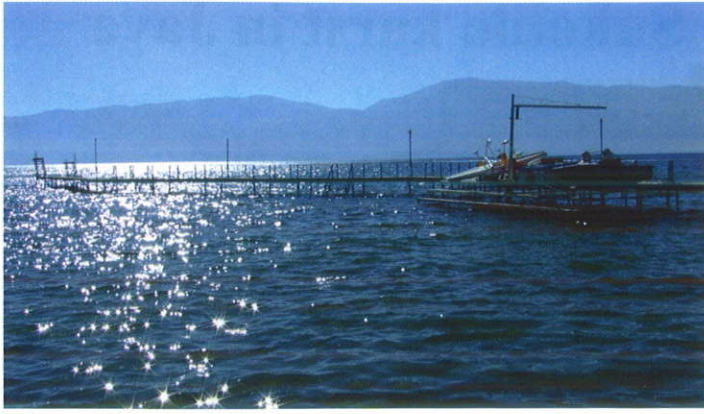


Figure 17. Lake Ohrid, the final station of our journey.

"Resurrection of the mummies" – extracting DNA from 110 years old samples

Our previous material, together with the material collected during this field trip, enabled an almost comprehensive molecular phylogenetic reconstruction, making the absence of *M. apfelbecki* especially disappointing. In utter desperation we tried another way, attempting to extract DNA from the almost 110 year-old syntype specimens kept in the Natural History Museum in Vienna.

Whether and how DNA is preserved in museum-stored materials depends on how these animals were dispatched and preserved. Fortunately, Buljubasić collected a large sample, including several living specimens. In some of the shells we found desiccated bodies coiling around the columella. This made us believe that the samples were well-ventilated after collecting and that the animals had slowly mummified after an aestivation period. This gave us hope that we would be able to extract usable DNA after 110 years.

We applied the method of Thomsen et al. (2009) optimized to gastropods by Páll-Gergely et al. (2015). DNA yield and quality was tested by agarose gel electrophoresis, indicating that we got a large amount, but of highly fragmented, DNA. After some unsuccessful attempts to amplify other standard DNA markers from the extracts, we finally got positive result with a short fragment of the 16S rRNA gene. This sequence helped us to approximate the position of this species in the phylogenetic tree, and with the DNA knowledge of the presumed relatives, we designed internal primers for the widest used barcoding marker, the subunit I of the mitochondrial cytochrome c oxidase (*COI*) gene. Internal primers were designed based on sequences of related taxa, in the way that three overlapping sections of 230–250 base pairs were defined by three primer pairs. All three of these sections were successfully amplified and sequenced, and then, like the pieces of a puzzle, they were fit together. *COI* showed (reinforcing what the short 16S fragment indicated) that the closest relatives of *M. apfelbecki* are far in the south, in Greece, and *M. apfelbecki*

is not conspecific with any of the previously presumed "*M. apfelbecki*" subspecies in Northern Albania.

Although a lot of knowledge has been gained recently, there is still information missing. For example, it is not known where the species lives within the Shënt Mts., nor if it still exists. Another conundrum is how it got there in the first place.

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- Photos: Dorottya Angyal, Zoltán Fehér, Jozef Grego, & Maroš Grego**