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Characterization of an allotriploid strawberry *Fragaria* × *bifera* Duchesne (Rosaceae) from Europe

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Abstract. Allopolyploidy has played an important role in the plant evolution. To assess its role in speciation, it is necessary to examine fertility and crossability of hybrids. A hybrid clone of the genus *Fragaria* with different and complex morphology compared to *F. vesca*, *F. viridis* and *F. moschata*, was detected in Germany (in Bayreuth, Bavaria). The genome size of these plants was measured using flow cytometry and their fertility was tested in experimental crossing. The parental origin of the hybrid was revealed using RAPD approach. From the mean intensity of fluorescence emitted by PI-stained nuclei for *F. moschata*, *F. vesca*, *F. viridis* and the hybrid, triploidy of the hybrid could be indicated. The hybrid shared an 1800bp and 880bp long species-specific RAPDs bands with *F. viridis* and *F. vesca*, respectively, indicating them as the parental species of the hybrid. The hybrid did not produce any fruit in selfing, open pollination and when crossed by pollen of *F. vesca* and *F. viridis*, all showing female sterility of the hybrid. The hybrid had 78% pollen sterility, however, pollinating *F. vesca* by pollen of the hybrid produced viable seed and F_1 plants, indicating its male fertility. This work shows allopolyploidy role in the evolution and speciation of *Fragaria*, and may suggest the study site as potential new centre of *Fragaria* speciation.

Key words: allopolyploidy, allotripolyploid strawberry, *Fragaria*, *Fragaria* × *bifera*, strawberry genome content

Izvleček. Karakterizacija alotriploidne jagode *Fragaria* × *bifera* Duchesne (Rosaceae) iz Evrope – Alopoliploidija ima pomembno vlogo v evoluciji rastlin. Kljub temu je za potrditev njene vloge pri speciaciji potrebno poznati plodnost in možnost križanja pri hibridih. V Nemčiji (Bayreuth, Bavarska) najden hibridni klon rodu *Fragaria* izkazuje drugačno in kompleksnejšo morfologijo kot vrste *F. vesca*, *F. viridis* in *F. moschata,* zato smo v analizi njegovega izvora uporabili molekulske pristope. Staršev izvor hibridov smo tako ugotavljali z metodo RAPD. Velikost genoma teh rastlin smo merili s pretočno citometrijo, plodnost hibridov pa smo testirali z eksperimentalnim križanjem. Povprečna intenziteta fluorescence *F. moschata*, *F. vesca*, *F. viridis* in hibrida, merjena s pretočno citometrijo, nakazuje triploidijo hibrida. Delitev 1800 in 880 bp dolgih vrstno specifičnih RAPD pasov z *F. viridis* in *F. vesca* pa nakazuje, da sta ti dve vrsti starševski hibridu. Hibrid ni proizvajal plodov pri samooploditvi, odprti oploditvi ali če je bil križan s pelodom *F. vesca* in *F. viridis*, kar nakazuje sterilnost ženskih hibridov. Hibridi so imeli 78 0% sterilnost peloda, opraševanje *F. vesca* s pelodom hibrida pa je rezultiralo v viabilnih semenih in F_1 rastlinah, kar nakazuje na moško plodnost. Delo kaže na pomen alopoliploidije v evoluciji in speciaciji rodu *Fragaria* ter nakazuje možnost, da je lokacija študije nov center speciacije za rod *Fragaria.*

Ključne besede: alopoliploidija, alotripoliploidna jagoda, *Fragaria*, *Fragaria* × *bifera*, genom jagode

Introduction

Polyploidy has played an important role in the evolution of the flowering plants through adaptation and speciation (Grant 1981). Based on different estimations, 47% to 70% of flowering plants have polyploid origin (Grant 1981). Polyploidy can arise directly within single species (autopolyploidy) or through interspecific hybridisation involving duplication of chromosomes (allopolyploidy). Allopolyploidy is thought to be a predominant mode of speciation in flowering plants (Soltis & Soltis 1993, Rieseberg 1997). The formation and consequently fertilization of unreduced gametes during micro- and megasporogenesis is one of the main mechanisms creating polyploidy (Harlan & de Wet 1975, Bretagnolle & Thompson 1995).

The genus *Fragaria* comprises about 22 species (Staudt 2009), and includes different ploidy levels of diploids, tetraploids, hexaploid, octaploids (Staudt 1989) and decaploid along with several intermediate ploidy levels of interspecific hybrids (Bringhurst & Gill 1970, Staudt et al. 2003, Hummer et al. 2009, Lundberg 2011, Nosrati et al. 2011a, 2013). Three *Fragaria* species, i.e. the diploids *F. vesca* L., *F. viridis* Weston and the hexaploid *F. moschata* Weston, are distributed in Europe (Staudt 1962, Tutin et al. 1968) along with several interspecific hybrids originating between them, including tetraploids *F.* × *intermedia* (Bach) Beck (between *F. moschata* and *F. vesca*) and *F.* × *neglecta* Lindem (between *F. moschata* and *F. viridis*) and diploid *F.* × *hagenbachiana* K. H. Lang ex W. D. J. Koch (between *F. viridis* and *F. vesca*), triploid *F.* × *bifera* Duchesne between *F. vesca* and *F. viridis* (Staudt et al. 2003), pentaploid (Nosrati et al. 2011a) and heptaploid (Nosrati et al. 2013) hybrids both between *F. vesca* and *F. moschata*. Moreover, interspecific tetra-, penta- and octoploid hybrids have also been artificially produced between European species (Lippert 1985).

Morphological recognition among species in the genus *Fragaria* is very difficult even at interploidy levels because of high morphological variations and similarities (Ichijima 1930). This difficulty is even higher in interspecific hybrids, especially hybrids between *F. vesca* and *F. viridis* due to close genetic relationship and higher morphological similarities (Potter et al. 2000).

To assess the likelihood that a new interspecific polyploid hybrid will be successfully established, it is necessary to have information on the viability and fertility of the hybrids (Ramsey & Schemske 1998). In almost all cases, the fertility and crossability of the interspecific hybrids reported so frequently in literature and papers have not been documented.

This work is aimed at documenting a putative interspecific triploid hybrid *Fragaria* from Europe on the basis of fertility, crossability, genome size, and DNA fingerprinting.

Materials and methods

Plant material

A clone of *Fragaria* with morphology different from the three European species *F. vesca*, *F. viridis* and *F. moschata* was detected in Germany, Bavaria (around Bayreuth, between Destuben and Rodensdorf; latitude: 49° 54' 10" N; longitude: 11° 34' 16" E), where all three species grow together. The preliminary investigations introduced it as a potential putative interspecific hybrid plant. This putative hybrid *Fragaria* had complex morphology so that its definite recognition was not possible on the basis of morphological characters. For example, runners in *F. viridis* are always monopodial, short and filiform, while in *F. vesca* they are sympodial and long (Tutin et al. 1968). The putative allotriploid hybrid *Fragaria* had runners of both types. The putative hybrid along with some samples of all the species growing in the site of the hybrid were transferred to the glasshouse at the University of Aberdeen, UK, for further analyses of total genome size to reveal the ploidy level using flow cytometry, fertility levels for understanding the extent of reproductive isolation between putative hybrid and its parental species using crossing experiments, and recognition of parental origin using RAPDs.

Genomic DNA measurement

The total amount of nuclear DNA of the putative hybrid along with several samples of *F. vesca, F. viridis* and *F. moschata* as putative parental species was measured using flow cytometry. We used the well-known cultivated octoploid strawberry *F.* × *ananassa* cv. Vivorosa as internal DNA reference standard. The chicken erythrocytes were used to test the linearity of the system. Approximately 100mg young leaf tissue was chopped with sharp scalpel in 1ml of ice-cold nuclear isolation buffer, LB01 (Dolezel et al. 1992). This buffer consisted of 15 mM Tris, 2 mM Na_2EDTA, 80 mM KCl, 20 mM NaCl, 0.5 mM spermine, 15 mM b-mercaptoethanol, 1 ml/l Triton X-100 with the modification of adding PVP-40 at the proportion of 10 g/l (Yokoya et al. 2000) as (1%) PVP-40 in the chopping solution which has been shown to increase the number of intact nuclei isolated in flow cytometric studies of Rosoideae (Dickson et al. 1992). Final pH was adjusted to 7.5. A volume of 0.5 ml lysate was recovered, after filtering through nylon gauze (pore size 50 µm). Ribonuclease A (2.9 µl of a 34 mg/l solution) and propidium iodide (PI) (10 µl of a 20 mg/l solution) were added and the lysate incubated in the dark for 1–1.5 h on ice. The samples were filtered through nylon gauze (pore size 50 or 20 µm) just before measuring, and fluorescence intensity was measured with a Becton Dickinson FACSCalibur Benchtop Cytometry Analyser. Domestic chicken lymphocytes were used as an internal DNA reference standard of known genome size following Galbraith et al. (1983). The PI-stained nuclei were excited by a 488 nm laser, and mean fluorescence intensity (MFI) of fluorescence emitted by nuclei was recorded. The total amount of nuclear DNA was assessed relative to that of cultivated *F.* × *ananassa* cv. Vivorosa (2n = 8x = 56) setting the MFI peak at 800.

Fertility test and crossing experiments

The pollen sterility of putative hybrid and parental species were tested on the basis of percentage of unstained pollen with 0.05% cotton blue in lactophenol by examining a minimum of 520 pollen grains. The fertility of putative hybrid along with putative parental species was measured on the basis of the fruit set under open pollination in the glasshouse over a period of 2.5 years. In addition, male and female fertility of the putative hybrid were investigated in artificial reciprocal experimental crosses between the putative hybrid and the three *Fragaria* species growing in the hybrid site. For hand-crossing experiments, floral buds on seed parents were emasculated approximately 3—4 days before anthesis by removing the indehiscent anthers with a sharp scalpel. These anthers-emasculated flowers were inspected using a *x*10 hand lens to ascertain that they were un-dehisced. The anther-emasculated flowers were gently washed with a little water and, after air-dried, pollinated by directly rubbing the anthers from the pollen parent onto the exposed stigmas. The hand-pollinated flowers were immediately covered by the double-layer of fibre fleece in order to prevent uncontrolled open-pollination and desiccation. Hand pollination was repeated a second time after 1—3 days to ensure the presence of viable pollen on stigma at the time of stigmatic receptivity.

Artificial hand-crossing was also carried out between putative parental species in order to measure the fertility levels and genome size among originated F_1 hybrids. Consequently, the fertility and genome size of the artificial hybrids were compared with those of the natural putative hybrid under study.

RAPDs analysis for parentage identification

First, species-specific RAPD markers were established for the three *Fragaria* species growing in the site of the putative hybrid using some 10 arbitrary RAPD primers. Consequently, the occurrence of the species-specific RAPD markers was investigated in the putative hybrid (Tab. 1). The RAPDs patterns in each case were repeated to ensure the reproducibility of the PCR profiles. The documentation of the hybrid and its parental origin was based on the presence of species-specific RAPDs markers of two different species in the putative hybrid.

Results

Genomic DNA content

The genomic DNA content of the putative hybrid and the three species of *F. vesca*, *F. viridis* and *F. moschata* growing in the site of the hybrid, along with the octoploid *F.* × *ananassa* cv. Vivorosa used as a control are shown in Fig. 1 and Tab. 1. In the flow cytometric assessment, when the MFI for the octoploid *F.* × *ananassa* cv. Vivorosa set at 800, this value, on average, for hexaploid *F. moschata*, diploids *F. vesca* and *F. viridis* was 613.64, 228.5 and 232.4, respectively, while the MFI value for the putative hybrid was found to be 309.9.

Table 1. Flow-cytometric mean fluorescence intensity (MFI) of genomic DNA content indicating the ploidy level in putative allotriploid hybrid *Fragaria* and samples of the three putative parental species from the study site.
Tabela 1. Povprečna intenziteta fluorescence (MFI) pri pretočni citometriji vsebine genomske DNA, ki nakazuje na stopnjo plidnosti v verjetnem alotriploidnem hibridu rodu *Fragaria* in za vzorce treh domnevnih starševskih vrst z istega območja raziskave ter kultiviranega oktoploida *F.* × *ananassa*.

Taxon	Mean MFI (CI*)
F. moschata Duchesne	613.64 (4.71)
F. viridis Watson	228.50 (8.08)
F. vesca L.	232.40 (8.00)
putative hybrid	309.90 (7.45)
F. × ananassa cv. Vivorosa	800

*CI (95%): confidence interval

RAPDs patterns

Out of the ten RAPD primers applied for establishing species-specific markers for the three species growing in the hybrid site, only four primers produced unique bands for the species. The primer D produced one unique band specific to *F. viridis*, and one unique band specific to *F. vesca*. In addition, the primer A produced one unique band specific to *F. vesca*. Two species-specific bands were detected in the RAPD patterns of the putative triploid hybrid, of which one band specific to *F. viridis* with band size of 1800bp and one band specific to *F. vesca* with band size 880 bp (Fig. 2).

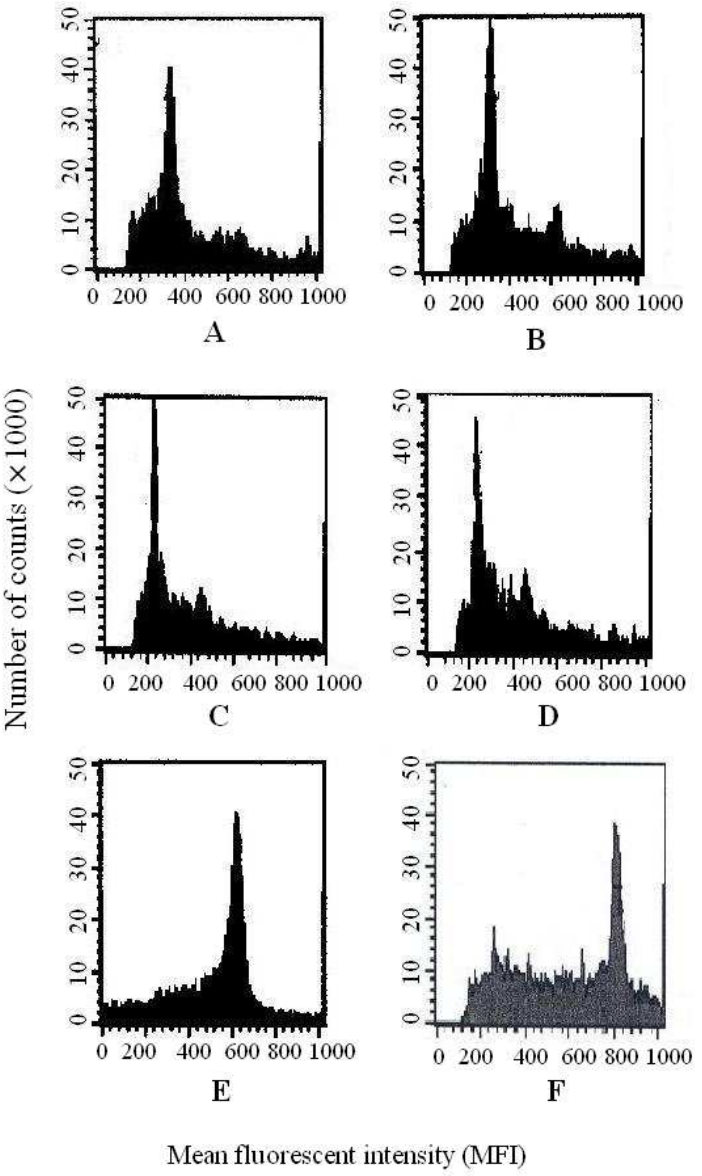

Figure 1. Samples of flow cytometry histograms showing genomic DNA content in the putative hybrid *Fragaria* (A, B) having originated from between *F. vesca* (C) and *F. viridis* (D) in Bayreuth, Bavaria, Germany along with hexaploid *F. moschata* (E) and the cultivated octoploid *F. × ananassa*.

Slika 1. Primeri historamov pretočne citometrije, ki kažejo vsebino genomske DNA v domnevnem hibridu *Fragaria* (A, B), ki izvira iz križanja med *F. vesca* (C) in *F. viridis* (D) v kraju Bayreuth, Bavarska, Nemčija, skupaj s heksaploidnim *F. moschata* (E) in kultiviranim oktoploidom *F. × ananassa* (F).

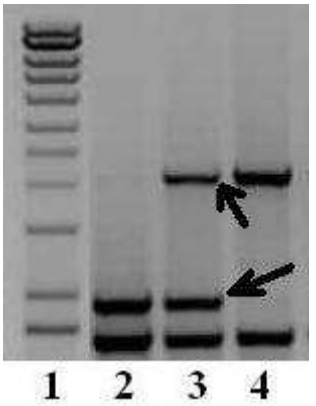

Figure 2. RAPDs pattern of the allotriploid hybrid (lane 3) and its putative parental species, i.e. *F. vesca* (lane 2) and *F. viridis* (lane 4), using primer D (sequence: 5'GTCCTTAGCG3'). The hybrid shared a band of 880 bp size (lower arrow) with *F. vesca* and a band of 1800 bp size (upper arrow) with *F. viridis* (The lane codes: 1 = standard size markers, 2 = *F. vesca*, 3 = the allotriploid hybrid, 4 = *F. viridis*).

Slika 2. Vzorec RAPD alotriploidnega hibrida (tretja kolona) in njegovih domnevnih starševskih vrst, t. j. *F. vesca* (stolpec 2) in *F. viridis* (stolpec 4), z uporabo primerja D (sekvenca: 5'GTCCTTAGCG3'). Hibrid si je delil pas 880 baznih parov (spodnja puščica) s *F. vesca* in pas 1800 baznih parov (zgornja puščica) s *F. viridis*. Stolpec 1 so standarizirane velikosti markerjev.

Fertility and crossing experiments

The putative hybrid had at least 78% pollen sterility, while the values for putative parental species of *F. vesca* and *F. viridis* were 6.8% and 23%, respectively. The pollen sterility for hybrids artificially obtained from crossing *F. vesca* with pollen of *F. viridis* was on average 34% (Tab. 2). These artificial interspecific hybrids were found to be diploid using flow cytometry.

Table 2. Pollen sterility in putative allotriploid hybrid and putative parental species.
Tabela 2. Sterilnost peloda pri domnevnem alotriploidnem hibridu in pri domnevnih starševskih vrstah.

Species and hybrids	No. of pollen examined	% pollen sterility	C.I. (95%)
F. vesca	703	6.8	1.9
F. viridis	521	23	3.6
Artificial hybrids*	843	34	3.2
Putative allotriploid	688	78	3.0

* Artificial hybrids were made between *F. vesca* and *F. viridis*.

In crossing experiments, crossing the putative hybrid by pollen of both *F. vesca* and *F. viridis* did not result in any fruit (cross types 1 and 2 in Tab. 3). In addition, hand-selfing of 24 flowers of the allotriploid hybrid also did not set any fruit, and similarly, 172 flowers of the hybrid plant did not produce any berry in open pollination at the glasshouse in a period of over 2.5 years (Tab. 3). However, crossing 7 flowers of *F. vesca* by pollen of the hybrid produced 2 berries (cross type 3 in Tab. 3). These two berries producing the total number of 21 seeds (achenes) had 15% and 17% levels of seed set. Only 9 out of 21 seeds germinated and produced seedlings, of which 5 matured in the glasshouse.

Table 3. Artificial crosses made between the putative hybrids and the putative parental species from the site of the hybrid, as well as among putative parents.
Tabela 3. Rezultati umetnih križanj med domnevnimi hibridi in domnevnimi starševskimi vrstamu z najdišča hibrida, kot tudi med domnevnimi starševskimi vrstami.

Cross type	Crosses (seed parent × pollen parent)	No. of crosses	No. of berries	% fruit set	% seed set	No. of achenes		No. of mature plants
						produced	germinated	
1	putative hybrid × *F. vesca*	5	0	-	-			
2	putative hybrid × *F. viridis*	1	0	-	-			
3	*F. vesca* × Putative hybrid	7	2	29	22 & 23	21	9	5
4	selfing of Putative hybrid	24	0	-	-			
5	putative hybrid in Open pollination	172	0	-	-			
6	*F. vesca* × *F. viridis*	12	9	75				
7	*F. viridis* × *F. vesca*	4	0	-	-			

Discussion

In this study, the ploidy level, fertility and parental origin of an allotriploid hybrid strawberry between diploids *F. vesca* and *F. viridis* was documented in Bayreuth, Bavaria (Germany) based on flow cytometric measurement of the genome size, the artificial crossing experiments, monitoring the outcome of open pollination during 2.5 years at the glasshouse, and detection of parental origin using RAPD markers. The triploidy of the hybrid indicates that an unreduced gamete was most likely involved in the formation of this allotriploid hybrid through sexual reproduction.

The artificial reciprocal crosses made in this work between putative parental species, i.e. *F. vesca* and *F. viridis*, were almost only successful in one direction, when *F. vesca* was used as seed parent. This is consistent with our previous work based on tremendous mutual crosses between these diploids, which showed that interspecific crosses between *F. vesca* and *F. viridis* were successful almost always when *F. vesca* was used as seed parent (Nosrati et al. 2011b). Therefore, it can be concluded that in the formation of allotriploid hybrid characterized in the current study between *F. vesca* and *F. viridis*, the latter species played as pollen donor parent.

The interspecific allotriploid hybrid reported in the current work was female sterile, as crossing it by viable pollen of *F. vesca* and *F. viridis* was unsuccessful. Moreover, the hybrid did not set any fruit in many hand-selfing crosses neither under open pollination over 2.5 years at the glasshouse. However, backcrossing *F. vesca* by pollen of the allotriploid hybrid produced fruit with viable seeds and offspring.

As we previously showed that hybrids originating from artificial hand-crossing experiments between *F. vesca* and *F. viridis* were almost always homoploid hybrids, i.e. diploids (Nosrati et al. 2011b), it can be concluded that naturally occurring allotriploid hybrid between these two species is, in fact, very rare.

The putative allotriploid hybrid reported in the current work had very high pollen sterility (78%), while this value for the artificial diploid interspecific hybrid obtained in the current work between *F. vesca* and *F. viridis* was low (34%). However, using the same technique and the other accessions of *F. vesca* and *F. viridis*, the level of pollen sterility among F_1 interspecific hybrids originated between these species was on average 65% with standard deviation of 1.8 (Nosrati et al. 2011b). This indicates that allopolyploids and homoploid hybrids may have the same level of male sterility.

The two natural hybrids, i.e. diploid *F.* × *hagenbachiana* K. H. Lang ex W. D. J. Koch and triploid *F.* × *bifera* Duchesne (Staudt et al. 2003), have already been reported between *F. vesca* and *F. viridis*, however, the levels of sterility in the hybrids and the occurrence and levels of reproductive isolation between hybrids and their parental species were not investigated. Therefore, the possibility that the previously reported hybridization between *F. vesca* and *F. viridis* will result in speciation, cannot be interpreted.

In the evolution and speciation of *Fragaria*, allopolyploidy has already played a vital role in different events, such as the formation of hexaploid *F. moschata* via two allopolyploid occasions among three diploids *F. vesca*, *F. viridis*, and *F. iinumae* Makino, and in the formation of octoploid *Fragaria* via allopolyploidy between *F. moschata* and *F. iinumae* (Lundberg 2011). Similarly, we have previously reported a natural allopentaploid and alloheptaploid hybrids both between *F. moschata* and *F. vesca* from this site (Nosrati et al. 2011a, 2013).

Hybrid formation involving contribution of functioning unreduced gametes have been frequently reported in the genus *Fragaria* from both wild (Bringhurst & Senanayake 1966, Bringhurst & Gill 1970, Staudt et al. 2003) and artificial crossing experiments (Fedorova 1934, Scott 1951, Ellis 1962, Staudt 1962). They indicated the vital role of the functioning unreduced gametes in the evolution of polyploidy in *Fragaria*. The frequency of functioning unreduced pollen in flowering plants was estimated to be 0.05% (Ramsey & Schemske 1998). A higher levels of polyploid F_1 progeny originating from artificial intraploid crosses have been reported in the flowering plants, e.g. onion (Jones & Clarke 1942), orchids (Storey 1956) and cassava (Hahn et al.1990). These may suggest that unreduced gametes may play an important role in interspecific hybridization, and consequently allopolyploidy evolution.

The results of the current study confirming our previous reports (Nosrati et al. 2011a, 2013) show that allopolyploidy still plays an important role in the evolution and speciation of the genus *Fragaria*, and that the study site could act as a new centre for speciation of *Fragaria*.

Povzetek

Ker alopoliploidija igra pomembno vlogo v evoluciji in speciaciji rastlin, je potrebno raziskati tudi plodnost in možnost križanja hibridov. Klon rodu *Fragaria* z drugačno morfologijo je bil najden v Nemčiji (Bayreuth, Bavarska), na rastišču, kjer sicer uspevajo tri evropske jagode, t.j. *F. vesca*, *F. viridis* in *F. moschata.* Analiza RAPD je pokazala, da je nova jagoda hibrid in da izvira kot vmesna oblika med *F. vesca* in *F. viridis*, saj z obema evropskima diploidoma deli vrstno specifične lokuse. Meritve velikosti genoma hibrida z uporabo pretočne citometrije je pokazala triploidno garnituro kromosomov hibrida. Kot kaže ima hibrid sterilne ženske rastline, saj ni sposoben proizvajanja plodov pri prostem opraševanju, umetno samooploditvijo ali pri križanju s pelodom predvidenih starševskih vrst. Moške rastline so plodne, saj je oprašitev *F. vesca* s pelodom hibrida rezultirala v viabilnih semenih in F_1 rastlinah. Študija potrjuje pomen alopoliploidije v evoluciji in speciaciji rodu *Fragaria*, rastišče hibrida pa bi bilo lahko nov center speciacije rodu *Fragaria*.

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First records of two *Cuthona* species (Gastropoda: Nudibranchia) in the Adriatic Sea

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Abstract. Authors are reporting on the first records of two nudibranch species of the genus *Cuthona* in the Slovenian part of the Adriatic Sea. In the period from September 2014 to March 2015, specimens of *Cuthona genovae* and *C. miniostriata* were found at different localities in the mediolittoral and upper infralittoral belts. The findings of both *Cuthona* species represent the first records in the Adriatic Sea.

Key words: first records, *Cuthona genovae, Cuthona miniostriata,* Gastropoda, Nudibranchia, Adriatic Sea

Izvleček. Prvi zapis o pojavljanju dveh vrst iz rodu *Cuthona* (Gastropoda: Nudibranchia) v Jadranskem morju – Avtorji poročajo o prvi najdbi dveh vrst polžev gološkrgarjev iz rodu *Cuthona* v slovenskem delu Jadranskega morja. V obdobju od septembra 2014 do marca 2015 so bili v mediolitoralu in zgornjem infralitoralu na različnih lokalitetah najdeni primerki vrst *Cuthona genovae* in *C. miniostriata*. To je tudi prvi zapis o pojavljanju obeh vrst iz rodu *Cuthona* v Jadranskem morju.

Ključne besede: prvi zapisi, *Cuthona genovae, Cuthona miniostriata,*Gastropoda, Nudibranchia, Jadransko morje

Introduction

The marine opisthobranch fauna of Slovenia gained increased scientific attention only during the last few decades when first checklists of this particular group were published by De Min & Vio (1997). The first checklist of opisthobranchs in the area was presented by Turk (2000). This list was further complemented by later works of Turk (2005a, b), Lipej et al. (2008, 2012), Mavrič & Lipej (2012), Lipej et al. (2014) and Zenetos et al. (2015a). Certain species, such as *Cumanotus beaumonti* (Turk 2005a, b) and *Piseinothecus sphaerifera* (Mavrič & Lipej 2012), had previously been found only in very few cases in the Mediterranean and other parts of world oceans.

This paper deals with two nudibranch species (Gastropoda: Heterobranchia) found in the coastal waters of Slovenia. They represent new records for the entire Adriatic Sea in general.

Material and methods

The nudibranchs of the genus *Cuthona* were found while inspecting the samples of low vegetation belt (known as turf) in the mediolittoral and infralittoral belts from different localities along the Slovenian coastline (Gulf of Trieste, northern Adriatic) (Fig. 1). The specimens were measured alive and photographed under the stereomicroscope Olympus SZX16. Afterwards, the nudibranchs were identified with the help of the determination keys for opisthobranchs (Pruvot-Fol 1954, Barletta 1980, Schmekel & Portmann 1982, Bielecki 2011, Trainito & Doneddu 2014). Specialized web sites such as www.seaslugforum.net were helpful as well. The taxonomy and nomenclature are in accordance with the World Register of Marine Species (WoRMS; www.marinespecies.org/). Subsequently, the specimens were fixed in 70% alcohol solution and deposited in the collection of the Marine Biology Station (MBS) of the National Institute of Biology.

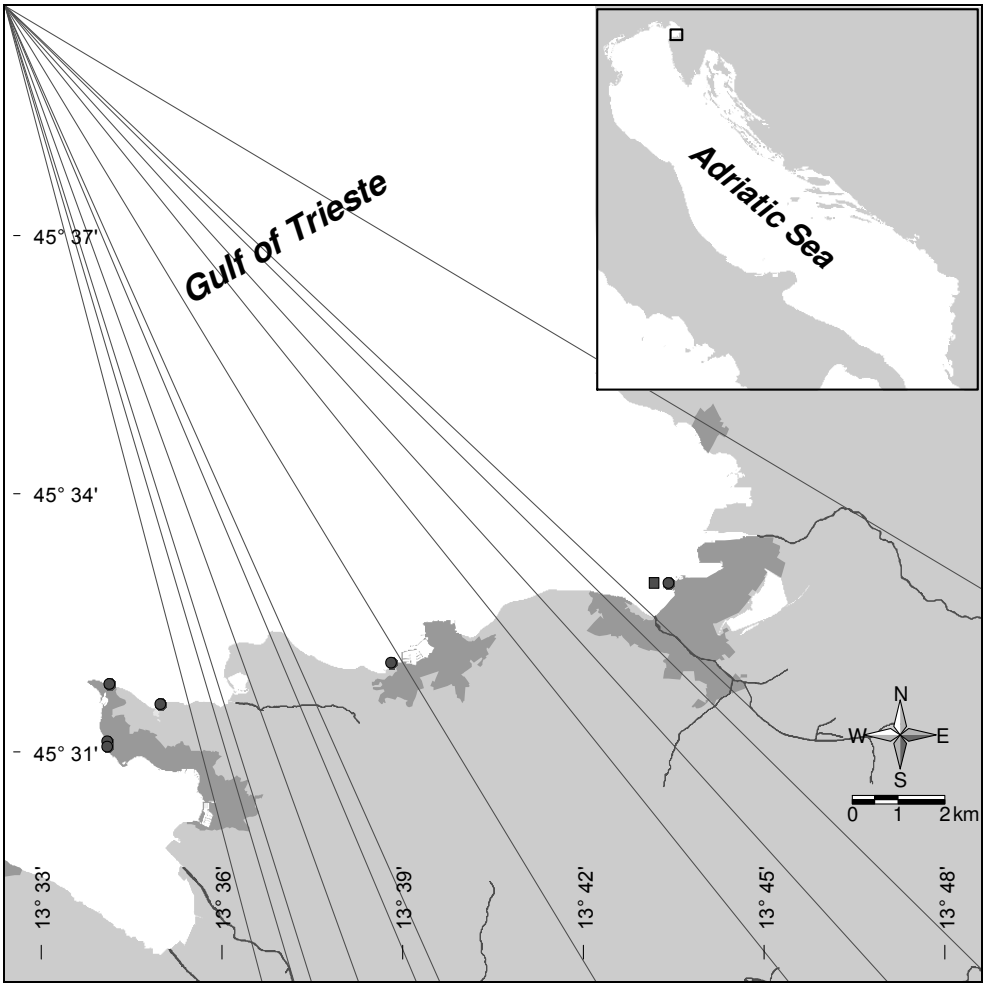

Figure 1. Study area with localities where nudibranchs *Cuthona genovae* (black circles) and *C. miniostriata* (black square) were recorded for the first time in the Adriatic Sea.

Slika 1. Zemljevid obravnavanega območja z lokalitetami, na katerih so bili prvič v Jadranskem morju najdeni gološkrgarji *Cuthona genovae* (črni krogci) in *C. miniostriata* (črni kvadrat).

Results

***Cuthona genovae* (O'Donoghue, 1928)**

Material:

- 24. 9. 2014, 2 specimens, Fiesa, 1 m depth, on *Cystoseira barbata*;
- 5. 10. 2014, 2 specimens, Atlantida – Izola, 1 m depth, on turf;
- 19. 1. 2015, 2 specimens, under Piran church, Piran, 1 m depth, algal belt;
- 3. 2. 2015, 1 specimen, Port of Koper, Koper, 1 m depth, algal belt;
- 19. 2. 2015, 1 specimen, Morgan, Piran, 1 m depth, turf on rocks;
- 3. 3. 2015, 2 specimens at 2 m depth and 7 specimens at 3 m depth, in front of the Marine Biology Station of the National Institute of Biology, Piran.

The specimens were recognized by parallel orange lines running from oral tentacles to rhinophores and to the base of the first cerata, forming a rhomboid shape on the head. Additional orange line is evident between the oral tentacles. Another characteristic is a yellow band running from rhinophores back to the heart (Schmekel & Portmann 1982, Rudman 2008, Trainito & Doneddu 2014). The body is more or less transparent, whereas up to seven groups of cerata are brownish.

Up to date, the species has been recorded in the entire Mediterranean Sea and British waters (Picton & Morrow 2010), Portugal (Calado et al. 1999), in waters off the Canarian Archipelago and in the Caribbean (Ballesteros et al. 2012-2015). In the Mediterranean Sea, it has been recorded in Genoa (locus typicus), more or less along the whole Mediterranean coast of Spain and along the Balearic Islands (Cervera et al. 2004) and in the waters off Malta (Sammut & Perrone 1998, Sammut 2011-2014a).

***Cuthona miniostriata* Schmeckel, 1968**

Material:

- 3. 2. 2015, 1 specimen, Port of Koper, Koper, 1 m depth, algal belt.

The main distinguishing character of this species is the orange line on the backside of rhinophores, which extends to the eyes. Rhinophores are smooth and somehow longer than oral tentacles. The body is whitish and translucent, whereas the 5 or 6 groups of thin cerata are brownish at the base and white on the top. The distal part of the cerata is rounded. According to Sammut & Perrone (1998), some specimens have a large amount of white pigment, especially on the cerata.

This species was found within a turf microhabitat in the mediolittoral belt (< 1 m depth). Samut & Perrone (1998) mentioned this species as fairly common on algae in shallow waters. Up to date, the *C. miniostriata* has been considered an endemic species in the Mediterranean Sea. It has been recorded in waters off the eastern Spanish coast (Cervera et al. 2004), Maltese Islands (Sammut & Perrone 1998, Sammut 2011-2014b) and in the Gulf of Naples (Schmekel & Portmann 1982), where it was described from.

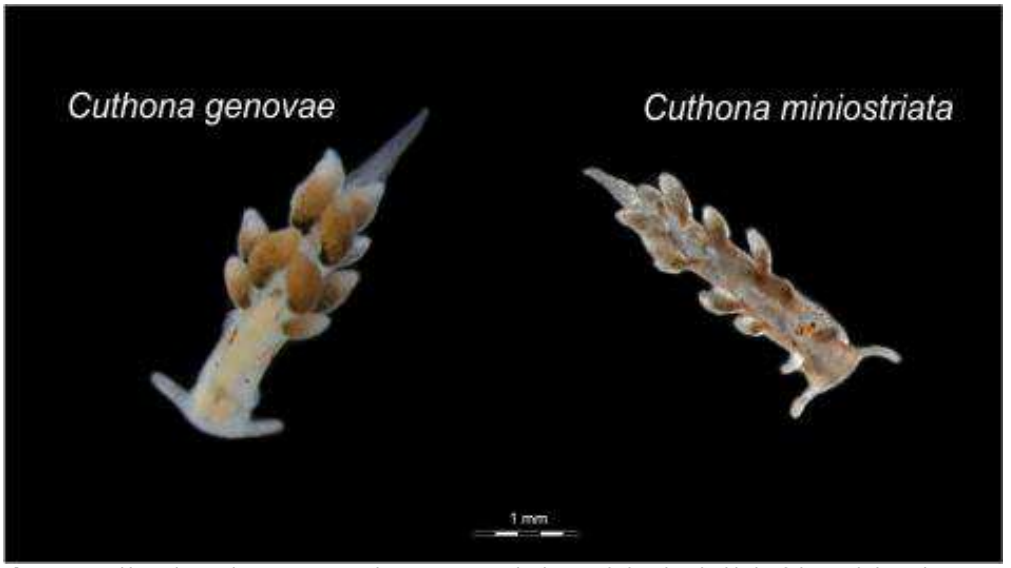

Figure 2. Nudibranchs *Cuthona genovae* and *C. miniostriata,* both recorded in the algal belt of the mediolittoral zone on 3. 2. 2015 in the Port of Koper (photo: B. Mavrič).
Slika 2. Polža gološkrgarja *Cuthona genovae* in *C. miniostriata,* najdena v algalni zarasti bibavičnega pasu v koprskem pristanišču 3. 2. 2015 (foto: B. Mavrič).

Discussion

Both species of the genus *Cuthona* were found in mediolittoral and infralittoral zones on turf or algal belts. Their small size (both species ± 5 mm in length) and peculiar habitat type in which they are living are probably the main reasons why these species have been overlooked in many Mediterranean areas (*sensu* Sammut 2011-2014a, b). So far, 5 species of the genus *Cuthona* have been recorded in the Adriatic Sea: *C. caerulea*, *C. foliata*, *C. ocellata*, *C. gymnota* and *C. perca* (sensu Zenetos et al. 2015b).

Although the coastal sea of Slovenia constitutes only a very small portion of the Adriatic Sea, at least 75 opisthobranch species (nudibranchs and other seaslug groups) have been recorded in this area (see Lipej et al. 2014). However, the checklist of opisthobranch fauna should be considered far from complete, since many species found in the Italian part of the Gulf of Trieste have not yet been confirmed in the Slovenian part of the Gulf (see Graeffe 1902, Zenetos et al. 2015b). In addition, certain habitat and microhabitat types were poorly studied. Interstitial habitats have not been studied at all, while muddy areas are probably hiding many not yet recorded fossorial opisthobranchs. Among the poorly studied habitats is also the low vegetation in shallow coastal areas (known as turf).

During sampling in specific habitat and microhabitat types in different seasons, certain rare or less known opisthobranchs were detected for the very first time in Slovenian coastal waters and adjacent areas along the west Istrian coasts. For instance, recent findings of *Aplysiopsis elegans* (Mavrič et al. 2014) and *Thordisa filix* (Zenetos et al. 2015a) represent the first Adriatic records for both species. Taking into consideration such records of small and thus easily overlooked opisthobranch species, it seems possible that the list of seaslugs will probably be enlarged even more in the near future.

Povzetek

Avtorji poročajo o prvi najdbi dveh vrst polžev gološkrgarjev iz rodu *Cuthona* v slovenskem delu Jadranskega morja. Vrste iz tega rodu so navadno majhne, zato jih je v njihovem okolju težko opaziti. V obdobju od septembra 2014 do marca 2015 so bili v različnih predelih slovenskega dela Jadrana najdeni primerki vrst *Cuthona genovae* in *C. miniostriata*. Prva vrsta je bila najdena na šestih različnih lokalitetah (Bernardin, Piran, med Piranom in Fieso, Fiesa, Izola, pristanišče Koper) v mediolitoralu (bibavičnem pasu) in infralitoralu do 3 m globine. Vsi primerki te vrste so bili najdeni v blazinasti algalni obrasti (turf) in na manjših algah. Druga vrsta, *C. miniostriata*, je bila najdena le v enem primeru februarja 2015 v koprskem pristanišču, in sicer v algalni zarasti bibavičnega pasu (±1 m globine).

Pričujoči prispevek je prvi zapis o pojavljanju obeh vrst iz rodu *Cuthona* v Jadranskem morju. Tudi sicer ni veliko znanih zapisov o pojavljanju teh vrst v Sredozemskem morju, prav vsi pa izvirajo iz njegovega zahodnega dela. Vrsta *C. genovae* se poleg Sredozemskega morja pojavlja še v severnem delu vzhodnega Atlantika in v Karibskem morju, vrsta *C. miniostriata* pa je endemit Sredozemskega morja.

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Results of the pioneer survey of potential bat hibernacula in Albania (2012–2015)

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Abstract. For the first time at a national scale in Albania, a winter bat population census in potential hibernacula has been implemented during the four winters (early 2012, 2012/2013, 2013/2014 and 2014/15). 178 potential hibernation sites have been visited. During the visits of natural caves, bunkers, tunnels, buildings and mines we recorded at least 9 bat species: *Rhinolophus ferrumequinum* (28 sites), *R. hipposideros* (36 sites), *R. blasii* (1 site), *R. euryale* (3 sites), *Myotis myotis/oxygnatus (blythii)* (4 sites), *M. capaccinii* (6 sites), *Pipistrellus* sp. (2 sites), *Hypsugo savii* (1 site) and *Miniopterus schreibersii* (9 sites). The data presented are substantial additions to knowledge on the distribution of these species and their roosts in Albania, and will form a basis for bat population monitoring and, at the same time, for improving conservation measures in Albania and the wider region.

Key words: Albania, bats, Chiroptera, hibernacula, monitoring, survey

Izvleček. Rezultati prvih popisov možnih prezimovališč netopirjev v Albaniji (2012-2015) – V štirih zimah (začetek 2012, 2012/2013, 2013/2014 in 014/15) je bil v Albaniji prvič na državnem nivoju opravljen popis prezimujočih netopirjev. Pregledanih je bilo 178 možnih prezimovališč. Med obiski naravnih jam, bunkerjev, tunelov, zgradb in rudnikov je bilo opaženih najmanj 9 vrst netopirjev: *Rhinolophus ferrumequinum* (28 lokacij), *R. hipposideros* (36 lokacij), *R. blasii* (1 lokacija), *R. euryale* (3 lokacije), *Myotis myotis/oxygnatus (blythii)* (4 lokacije), *M. capaccinii* (6 lokacij), *Pipistrellus* sp. (2 lokaciji), *Hypsugo savii* (1 lokacija) in *Miniopterus schreibersii* (9 lokacij). Predstavljeni rezultati v veliki meri prispevajo k poznavanju razširjenosti teh vrst in njihovih zatočišč v Albaniji ter bodo osnova za monitoring populacij netopirjev in za izboljšanje ohranitvenih ukrepov tako v Albaniji kot v širši regiji.

Ključne besede: Albanija, netopirji, Chiroptera, prezimovališča, monitoring, raziskava

Introduction

Since the first data on bats collected in Albania a century ago, with a female *P. auritus* caught in 1914 (Spitzenberger et al. 2001), surveys on bats in Albania have been organised during spring, summer or autumn (Hanak et al. 1961, Hurka 1962, Hanak 1964, Lamani 1970, Bego & Griffiths 1994, Chytil & Vlašin 1994, Uhrin et al. 1996, Sachanowicz & Ciechanowski 2006, Sachanowicz et al. 2006, Schieffler et al. 2013, Théou & Bego 2013). Thanks to all these surveys carried out mostly by foreign researchers, 32 bat species were confirmed to occur in Albania (Bego & Théou 2014). With the exception of three records concerning *R. hipposideros*, *M. capaccinii* and *Plecotus* sp., coming from bat surveys of a few caves around Lake Prespa in February 2011 (Papadatou et al. 2011), no other winter data on bats had been collected in this country, even though there are numerous potential bat hibernacula, e.g. thousands of caves and hundreds of former military buildings (Théou 2014). In 2012, a pilot bat monitoring programme focusing on bunkers around Tirana was started by Théou & Bego (2014), and since then at least a few hibernacula have regularly been monitored for the first time in Albania. Even at the scale of wider region, the lack of knowledge about bat hibernacula is apparent, with rare winter observations available for Bosnia and Herzegovina (Pašić et al. 2013), Montenegro (Presetnik et al. 2014), Macedonia (Kryštufek et al. 1992) and Greece (Papadatou et al. 2011). Nevertheless, winter data on bats and their roosts are key elements to understand the bat species conservation status and for implementing a successful management at local and regional level (Dietz et al. 2009). Therefore we hope that the reported results of bat winter surveys in Albania have improved the knowledge on bats not only for Albania, but for the neighbouring countries as well, contributing to the clearer status overview for the south-west Balkan area.

Material and methods

Surveys were organized between mid-November and end of February, spanning four winters (early 2012, 2012/2013, 2013/14 and 2014/15) in several parts of Albania (Fig. 1). In these areas, as in most of Albania, winters are known to be severe, with possible important snow covering, and long period of low temperatures. For some of these areas, annual monitoring programs have been on-going. In general, one visit per winter has been implemented for most of the sites. Natural caves (horizontals and pits) but also bunkers, tunnels, buildings (church, castle, private house, school) and mines were visited in order to identify possible bat hibernacula. These sites have been selected following previous surveys during spring or summer, but several sites have been randomly visited following indications on presence of bats by local inhabitants. For each site visited, the geographic latitude and longitude coordinates were recorded. Bats were counted visually thanks to head lamps and determined down to the species level when possible or attributed to higher taxon following Dietz et al. (2009). In some cases bat detectors (D1000X Petterson Elektronik AB, Sweden) were used at the site to record any bat ultrasound calls, which were eventually analysed with Bat Sound, v4.1 programme (Pettersson Elektronik AB, Sweden) and calls identified according to Barataud (2014). When larger bat groups were encountered, pictures were taken and the

number of individuals of each species determined later on the computer. During all surveys, a strict protocol was implemented in order to limit the disturbance of bats with, for example, a limited number of persons checking the roosts to avoid making any noise. No bats were manipulated by hand for direct measurements.

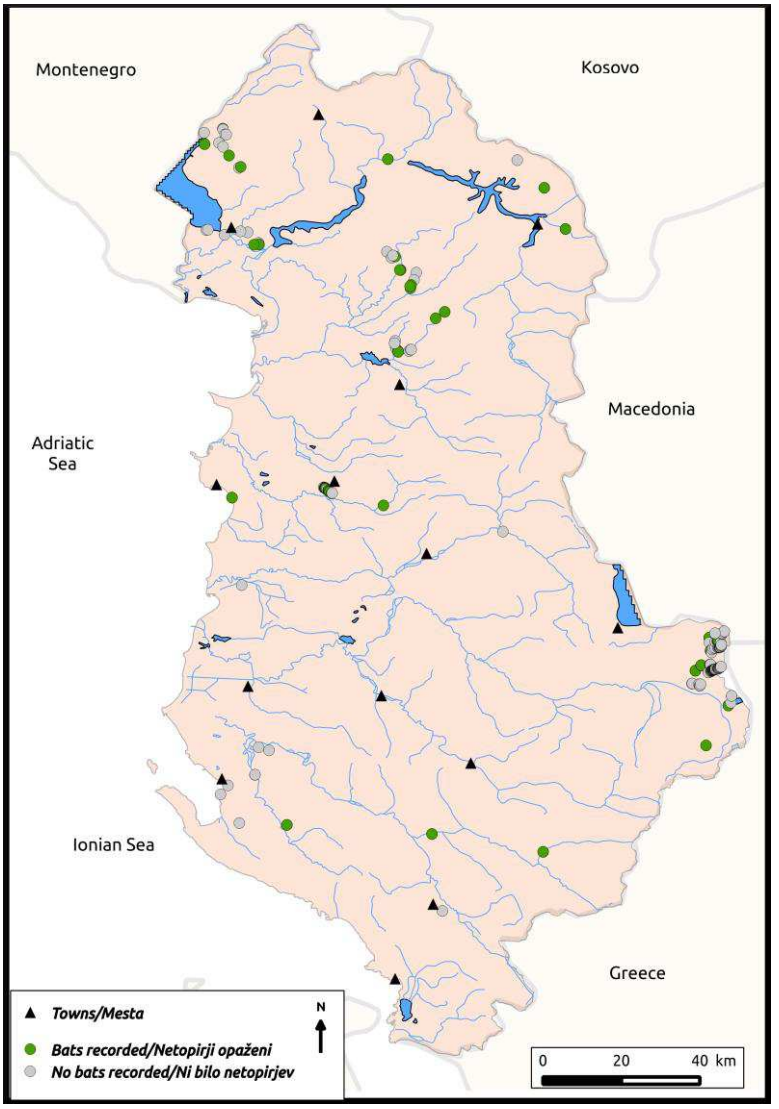

Figure 1. Potential bat hibernacula visited in Albania in winters from early 2012 to 2014/2015.
Slika 1. Možna prezimovališča netopirjev v Albaniji v zimah od začetka 2012 do 2014/2015.

Results and discussion

General results

In total, 178 potential bat hibernacula have been visited in Albania (Fig. 1), and 245 visits made thanks to the implementation of monitoring protocols (Tab. 1).

Table 1. Number of potential bat hibernacula in Albania visited each winter, from early 2012 to 2014/2015. Numbers in brackets refer to sites visited for the first time in that winter.
Tabela 1. Število potencialnih prezimovališč v Albaniji, obiskanih na zimo, od začetka 2012 do 2014/2015. Števila v oklepaju so lokalitete, ki smo jih prvič obiskali tisto zimo.

Winter	Number of potential bat hibernacula visited (first visit)
Early 2012	1 (1)
2012/13	75 (75)
2013/14	72 (44)
2014/15	77 (58)

Of the 178 sites, 114 were caves, 28 bunkers, 22 tunnels, 9 buildings and 5 mines. In 51 (28%) of these sites (27 caves, 8 bunkers, 14 tunnels, 1 building and 1 mine, Tab. 2) at least 9 bat species have been observed (Tab. 3). A maximum of five species were recorded in the same hibernacula, whereas a maximum number of 2,431 individuals from three species were recorded in one site (Tab. 2). 72% of the hibernacula hosted less than 10 individuals, whereas at 15% of the sites more than 100 individuals were found. Most important bat groups composed of more than 500 individuals have been observed in 5% of the hibernacula.

Although hibernating bats were recorded in all types of sites, caves hosted the most important diversity (a maximum of 5 species) and the most numerous groups of bats (maximum of 2,431 individuals) (Tab. 2). In comparison, bunkers hosted a maximum of 4 species and 71 individuals, tunnels 4 species and 23 individuals, mines 1 species and 1 individual, buildings 1 species and 1 individual. However, despite this general observation, former military buildings and mines are interesting roosts for some bat species on a local scale, especially for hibernacula, when natural caves are missing in the area (Théou & Bego 2014), which is the case in most of the western part of Albania.

Bat species are distributed differently in Albanian hibernacula. Whereas *Rhinolophus ferrumequinum* or *R. hipposideros* have been commonly recorded in hibernacula throughout the country, not only in caves but also bunkers, buildings and mines, other species as *Miniopterus schreibersii* have been encountered only in a few caves (Tab. 2, Figs. 2, 5). This observation seems to be similar to other data collected in other countries of the region (Kryštufek et al. 1992, Papadatou et al. 2011, Pašić et al. 2013, Presetnik et al. 2014). Also, the repeated visits made within the framework of this study showed that identified roosts had been used for several years in a row, especially the roosts hosting more than 50 individuals. Small fluctuation in the number of bats using these sites have been observed, but with data collected during three winters at maximum we cannot give any reliable interpretation of potential trends. However, these results confirm the importance to establish the monitoring protocols for all hibernacula, in order to be able to observe possible changes at the local but also national scale in the future.

It is also important to underline that, contrary to other types of surveyed sites, most of the caves used during the winter were unused by bats during the spring and summer (Théou, unpublished). This aspect has to be taken in account by managers and for the redaction of future management plans, in order not to base management measures only on data collected during spring and summer. The use of several roosts during the year underlines also the importance of creating protected cave networks at a local, national and regional scale.

Table 2. Bat hibernacula found in Albanian in winters from early 2012 to 2014/2015.
Tabela 2. Prezimovališča netopirjev, najdena v Albaniji v zimah od začetka 2012 do 2014/2015.

Site code	**Name** (NN – the site has no designated name)	**Closest village**	**Coordinates** (WGS 84)	**Type of site**	**Number of hibernating bat species recorded**	**Max. sum of individuals for one survey** (winter years)
AL0005	Shkëmbi i Kavajës	Golem	41.27°N 19.51°E	bunker	4	36 (2013/14)
AL0063	NN	Lajthizë	40.77°N 20.88°E	tunnel	4	23 (2014/15)
AL0069	NN	Liqenas	40.78°N 20.90°E	tunnel	1	1 (2013/14)
AL0073	NN	Gorica e vogël	40.87°N 20.92°E	cave	2	270 (2014/15)
AL0080	NN	Gollomboç	40.83°N 20.93°E	cave	4	4 (2014/15)
AL0108	NN	Zaroshkë	40.77°N 20.94°E	cave	1	4 (2014/15)
AL0113	NN	Zaroshkë	40.77°N 20.94°E	cave	1	1 (2013/14)
AL0129	NN	Kallamas	40.87°N 20.94°E	cave	1	1 (2014/15)
AL0145	NN	Gollomboç	40.86°N 20.95°E	cave	1	2 (2013/14)
AL0146	NN	Gollomboç	40.86°N 20.95°E	cave	1	1 (2012/13)
AL0159	NN	Gollomboç	40.85°N 20.95°E	cave	3	3 (2012/13)
AL0171	NN	Gollomboç	40.84°N 20.96°E	cave	1	4 (2013/14)
AL0203	Treni cave	Treni	40.67°N 20.98°E	cave	5	108 (2013/14)
AL0205	NN	Shueç	40.70°N 20.99°E	tunnel	1	1 (2012/13)
AL0270	Zef Toma cave	Bajzë	42.31°N 19.43°E	cave	3	14 (2014/15)
AL0282	NN	Juban	42.01°N 19.58°E	bunker	1	70 (2014/15)
AL0285	Ali Dedes cave	Juban	42.01°N 19.59°E	cave	1	7 (2014/15)
AL0293	Velce cave	Velce	40.32°N 19.67°E	cave	5	313 (2014/15)
AL0303	NN	Tirana	41.30°N 19.78°E	bunker	1	1 (2012/13)
AL0306	NN	Tirana	41.30°N 19.78°E	bunker	1	3 (2013/14)
AL0308	NN	Tirana	41.30°N 19.79°E	tunnel	4	4 (2012/13)
AL0310	NN	Tirana	41.30°N 19.79°E	tunnel	1	1 (2012/13)
AL0313	NN	Tirana	41.30°N 19.79°E	bunker	2	23 (2012/13)
AL0314	NN	Tirana	41.30°N 19.79°E	tunnel	2	3 (2012/13)
AL0318	NN	Tirana	41.30°N 19.79°E	tunnel	1	1 (2012/13)
AL0321	NN	Tirana	41.29°N 19.79°E	tunnel	1	1 (2013/14)
AL0322	NN	Tirana	41.29°N 19.80°E	bunker	2	3 (2013/14)
AL0325	NN	Tirana	41.30°N 19.80°E	tunnel	1	2 (2013/14)
AL0326	NN	Tirana	41.30°N 19.80°E	tunnel	3	9 (2013/14)
AL0327	NN	Tirana	41.29°N 19.80°E	tunnel	2	8 (2012/13)
AL0330	NN	Tirana	41.29°N 19.80°E	tunnel	1	1 (2013/14)
AL0334	NN	Tirana	41.29°N 19.81°E	tunnel	2	6 (2012/13)
AL0341	Black cave	Ibë	41.25°N 19.96°E	cave	1	79 (2011/12)
AL0347	Keputes cave	Urakë	41.70°N 20.00°E	cave	3	563 (2013/14)
AL0348	Blazi cave	Urakë	41.70°N 20.00°E	cave	2	619 (2014/15)

Site code	**Name** (NN – the site has no designated name)	**Closest village**	**Coordinates** (WGS 84)	**Type of site**	**Number of hibernating bat species recorded**	**Max. sum of individuals for one survey** (winter years)
AL0363	NN	Luadh	40.29°N 20.10°E	cave	3	8 (2014/15)
AL0392	Bat cave	Kishaj	42.18°N 20.44°E	cave	3	2431 (2014/15)
AL0397	Jezim cave	Belje	42.06°N 20.50°E	cave	4	486 (2014/15)
AL0411	NN	Benjë	40.24°N 20.43°E	cave	3	125 (2014/15)
AL0413	NN	Ziçisht	40.55°N 20.92°E	cave	2	8 (2014/15)
AL0424	NN	Gojan	41.98°N 19.99°E	building	1	1 (2014/15)
AL0429	NN	Gjegjan	41.94°N 20.01°E	bunker	1	2 (2014/15)
AL0431	NN	Reps	41.89°N 20.04°E	cave	1	1 (2014/15)
AL0433	NN	Reps	41.89°N 20.04°E	mine	1	1 (2014/15)
AL0435	NN	Reps	41.89°N 20.04°E	tunnel	1	2 (2014/15)
AL0436	NN	Mërkurth	41.82°N 20.14°E	cave	1	12 (2014/15)
AL0437	NN	Mërkurth	41.80°N 20.11°E	cave	2	53 (2014/15)
AL0441	NN	Breglum	42.26°N 19.97°E	cave	2	7 (2014/15)
AL0487	Muriqit cave	Lukaj	42.35°N 19.48°E	cave	2	5 (2014/15)
AL0493	NN	Lohja	42.27°N 19.50°E	bunker	2	71 (2014/15)
AL0494	Gurrës cave	Qafe Gradë	42.24°N 19.54°E	cave	1	1 (2014/15)

Table 3. Bat species recorded in different types of hibernacula in Albania during winters from early 2012 to 2014/2015.
Tabela 3. Vrste netopirjev, opažene v različnih prezimovališčih v Albaniji v zimah od začetka 2012 do 2014/2015.

Species	**No. of hibernacula sites** (caves / bunkers / tunnels / buildings / mines)	**Max. no. of bats at a site**	**Max. sum of bats in one winter session** (winter census year)
Rhinolophus ferrumequinum	28 (15/3/8/1/1)	304	413 (2014/15)
Rhinolophus hipposideros	36 (21/4/11/0/0)	20	62 (2014/15)
Rhinolophus blasii	1(1/0/0/0/0)	254	254 (2014/15)
Rhinolophus euryale	3 (2/0/1/0/0)	261	262 (2014/15)
Rhinolophus spp. (middle sized)	10 (7/2/1/0/0)	617	1,178 (2014/15)
Myotis myotis/blythii	4 (3/0/1/0/0)	1	4 (2014/15)
Myotis capaccinii	6 (4/1/1/0/0)	100	103 (2013/14)
Pipistrellus sp.	2 (0/2/0/0/0)	70	140 (2014/15)
Hypsugo savii	1 (0/1/0/0/0)	1	1 (2014/15)
Miniopterus schreibersii	9 (7/1/1/0/0)	2,409	2,666 (2014/15)

Four years of winter surveys and monitoring of selected sites have substantially increased the knowledge on several bat species in Albania, and in the south-west Balkan region in general. This is the case for the usual cave-dwelling species and also for bat species that are not often found in Albania during spring or summer, as they are using, during these particular periods, human-made habitats that have till recently been surveyed to a lesser extent in the country (as *R. ferrumequinum* and *R. hipposideros*). Winter represents an interesting period for the survey of such species, as they can be observed often in caves, bunkers and tunnels. This study, however, represents only a start and the results provided herewith should be used as a base for planning research in the next years in the country. The search for new hibernacula should be intensified and monitoring network expanded according to new knowledge. Regular monitoring of a large number of sites will enable us to follow the trends in bats population size and status of habitats in Albania. Such data can easily be incorporated into regional and European bat population monitoring protocols (Van der Meij et al. 2015).

Species part

***Rhinolophus ferrumequinum* (Schreber, 1774)**

Most of the 34 observations in the 28 hibernacula concern only a few individuals (Fig. 2a). 35% of the records are solitary individuals, whereas 68% of the data are from sites hosting less than 5 individuals. Sites with more than 10 individuals represent 9% of all the hibernacula identified. One cave hibernaculum (site AL0397) hosted 74% of all the *R. ferrumequinum* recorded during the winter 2014-2015.

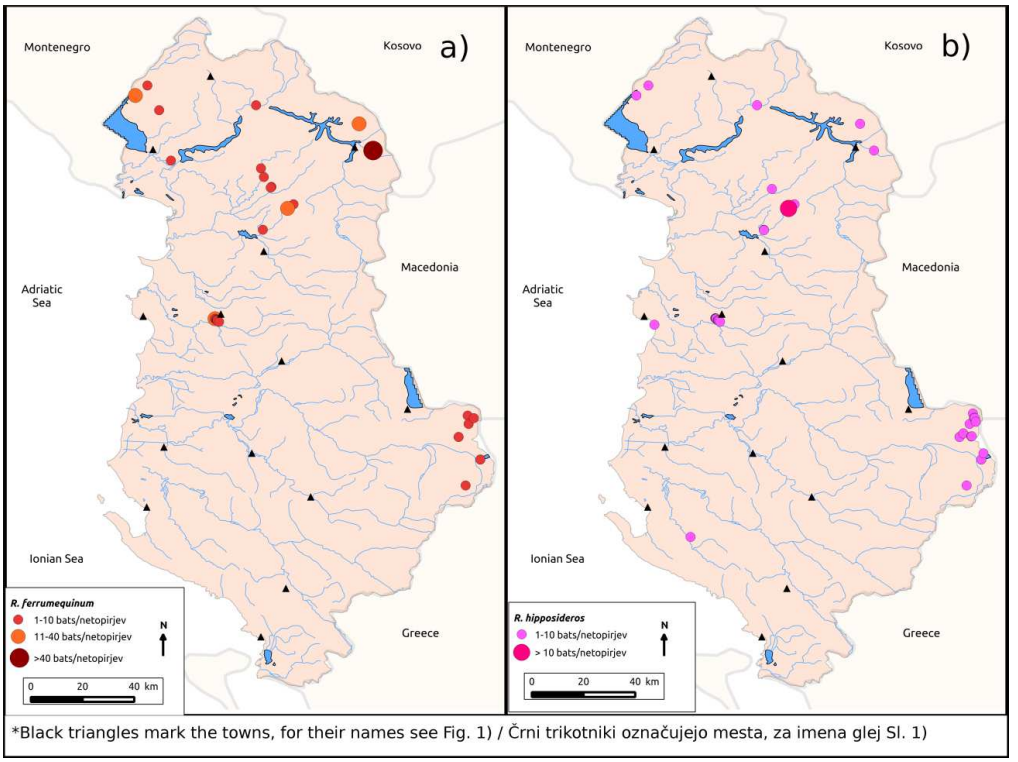

Figure 2. Hibernation sites of a) *Rhinolophus ferrumequinum* and b) *R. hipposideros* in Albania in winters from early 2012 to 2014/2015.
Slika 2. Lokalitete prezimovališč vrst a) *Rhinolophus ferrumequinum* in b) *R. hipposideros* v Albaniji v zimah od začetka 2012 do 2014/2015.

Observations (ex. is used as abbreviation for individual(s)): – AL0063: 23.2.2014, 6 ex.; 13.11.2014, 6 ex. – AL0073: 12.11.2014, 9 ex. – AL0080: 22.2.2014, 1 ex. – AL0159: 24.11.2012, 1 ex. – AL0203: 1.2.2013, 4 ex.; 21.2.2014, 1 ex.; 13.11.2014, 4 ex. – AL0270: 29.1.2015, 12 ex. – AL0285: 27.1.2015, 7 ex. – AL0313: 16.2.2013, 22 ex. – AL0314: 16.2.2013, 1 ex. – AL0318: 16.2.2013, 1 ex. – AL0322: 16.2.2013, 1 ex.; 7.12.2013, 2 ex. – AL0325: 16.2.2013, 2 ex.; 7.12.2013, 2 ex. – AL0326: 16.2.2013, 3 ex.; 7.12.2013, 5 ex. – AL0327: 16.2.2013, 7 ex. – AL0334: 16.2.2013, 2 ex. – AL0347: 12.12.2013, 1 ex. – AL0392: 12.12.2014, 18 ex. – AL0397: 13.12.2014, 304 ex. – AL0413: 13.11.2014, 2 ex. – AL0424: 9.12.2014, 1 ex. – AL0429: 9.12.2014, 2 ex. – AL0433: 10.12.2014, 1 ex. – AL0435: 10.12.2014, 2 ex. – AL0436: 11.12.2014, 9 ex. – AL0437: 11.12.2014, 33 ex. – AL0441: 12.12.2014, 1 ex. – AL0487: 28.1.2015, 1 ex. – AL0494: 30.1.2015, 1 ex. – AL0494: 30.1.2015, 1 ex.

***Rhinolophus hipposideros* (Bechstein, 1800)**

Most of the 51 observations in the 36 hibernacula identified for this species concern only few individuals (Fig. 2b). 37% of the records are solitary individuals, whereas 86% of the records are from sites hosting less than 5 individuals. Only one cave site (AL0437) hosted more than 10 individuals (2%).

Observations (ex. is used as abbreviation for individual(s)): – AL0005: 3.2.2014, 2 ex. – AL0063: 23.2.2014, 5 ex.; 13.11.2014, 3 ex. – AL0069: 3.3.2013, 1 ex.; 22.2.2014, 1 ex. – AL0080: 22.2.2014, 2 ex. – AL0108: 3.2.2013, 1 ex.; 22.2.2014, 2 ex.; 11.11.2014, 4 ex. – AL0113: 22.2.2014, 1 ex. – AL0129: 12.11.2014, 1 ex. – AL0145: 22.2.2014, 2 ex. – AL0146: 2.2.2013, 1 ex. – AL0159: 24.11.2012, 2 ex. – AL0171: 2.2.2013, 4 ex.; 22.2.2014, 4 ex. – AL0203: 24.11.2012, 7 ex.; 1.2.2013, 2 ex.; 21.2.2014, 5 ex.; 13.11.2014, 4 ex. – AL0205: 3.2.2013, 1 ex. – AL0270: 29.1.2015, 1 ex. – AL0293: 6.12.2014, 1 ex. – AL0306: 16.2.2013, 1 ex.; 7.12.2013, 3 ex. – AL0308: 16.2.2013, 2 ex.; 7.12.2013, 3 ex. – AL0310: 16.2.2013, 1 ex. – AL0313: 16.2.2013, 1 ex. – AL0314: 16.2.2013, 2 ex.; 7.12.2013, 1 ex. – AL0321: 7.12.2013, 1 ex. – AL0322: 7.12.2013, 1 ex. – AL0326: 16.2.2013, 2 ex.; 7.12.2013, 3 ex. – AL0327: 16.2.2013, 1 ex.; 7.12.2013, 1 ex. – AL0330: 7.12.2013, 1 ex. – AL0334: 16.2.2013, 4 ex. – AL0347: 12.12.2013, 1 ex. – AL0348: 12.12.2013, 2 ex.; 11.12.2014, 2 ex. – AL0392: 12.12.2014, 4 ex. – AL0397: 13.12.14, 2 ex. – AL0413: 13.11.2014, 6 ex. – AL0431: 10.12.2014, 1 ex. – AL0436: 11.12.2014, 3 ex. – AL0437: 11.12.2014, 20 ex. – AL0441: 12.12.2014, 6 ex. – AL0487: 28.1.2015, 4 ex.

***Rhinolophus* spp. (middle sized)**

During the surveys, it was sometimes possible to identify bats as *Rhinolophus euryale* (Blasius, 1853) or *Rhinolophus blasii* (Peters, 1866), using bat-detectors and/or pictures. At most of the sites, however, the identification was possible just down to the taxon *Rhinolophus* of middle size. In Albania, most of the data concerning these species come from the winter census, whereas the number of known maternity colonies is still low (Théou, unpublished). During our study, important winter groups have been recorded, sometimes with less than 200 metres between two caves hosting more than 500 individuals each (sites AL0347 and AL0348). This situation may underline the possible importance of a network of roosts used by bats, with exchanges between the two groups (Bagrowska-Urbańczyk & Urbańczyk 1983). Additionally to the above mentioned species, *Rhinolophus mehelyi* (Matschie, 1901) could also have been using the hibernacula, as this species was recently identified in the country (Bego & Théou 2014). 42% of the 19 observations concern sites with less than 10 individuals, whereas 26% of the data concern sites hosting more than 200 individuals (Fig. 3).

Observations (ex. is used as abbreviation for individual(s); *–*R. euryale* ; ** –*R. blasii*): – AL0005: 3.2.2014, 28 ex. – AL0063: 23.2.2014, 8 ex.; 13.11.2014, 13 ex. – AL0073: 23.2.2014, 65 ex.; 12.11.2014, 261 ex.* – AL0159: 22.2.2014, 1 ex. – AL0203: 24.11.2012, 7 ex.; 21.2.2014, 1 ex. – AL0293: 6.12.2014, 254 ex.** – AL0303: 16.2.2013, 1 ex. – AL0326: 7.12.2013, 1 ex.* – AL0341: 19.2.2012, 79 ex.; 26.01.2014, 49 ex. – AL0347: 12.12.2013, 563 ex.; 11.12.2014, 460 ex. – AL0348: 12.12.2013, 2 ex.; 11.12.2014, 617 ex. – AL0363: 18.11.2014, 1 ex.* – AL0411: 19.11.2014, 88 ex.

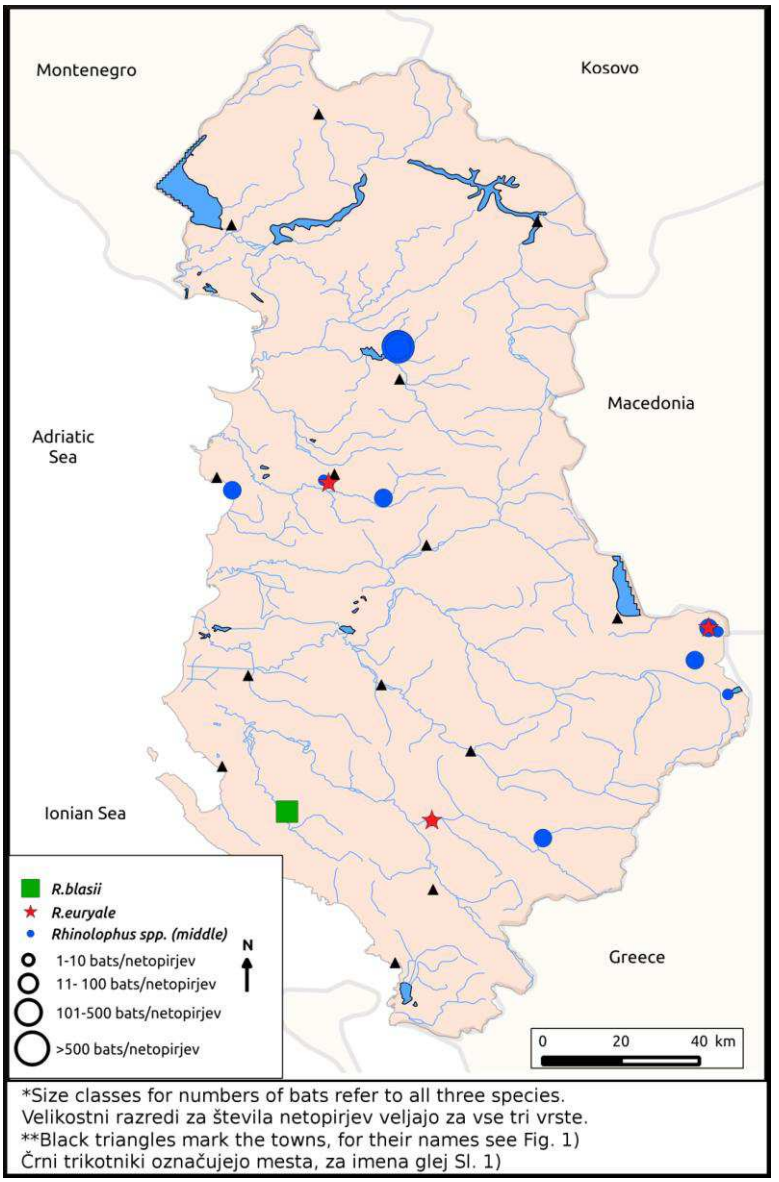

Figure 3. Hibernation sites of *Rhinolophus blasii*, *R. euryale* and undetermined middle size *Rhinolophus* spp. in Albania during winters from 2012/2013 to 2014/15.
Slika 3. Lokalitete prezimovališč *Rhinolophus blasii*, *R. euryale* in nedoločenih srednje velikih *Rhinolophus* spp. v Albaniji v zimah od 2012/2013 do 2014/2015.

Myotis myotis (Borkhausen, 1797) / *Myotis oxygnatus* (Monticelli, 1885)

The two species cannot be clearly identified without taking detailed morphological measurements. In accordance with our strict protocol and in order not to disturb animals, we have referred to those few sightings of all large *Myotis* sp. as taxon *Myotis myotis/oxygnatus*. In official documents concerning bats protection in Albania, *M. oxygnatus* is descripted also as *M. blythii* (Bego & Théou 2014). During spring 2014, close to 9,000 *M. myotis/oxygnathus* were recorded during surveys (direct visits of roosts) organised in the entire country and concerning all types of roosts (buildings, caves, tunnels and bunkers). During the winter surveys, however, we observed only four animals at four sites scattered across the country. The difficulty of recording these species during winter seems to be similar in the entire region (Pavlinić et al. 2010), certainly due to the possible use of crevices by individuals (Dietz et al. 2009). However, these rare observations show, at least, that these species seems to hibernate all over Albania (Fig. 4).

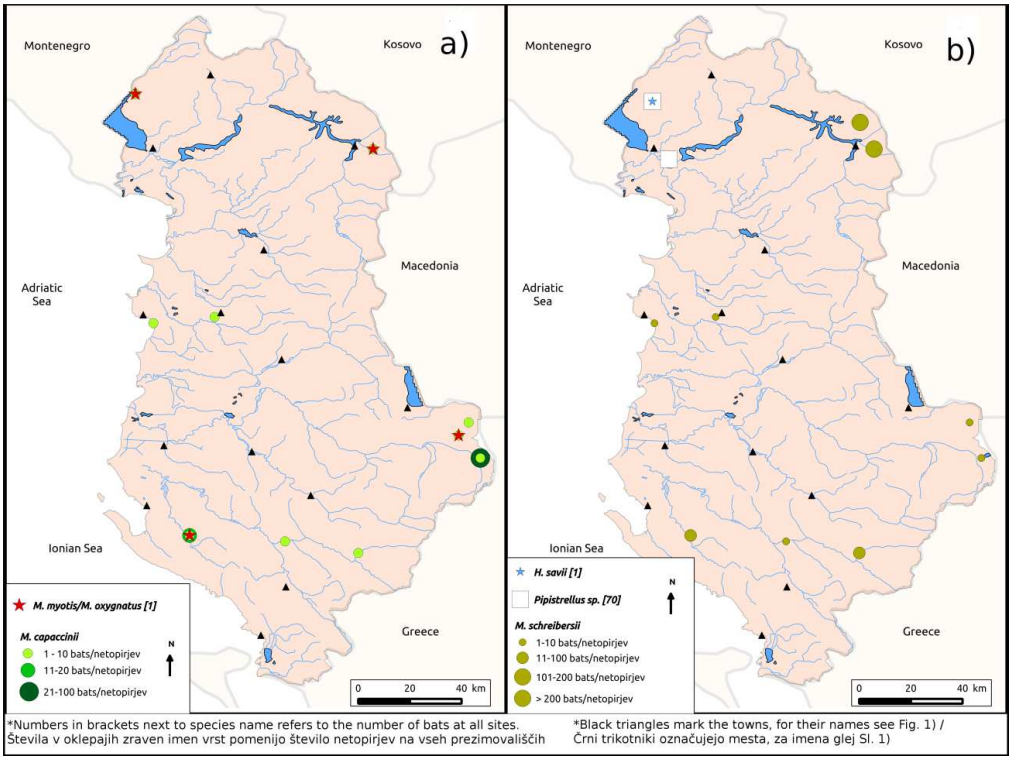

Figure 4. Hibernation sites of a) *Myotis myotis/oxygnatus* and *M. capaccinii*, b) *Hypsugo savii*, *Pipistrellus* sp. and *Miniopterus schreibersii* during winters from 2012/2013 to 2014/15.
Slika 4. Lokalitete prezimovališč vrst a) *Myotis myotis/oxygnatus* in *M. capaccinii*, b) *Hypsugo savii*, *Pipistrellus* sp. in *Miniopterus schreibersii* v Albaniji v zimah od 2012/2013 do 2014/2015.

Observations (ex. is used as abbreviation for individual(s)): – AL0063: 13.11.2014, 1 ex. – AL0293: 6.12.2014, 1 ex. – AL0397: 13.12.2014, 1 ex. – AL0270: 29.1.2015, 1 ex.

***Myotis capaccinii* (Bonaparte, 1837)**

For *Myotis capaccinii*, most of the maternity colonies are known from the area of Lake Prespa, where we have found the biggest winter congregation of the species (Fig. 4). This is concordant with Papadatou et al. (2011) data. Only mono-specific groups have been found at all these sites.

Observations (ex. is used as abbreviation for individual(s)): – AL0005: 3.2.2014, 2 ex. – AL0080: 22.2.2014, 1 ex. – AL0203: 24.11.2012, 1 ex.; 21.2.14, 100 ex.; 13.11.2014, 8 ex. – AL0293: 6.12.2014, 15 ex. – AL0308: 16.2.2013, 2 ex. – AL0411: 19.11.2014, 5 ex.

***Pipistrellus* sp.**

Considering the high possibility of wrong identification of *Pipistrellus* bats based on observations only, we considered all the individuals of this genus as *Pipistrellus* sp. It is highly likely that more than one species of this genus is hibernating in Albania, as all four species have been recorded during spring and summer (Bego & Théou 2014). The hibernating groups were recorded in the vicinity of Lake Shkodra, and underline the importance of bunkers as roosts for bats. The number of these roosts is now strongly decreasing due to illegal destructions, which represent a significant threat for several bat species in Albania (Théou & Bego 2014) (Fig. 4).

Observations (ex. is used as abbreviation for individual(s)): – AL0282: 27.1.2015, 70 ex. – AL0493: 28.1.2015, 70 ex.

***Hypsugo savii* (Bonaparte, 1837)**

The first record of this species during the winter period in Albania was made in the crevice of a bunker, where one animal was hiding among dozens of *Pipistrellus* sp. (Fig. 4). The individual was well visible, which allowed us to clearly identify it following Dietz et al. (2009). This is the first hibernaculum known for the species in the country, and the species is very likely common in the entire country (Uhrin et al. 1996). It also underlines yet again the importance of bunkers in bat conservation in Albania (Théou 2014).

Observation (ex. is used as abbreviation for individual(s)): – AL0493: 28.1.2015, 1 ex.

***Miniopterus schreibersii* (Kuhl, 1817)**

The data collected in Albania must be interpreted on a scale of the south-west Balkans. A colony in the cave (site AL0392) in the north-eastern part of the country represents the third biggest known winter group for this species in the south-west Balkans (Papadatou et al. 2011, Presetnik et al. 2014, Théou & Đurović, unpublished) (Fig. 4). However, the actual knowledge of winter roosts of this species is still unsatisfactory, especially when compared to the summer population in Albania (approximately 6,000 individuals in 2014 (Théou, unpublished data)). The main maternity colonies are located in the area of Lake Prespa. Considering the annual migratory behaviour of *M. schreibersii*, it is possible that many animals from these roosts migrate to Greek or Macedonian hibernacula. At the same time, some important hibernacula identified in north-east Albania may harbour a part of summer populations from Kosovo. The site AL0392 represents 91% of all the individuals recorded during the winter 2014/15 and

confirm the data collected in other parts of the continent, with important hibernation groups created by this species (Serra-Cobo et al. 1998).

Observations (ex. is used as abbreviation for individual(s)): – AL0005: 3.2.2014, 4 ex. – AL0080: 12.9.2014, 2 ex. – AL0203: 24.11.2012, 1 ex.; 21.2.2014, 1 ex. – AL293: 6.12.2014, 42 ex. – AL0308: 7.12.2013, 1 ex. – AL0363: 18.11.2014, 2ex. – AL0392: 12.12.2014, 2,409 ex. – AL0397: 13.12.2014, 179 ex. – AL0411: 19.11.2014, 32 ex.

Povzetek

Število zabeleženih vrst netopirjev v Albaniji se je pred kratkim povečalo na 32 (Bego & Théou 2014), predvsem zaradi ekspedicij tujih raziskovalcev, ki so potekale v obdobju več let od aprila do oktobra (Hanak et al. 1961, Hurka 1962, Hanak 1964, Lamani 1970, Bego & Griffiths 1994, Uhrin et al. 1996, Sachanowicz & Ciechanowski 2006, Sachanowicz et al. 2006, Schieffler et al. 2013, Théou & Bego 2013). Z izjemo dveh podatkov iz februarja 2011 (Papadatou et al. 2011), drugih informacij o prezimovanju netopirjev v državi ni bilo. V štirih zimah (začetek 2012, 2012/2013, 2013/2014 in 2014/15) smo skupno pregledali 178 potencialnih prezimovališč in odkrili 51 dejanskih prezimovališč netopirjev (27 jam, 8 bunkerjev, 14 tunelov, en rudnik in eno zgradbo). Potrdili smo prezimovanje vsaj devetih vrst netopirjev: *Rhinolophus ferrumequinum* (na 28 mestih), *R. hipposideros* (36), *R. blasii* (1), *R. euryale* (3), *Myotis myotis/oxygnatus* (4), *M. capaccinii* (6), *Pipistrellus* sp. (2), *Hypsugo savii* (1) in *Miniopterus schreibersii* (9). Na posamičnem prezimovališču smo našli največ pet vrst netopirjev in največ 2.431 osebkov (treh vrst). V 72 % prezimovališč smo opazili manj kot 10 osebkov, v 15 % prezimovališč pa je prezimovalo več kot 100 osebkov. Čeprav so bili prezimujoči netopirji opaženi v vseh tipih zatočišč, je v jamah prezimovalo največ vrst, prezimovale pa so tudi največje skupine netopirjev. Izredno se je povečalo poznavanje nekaterih vrst, ki so bile v Albaniji redko najdene pomladi ali poleti, kot npr. za *R. ferrumequinum* in *R. hipposideros*. Rezultati te raziskave, ki je ena prvih, ki so jih opravili večinoma v Albaniji stanujoči raziskovalci, potrjujejo, da je v tej državi tudi pozimi mogoče najti pestro združbo netopirjev. Nekatere populacije so pomembne za širšo regijo, saj v kar nekaj prezimovališčih prezimuje več kot 300 osebkov. Zbrani podatki so pomemben korak naprej pri vključitvi Albanije v regionalno in evropsko mrežo raziskav in varstva netopirjev, a tudi za uresničevanje pravnih in praktičnih zavez za ohranitev netopirjev.

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SHORT COMMUNICATION

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First confirmations of the greater noctule bat *Nyctalus lasiopterus* (Schreber, 1780) presence in Slovenia after more than 85 years

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Abstract. During a transect count using ultrasound detectors within the framework of the national bat monitoring scheme, three foraging *Nyctalus lasiopterus* were observed on 27. 6. 2014, and eventually confirmed by an analysis of their echolocation calls recorded in the forest clearing Šetinov laz near the hamlet of Leskova dolina close to Mt. Snežnik in southern Slovenia. The re-examination of recordings made at the same site during 15 other transect counts in the 2007–2014 period revealed that *N. lasiopterus* was also present there on 14. 10. 2013. These observations are the first confirmations of this rare species' occurrence in over 85 years, and opens up questions about the regular presence of *N. lasiopterus* in Slovenia.

Key words: *Nyctalus lasiopterus*, foraging area, echolocation, Slovenia

Izvleček. Prve potrditve prisotnosti velikega mračnika *Nyctalus lasiopterus* (Schreber, 1780) v Sloveniji po več kot 85 letih – 27. 6. 2014 sva v okviru državnega monitoringa netopirjev med transektnim popisom netopirjev z ultrazvočnim detektorjem na gozdni jasi Šetinov laz pri zaselku Leskova dolina blizu gore Snežnik v južni Sloveniji opazila tri prehranjujoče se velike mračnike *Nyctalus lasiopterus* in določitev vrste kasneje potrdila z analizo posnetkov njihovih eholokacijskih klicev. Ko sva pregledala posnetke, narejene med 15 ostalimi popisi na isti lokaciji v letih 2007–2014, sva ugotovila, da je bil veliki mračnik tam zagotovo prisoten tudi med popisom 14. 10. 2013. To so prva opazovanja te redke vrste po več kot 85 letih, ki odpirajo vprašanje, ali so veliki mračniki redno prisotni v Sloveniji.

Ključne besede: *Nyctalus lasiopterus*, prehranjevališče, eholokacija, Slovenija

Introduction

The greater noctule bat, *Nyctalus lasiopterus* (Schreber, 1780), is a rarely observed species in Europe (Dietz & Kiefer 2014). There was only one previous report of its occurrence in Slovenia, i.e. by Dal Piaz (1927), and all other general sources (e.g. Kryštufek 1991, Ibáñez et al. 2001, Presetnik et al. 2009) are based on this report. Even though over 80 years had passed from the only recorded observation, Petrinjak (2009) presumed that it continued to occur in Slovenia, even if only sporadically, and we are presenting results confirming this hypothesis.

Material and methods

One of the transects, which is part of the foot transect counts carried out using ultrasound detectors within the framework of the national bat monitoring scheme (Presetnik et al. 2007, Presetnik & Podgorelec 2008), is called »Leskova dolina«. It starts at the forest clearing called »Šetinov laz« (lat. 45.6230°N, long. 14.4366°E), 1.9 km E of the hamlet of Leskova dolina in the southern part of Slovenia close to Mt. Snežnik. Šetinov laz is an approximately 200×100 m wide meadow in a shallow depression at 820 m a. s. l, surrounded by extensive mixed Dinaric forests.

The protocol for the national bat monitoring foot transect reads as follows: i) be at the starting point (point A) at sunset, ii) wait for half an hour, iii) start transecting by listening for 3 minutes at point A, iv) move to point B and listen for 3 minutes, iv) walk to the next point, etc. until the 10th point (point J) is reached. Points are approximately 220 m from each other, and the total time to walk from point A to point J lasts approximately one hour. From sunset to the end of the walk along the transect, surveyors constantly listen with bat detectors (Pettersson D240x) and record all bat calls in 10× time expansion mode on a digital recorder, which was in our case Marantz PMD 670. Recordings are later analysed with the BatSound 4.0 program (Pettersson Elektronik).

Transect counts were done each year from 2007 until the autumn of 2014, 16 times in total. Surveys were usually performed once during summer months (end of June–start of August) and once in October. However, some summer counts are missing due to financial constraints.

Results and discussion

On 27. 6. 2014, approximately 20 min after sunset, we heard loud bat echolocation calls with unaided ears. A few moments later, we observed initially one bat, which was some minutes later joined by at least two more animals. The bats were foraging at times below treetops, and at times at approximately their height. On the heterodyne bat detector, we could clearly hear alternating echolocation calls (»plip-plop«), with the best listening frequency between 11–16 kHz (Fig. 1), and the animals were notably bigger in comparison to *Nyctalus noctula*.

Computer analysis of the echolocation calls of the first bat (when flying alone) were: maximum frequency 11.7–13.9 kHz, start frequency 15.7–22.5 kHz, end frequency 11.5–14.5 kHz, call duration 21–30 ms and interpulse interval 364–468 ms (N = 6, Fig. 1). The low maximum and end frequency of the echolocation calls, together with the long call duration (Fig. 2) are, according to Haquart & Disca (2007), Estók & Siemers (2009) and Haquart et al. (2010), characteristics of *N. lasiopterus* echolocation calls. Such characteristics are sufficient to separate *N. lasiopterus* from *N. noctula*, which does not have such a low echolocation call frequency, and from *Tadarida teniotis*, whose echolocation calls on the above given frequencies should be of shorter duration.

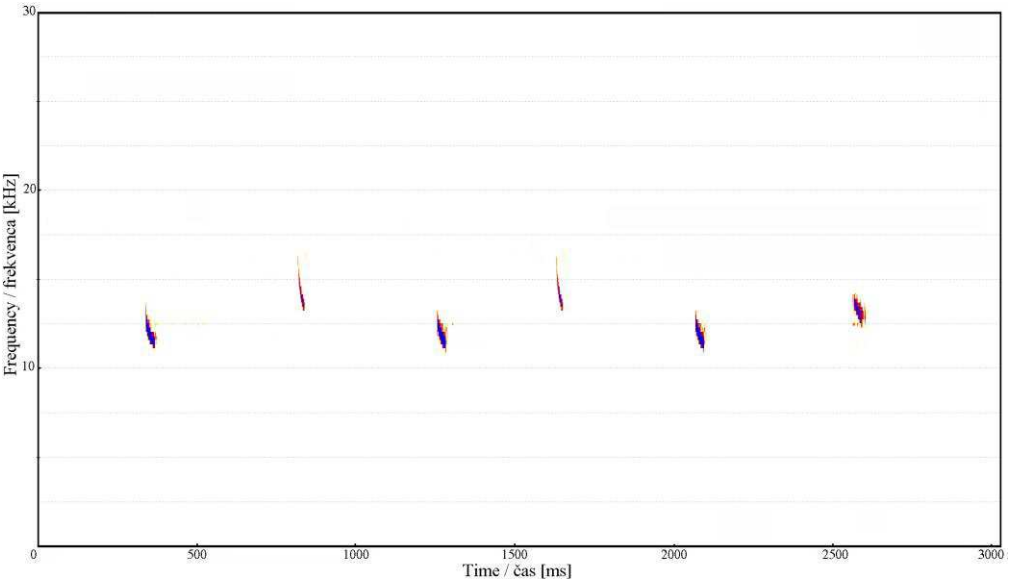

Figure 1. Spectrogram of the *Nyctalus lasiopterus* echolocation calls sequence made at the forest clearing Šetinov laz near the hamlet of Leskova dolina on the 27. 6. 2014.
Slika 1. Spektrogram serije eholokacijskih klicev velikega mračnika *Nyctalus lasiopterus*, posnetih na jasi v Šetinovem lazu pri Leskovi dolini 27. 6. 2014.

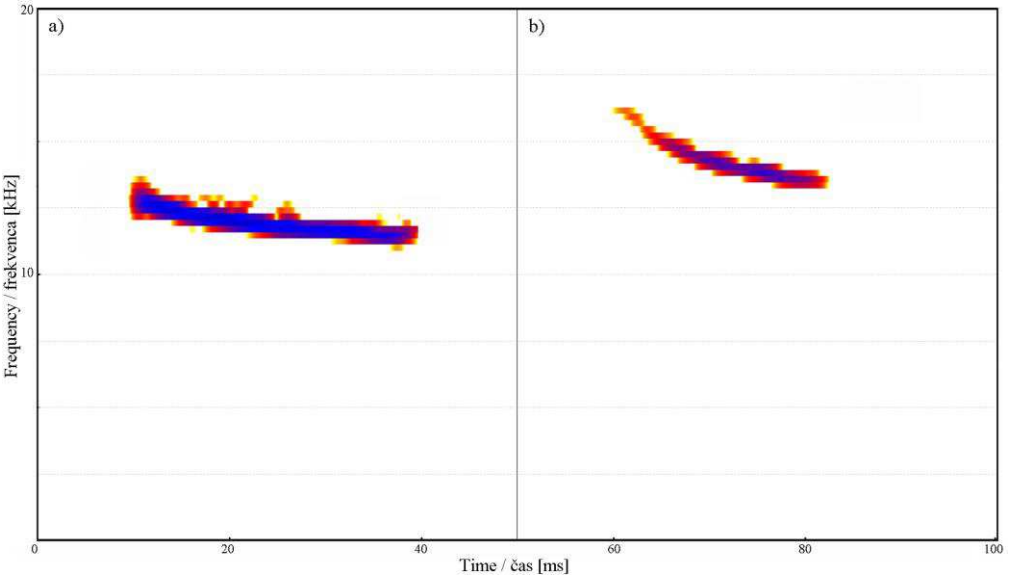

Figure 2. Spectrogram of the recorded a) lower and b) higher echolocation call types of *Nyctalus lasiopterus* (interpulse interval is not in scale, see Fig. 1).
Slika 2. Spektrogram posnetega a) nižjega in b) višjega tipa eholokacijskega klica velikega mračnika *Nyctalus lasiopterus* (medklicni presledek ni v merilu, glej. Sl. 1).

Consequently, we checked all previous echolocation recordings made on that transect as we had recorded, on several other occasions, echolocation calls attributed to taxa *Nyctalus noctula* / *lasiopterus* and/or *Nyctalus* / *Eptesicus* / *Vespertilio*. Only in a survey conducted on the 14. 10. 2013, could we unambiguously attribute a very short recording sequence (3 calls in total) belonging to *N. lasiopterus*, as all other echolocation recordings fell in the overlap zone with *N. noctula*.

After Dal Piaz's (1927) definite record of *Nyctalus lasiopterus* in Slovenia (specimen is kept by the Natural History Museum of the University of Pisa, a drawing of the skull and mandible was published by e.g. Lanza (2012)) more than 85 years ago, our observations are the first that confirm this species' current existence in Slovenia. The site, Šetinov laz, lies approximately 65 km E from Piran, where the first specimen was recorded in Slovenia. The location is approximately 30 km inland from the Adriatic Sea coast, from where the closest most current observations of *N. lasiopterus* originate: approximately 200 km NNW from the island of Kornat in Croatia (Kovač et. al. 2011), and approximately 190 km ENE from the town of Dolo in Venice, Italy (Vernier & Vedovat 2011). In these countries, *N. lasiopterus* is also very rarely found (Kovač et al. 2011, Vernier & Vedovat 2011, Lapini et al. 2014), similar as generally in the whole distribution area (Dietz & Kiefer 2014).

The environment close to Mt. Snežnik where *N. lasiopterus* was observed is not surprising as this species was, at middle geographic latitudes in Europe, often found in (sub)mountainous forests (Estók 2011, Dubourg Savage et al. 2013). These finds were often associated with standing or flowing waters which, due to limestone bedrock, are almost absent in the wider area of Mt. Snežnik, while the closest substantial watercourse is approximately 7 km NE of the site of our observation.

Obviously, *N. lasiopterus* does not commonly occur along the transect of Leskova dolina, as it was recorded on only 2 out of 16 possible occasions (12.5%). Nevertheless, more detector and mist netting work should be done in the wider area of our site to be certain whether our observation was merely coincidental or whether *N. lasiopterus* bats are in fact common inhabitants of the area.

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First record of the European pond turtle *Emys orbicularis* (Linnaeus, 1758) near Kočevje, SE Slovenia

Prva najdba močvirske sklednice *Emys orbicularis* (Linnaeus, 1758) pri Kočevju, JV Slovenija

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The European pond turtle (*Emys orbicularis*) is one of the two representatives of the family Emydidae (Vamberger et al. 2015). It has a very wide range, from northern Europe to some places in North Africa in the south. It occurs in most European countries and countries around the Black Sea in the east (Fritz et al. 2007, Pedall et al. 2011). In Slovenia, individuals of *E. orbicularis* belong to the subspecies *E. o. hellenica* (Vamberger et al. 2015). It is the only indigenous freshwater turtle in Slovenia, common in most regions except in the Alpine area (Krofel et al. 2009). Larger populations have been found at Ljubljansko barje, in Bela krajina, the Sava Basin and on the Slovene coast (see Krofel et al. 2009, Vamberger & Kos 2011). Presence of European pound turtles is often unknown or underestimated owing to their timidity and the fact they surface only if completely undisturbed (Mršić 1997). New findings are therefore expected as also confirmed by Grželj & Grželj (2012).

During the Biology Students Research Camp 2014 (Raziskovalni tabor študentov biologije 2014), which took place in Kočevje from 18. to 29. 7. 2014, we discovered a new locality of the European pond turtle in the Rinža River near Kočevje. We have no knowledge of this species being previously confirmed in the Rinža River system within the UTM VL85 (Krofel et al. 2009). However, the pond turtle has been noted in the neighbouring UTM VL84, near the village of Kočevska reka, although in a different river system – the Reka River (Krofel et al. 2009).

The Rinža is a slow flowing river with a few areas along its course that exhibit wetland conditions, which persist even during low water level. Near the village of Mahovnik, the river expands and forms a swampy area next to the river bank, surrounded by woodland as a suitable habitat for the pond turtle (Fig. 1b, Mršić 1997). The vegetation in this stretch of the river is typical of lowland rivers: *Carex* sp., *Juncus* sp., *Iris pseudacorus*, *Lythrum salicornia*, with water surface covered by *Nuphar lutea* and *Potamogeton* sp. In this area, we attempted to check for the presence of European pond turtle by placing baited funnel traps (under the license 35601-32/2010-6 issued by the Slovenian Environment Agency – ARSO).

In the afternoon of 26. 7. 2014, we placed seven funnel traps in the Rinža River west from the village of Mahovnik near Kočevje. As bait, we used chopped pork liver and aquarium fish food. The traps were left there for two days and checked daily. At the end, we removed them.

On the second trapping day, 28. 7. 2014, one 10-13 year-old European pond turtle male (Fig. 1a) was captured in the trap set at N 45°38'52.9" and E 14°50'31.9". We measured, photographed and marked the individual with a special code using the method by Kuchling (1987). The male weighed 609 g, the carapace was 15.3 cm long and 12.4 cm wide and the plastron was 13.9 cm long, 8.9 cm wide and 6.1 cm high. Later we returned it to the same location where captured.

This new record is significant for the knowledge on distribution of the European pond turtle in Slovenia owing to the following two reasons. It confirms the presence of this species in the Rinža River system, where it had not been found before. The finding in this slow flowing river is also a new information on habitat use by the European pond turtle in Slovenia, as the species had previously been

recorded only in channels and standing water bodies (e.g. at Ljubljansko barje: Vamberger & Kos 2011). Additional and systematic surveys using funnel traps along the Rinža are needed to ascertain its distribution here. Other similar rivers with wetland conditions in Slovenia may also be potentially occupied by the European pond turtle and should be checked for its presence in the future.

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Figure 1. a) The captured 10-13 year-old European pond turtle male, *Emys orbicularis* (photo: Tina Urek); and b) its suitable habitat in the Rinža River at Mahovnik near Kočevje (photo: Marijan Govedič).
Slika 1. a) Ujeti samec močvirske sklednice, *Emys orbicularis,* star med 10 in 13 let (foto: Tina Urek); ter b) reka Rinža, v bližini Mahovnika pri Kočevju, kot njegov ustrezen habitat (foto: Marijan Govedič).

First record of dicephalism in the four-lined snake *Elaphe quatuorlineata* Lacépède, 1789 (Serpentes: Colubridae) from Montenegro

Prva najdba dvoglavega primerka progastega goža (*Elaphe quatorlineata*) v Črni Gori

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Dicephalism, also known as polycephaly or dicephaly, is a phenomenon of an animal having two heads (Kompanje & Hermans 2008). The existence of two-headed animals is well documented in mammals and reptiles (Hoser & Harris 1995, Swanson et al. 1997, Diong et al. 2003, McAllister & Wallach 2006, Wallach 2007, Spadola & Insacco 2009, De Albuquerque et al. 2010, Pezdirc et al. 2013). The first known appearance of dicephalism in reptiles is the 120 million-year-old fossil found in China (Buffetaut et al. 2007). In a review of 950 cases of dicephalism in snakes, it has been shown that two-headed individuals occur in 169 species of 93 genera in 8 families (Wallach 2007). There are 116 reports on two-headed snakes from Europe (Wallach 2007).
Two-headed snakes are rare in nature, but can occur more frequently in captivity (Wallach 2007). There are many possible causes of dicephaly: incomplete division of a single embryo; partial fusion of two embryos; abnormally low or high temperatures during incubation or gestation; regeneration after an embryonic lesion; anoxia during embryonic development; toxic effects of metabolic secretions during a prolonged sojourn in the oviduct; inbreeding depression from small population gene pools, back-crossing, designer morphs, and albinos; hybridization; environmental pollution; chemical toxins in captivity or exposure to radiation (Wallach 2007).

On 29.10.2014, during our fieldwork study on reptiles and amphibians of the Montenegrin coast, we found a live two-headed four-lined snake (*Elaphe quatuorlineata* Lacépède, 1789) juvenile (Fig. 1). The snake was found on the car parking lot at Dobrota, Kotor (42° 26′ 32.40″ N, 18° 46′ 12.80″ E; 5 m a.s.l.) (Fig. 2), where a cat was playing with it. Heads were completely separated, well developed and both with two eyes. It was about 20 cm long, but we could not determine the gender. After taking the photo and coordinates we left the snake where we found it.

Wallach (2007) reports on two-headed *Elaphe quatuorlineata* individuals from Europe, but without exact location or country. Our observation represents a novel contribution to the knowledge on dicephalism in natural populations of snakes, and is the first reported record of *Elaphe quatuorlineata* dicephaly in Montenegro.

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Figure 1. *Elaphe quatuorlineata* with two heads found on 29.10.2014 at Dobrota, Kotor (photo: N. Čavor).
Slika 1. *Elaphe quatuorlineata* z dvema glavama, najden 29.10.2014 v Dobroti, Kotor (foto: N. Čavor).

Figure 2. Map with marked locality (red circle) where the two-headed *Elaphe quatuorlineata* was found.
Slika 2. Karta z označeno lokaliteto (rdeč krog), kjer je bil najden dvoglavi *Elaphe quatuorlinea*.

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