# ARTHROPOD SYSTEMATICS & PHYLOGENY

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# Further clarifications to the systematics of the cave beetle genera *Remyella* and *Rozajella* (Coleoptera: Leiodidae: Cholevinae: Leptodirini)

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## Abstract

The subtribe Leptodirina is one of the most species-rich subtribes of the tribe Leptodirini, comprising 36 genera and 103 species of beetles adapted to the subterranean environment and distributed in the West Paleartic. The genera of Leptodirina show potentially convergent morphological characters resulting from the adaptation to the subterranean environment. Two genera with uncertain systematic position, living in Sandžak, a geo-political region divided by the border of Serbia and Montenegro, *Rozajella* S. Ćurčić, Brajković & B. Ćurčić, 2007 and *Remyella* Jeannel, 1931, were recently tentatively placed in this subtribe. However, no molecular phylogenetic studies have been performed to support this placement. Both genera share morphological characters and live in neighbouring geographic areas (Pešter polje and surrounding massifs). We performed a molecular phylogenetic analysis to examine the phylogenetic position of these two genera. A more detailed molecular and morphological investigation of the taxa within *Remyella* clarified their systematics. The speciation events within *Remyella* occurred approximately from 5 to 2 million years ago, which corresponds to the end of the Miocene and the Pliocene, periods when Pešter was periodically filled by shallow lakes. The distribution area of the genus, confined to the periphery of Pešter, is probably contingent to the presence of these lakes that prevented species from spreading more uniformly over the entire area of the plateau. Additionally, the correlations between genetic and geographic distances were investigated using the Mantel test. Four synonymies inside *Remyella* are established and all species of the genus are briefly diagnosed. Identification tables and distribution maps of species of *Remyella* and *Rozajella* are provided, and the new species *Rozajella madzgalji* **n.sp.** is described.

### Key words

Leptodirini, speciation by distance, Montenegro, Serbia, troglobites.

## 1. Introduction

The tribe Leptodirini Lacordaire, 1854 is the most speciose tribe in the subfamilly Cholevinae of the family Leiodidae (Coleoptera) and comprises 195 genera and 925 (mostly polytypic) species (NEWTON 2016). Most species are more or less adapted to subterranean environments and mostly fully anophthalmic. The characters shared by all members of this tribe are: female protarsi tetramerous and spiculum gastrale of the abdominal IX segment in



Fragment	Name	Sense	Sequence 5'-3'	Reference
COla	LCOI-1490	F	GGTCAACAAATCATAAAGATATTG	FOLMER et al. (1994)
COla	HCOI-2198	R	TAAACTTCAGGGTGACCAAAAAATCA	FOLMER et al. (1994)
COIb	Jerry	F	CAACATTTATTTTGATTTTTTGG	SIMON et al. (1994)
COIb	Pat	R	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)
18S	5′	F	GACAACCTGGTTGATCCTGCCAGT	Shull et al. (2001)
<i>18S</i>	b5.0	R	TAACCGCAACAACTTTAAT	SHULL et al. (2001)
285	Ка	F	ACACGGACCAAGGAGTCTAGCATG	RIBERA et al. (2010)
285	Kb	R	CGTCCTGCTGTCTTAAGTTAC	RIBERA et al. (2010)

Table 1. Primers used in the study.

Table 2. PCR cycling conditions. Number of cycles: 40.

Temperature (°C)	Time (min)
95	3:00
94	0:15
54	0:30
72	0:40
72	5:00
12	∞

males fused with lateral epipleurites. Most species have a complete mesoventral processus separating the two mesocoxal cavities (except a few ones in which this structure secondarily disappeared). Except a single nearctic and myrmecophilous genus, Leptodirini have a Palearctic distribution: Iberian Peninsula, Appeninian Peninsula, Balkan Peninsula, southern Alps, Romania, southern Russia, the Caucasus, Middle East, and Iran, with the highest diversity in the Mediterranean basin (PERREAU 2000, 2015; RIBERA et al. 2010). The placement of extreme oriental genera is under discussion (FRESNEDA et al. 2011).

The phylogeny of Leptodirini remains poorly understood, although several systematic and evolutionary studies were recently performed on this group (RIBERA et al. 2010; FRESNEDA et al. 2011; CIESLAK et al. 2014). Since the pioneering work of CACCONE & SBORDONI (2001), molecular phylogenetic studies of Leptodirini focus on Western Mediterranean genera (Ribera et al. 2010; FRESNEDA et al. 2007) or Eastern Mediterranean genera (PERREAU & PAVIĆEVIĆ 2008b; NJUNJIĆ et al. 2016). The Leptodirini subtribe Anthroherponina shows the most pronounced troglomorphic morphological characters of all known Coleoptera: extremely long appendages, extreme elongation of the head and pronotum, and hemispherical elytra (NJUNJIĆ et al. 2015). The subtribe Anthroherponina was traditionally defined by the position of the antennal insertions on the posterior third of the head and the enlarged and apically blunt claws (JEANNEL 1924). JEANNEL (1924) used this combination to characterize the "Anthroherpona" composed of the "phyletic series of Anthroherpon" (today: subtribe Anthroherponina) and the "phyletic series of Spelaeobates" (today: subtribe Spelaeobatina sensu Guéorguiev 1974). The latter is nowadays generally considered to be phylogenetically more closely related to Bathysciina (CASALE et al. 1991) than Anthroherponina. JEANNEL (1931) placed the genus *Remyella* in the Anthroherponini (today: subtribe Anthroherponina) based on the insertion of the antennae on the posterior third of the head, and despite its claw conformation different from other genera of this subtribe (narrow in *Remyella* and wide in Anthroherponina). PERREAU & PAVIĆEVIĆ (2008b) reconstructed the phylogeny of the subtribe Anthroherponina based on morphological characters and excluded the genus *Remyella* from this subtribe, placing it tentatively into the subtribe Leptodirina. The monophyly of Leptodirina, however, is highly questionable (PERREAU & PAVIĆEVIĆ 2008b), especially because many species are highly troglomorphic and many characters are potentially homoplasious.

To allow a better understanding of the evolution and phylogeny of Leptodirini, we examined the phylogenetic positions of *Remyella* and *Rozajella*, two genera sharing morphological characters and living in neighbouring geographic areas (Pešter polje and surrounding massifs) using a molecular approach. A detailed molecular and morphological investigation of the taxa within *Remyella* is also provided, since up to now the morphological distinctions of species and subspecies are tenuous, their geographical distribution hardly coherent, and their validity disputable. Additionally, we describe a new species: *Rozajella madzgalji* **n.sp.** and the male of *R. deelemani*, of which only females were previously known.

#### 2. Material and methods

#### 2.1. Acronyms

The following abbreviations for collections and institutions are used: **CDPV**: Dragan Pavićević Collection, Belgrade, Serbia; **CINJ**: Iva Njunjić Collection, Leiden, The Netherlands; **CMPR**: Michel Perreau Collection, Paris, France; **MNHN**: Muséum National d'Histoire Naturelle, Paris, France; **Naturalis**: Naturalis Biodiversity Center, Leiden, The Netherlands. — The following abbreviations are used for type specimens: **HT**: holotype; **PT**: paratype. — In Table 3, **TL** means: type locality.

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Coordinates (WGS84)	N43°06'57.03" E20°22'15.36"		N43°20'38.86" E19°57'17.74"		N43°04'52.9" E19°55'23.2"	N43°04'35.2" E19°55'21.9"	N43°08'40.4" E19°59'26.8"				N43°04'39.5" E20°02'09.8"		N43°04'49.1" E20°00'11.7"	N43°03'54.0″ E20°02'48.2″	N43°02′46.5″ E20°02′14.0″	N43°02'36.7" E20°02'20.9"				N42°59'35.0″ E20°06'00.0″	N42°59'35.0" E20°06'00.0"	N42°59'35.0" E20°06'00.0"	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0"	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0"	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0" N42°58'51.0" E20°05'55.0"	N42°59'35.0° E20°05'00.0° N42°58'48.7° E20°05'55.0° N42°58'51.0° E20°07'13.0° N42°58'51.0° E20°07'13.0°	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0" N42°58'51.0" E20°05'55.0" N42°58'51.0" E19°54'26.1"	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0" N42°58'51.0" E20°05'55.0" N42°50'06.7" E19'54'26.1" N42°50'16.7" E19'54'26.1"	N42°59'35.0" E20°06'00.0" N42°58'48.7" E20°05'55.0" N42°58'51.0" E20°05'13.0" N42°50'06.7" E19'54'26.1" N42°52'15.7" E19°55'10.5"
Location	Novi Pazar	Ursule	Ušak	Grgaje	Crvsko	Đalovići	Lopužino brdo	Kapeš	Kapeš	Kapeš	Doliće	Doliće	Boljare	Ugao	Ugao	Ugao	Ugao	Suvi Do		Đerekare	Derekare	Đerekare Đerekare Đerekare	Berekare Berekare Berekare Konito	Berekare Derekare Derekare Konito Konito	Berekare Berekare Berekare Konto Konto Berekare	Berekare Berekare Derekare Korito Korito Berekare Petnik	Berekare Berekare Derekare Korito Korito Berekare Petnik Donje Biševo	Berekare Derekare Derekare Korito Korito Berekare Petnik Zagrađe	Berekare Derekare Derekare Korito Korito Berekare Petnik Donje Biševo Zagrađe Zagrađe
Cave	Pećina na Vrelu Raške	Baždarska pećina	Ušačka pećina	Velika pećina	Jagoševa pećina	Đalovića pećina = Pećina nad Vražjim Firovima	Lopužina jama	Uleva pećina III	Pećina u Anin Kapeš	Pećina Ispod Gluare	Jama bez dna	Pećina kod Jagodina Dola	Kaćunova Jama	Špela Bores	Špela Hajnet (= Špela Hajnit = Špela Maja Hajnet)	Špela Vogel (= Maja Vogelj)	Sinkhole of Boroštica	Doćina u Lamidavaj vrtači		seura u ramovoj vracu Sijera jama (= Sijera livada)	гесла и палномој у каси Sijera jama (= Sijera livada) Jama u Piskovoj livadi	recina u namuovoj vraci Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice	Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina	Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pećina u vrh Livade Radojeve	recina u namuvoj vracu Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pećina u vrh Livade Radojeve Pećina na Đerekarskom vrelu	recina u namuouoj vracu Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pečina u vrh Livade Radojeve Pečina na Đerekarskom vrelu Građa Pećina	recina u namiouvoj vracu Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pećina u vrh Livade Radojeve Pećina na Đerekarskom vrelu Građa Pećina Pećina u Dubokom potoku	Sijera jama (= Sijera livada) Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pećina u vrh Livade Radojeve Pećina na Đerekarskom vrelu Građa Pećina Mala pećina u Garaškom kršu	Sijera jama (= Sijera livada) Jama u Piskovoj livadi Ponor Ledenice Ledenica pećina Pećina u vrh Livade Radojeve Pećina na Đerekarskom vrelu Građa Pećina Pećina u Garaškom kršu Wala pećina u Garaškom kršu
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Table 3. Populations and localities investigated. Numbers in 1st column refer to the map of Fig. 39.

Species	voucner	LOCALITY	Source		Genbank	accession	
	code			18S	28S	COla	COIb
Anthroherpon cylindricolle cylindricolle (Apfelbeck, 1889)	IE3	BiH, Pale, Rogatica, Golubovića pećina	I. Njunjić	KU980189	KX121055	KX121089	KX121110
Anthroherpon gang/baueri gang/baueri (Apfelbeck, 1894)	IE11	BiH, Nevesinje, Novakuša	I. Njunjić	KU980190	KX121077	KX121090	KX121111
Anthroherpon hoermanni hoermanni (Apfelbeck, 1889)	187	BiH, Kalinovik, Miljevina, Borija pećina	I. Njunjić	KU980192	KX121061	KX121092	KX121113
Anthroherpon matzenaueri matzenaueri (Apfelbeck, 1907)	IB13	MNE, Golija, Latično, Jama u Rudinskom dolu	I. Njunjić	KU980193	KX121057	KX121086	KX121116
Anthroherpon pygmaeum stricticolle (Jeannel, 1930)	IE25	BiH, Bjelašnica, Opančak, Ledenjača	I. Njunjić	KU980178	KX121064	KX121085	KX121118
Anthroherpon stenocephalum stenocephalum (Apfelbeck, 1910)	IE44	BiH, Olovo, Bijambare	I. Njunjić	KU980195	KX121066	KX121096	KX121119
Anthroherpon taxi taxi (Müller, 1913)	IE55	MNE, Kameno more, Dvestotka	I. Njunjić	KU980185	KX121067	KX121097	KX121120
Antrocharis querithaci dispar Abeille de Perrin, 1878	MNCN-AI588	Ariège, Aigües-Juntes, Grotte Les Cloutets	A. Faille	GU356905	GU356942	GU356851	I
Apholeuonus nudus sturanyi Apfelbeck, 1906	IAP3	BIH, Kalinovik, Borija, Borija pećina	I. Njunjić	KU980171	KX121082	KX121100	I
Apholeuonus nudus sturanyi Apfelbeck, 1906	IAP4	BIH, Kalinovik, Borija, Borija pećina	I. Njunjić	KY397768	KY397806	1	I
Charonites sp. Apfelbeck, 1907	1033	BIH, Pale, Omladinska pećina	I. Njunjić	KU980172	KX121083	KX121101	KX121123
Hadesia vasiceki Müller, 1911	MP05	BIH, Popovo polje, Vjeternica	M. Perreau	KY397769	KY397804	1	KY397861
Hadesia vasiceki Müller, 1911	IH3	BIH, Popovo polje, Vjeternica	M. Perreau	KY397770	KY397805	KY397840	KY397860
Hadesia weiratheri Zariquiey, 1927	IH2	Crna Gora, Orjen, Kameno more, PT4	M. Perreau	KU980177	KX121076	KX121104	KX121128
Leptomeson bujasi Giachino, Bregović, Jalžić, 2011	IL6	Croatia, Brač, Jama kod Matešića stana	B. Jalžić & P. Kutleša	KY397772	KY397808	KY397842	KY397862
Leptomeson radjai Giachino, Bregović, Jalžić, 2011	115	Croatia, Šibenik, Perković, Zvekača	P. Bregović	KY397771	KY397807	KY397843	KY397863
Parapropus sericeus muelleri Jeannel, 1924	IE35	BIH, Sanski most, Hrustovačka pećina	I. Njunjić	KY397773	KY397809	KY397841	KY397864
Parapropus sericeus muelleri Jeannel, 1924	1026	BIH, Sanski most, Hrustovačka pećina	I. Njunjić	KU980173	KX121084	KX121087	KX121133
Parapropus pfeiferi Apfelbeck, 1908	IE34	BiH, Sanski most, Donji Kamičak, pećina u Donjem Kamičaku	I. Njunjić	KY397774	KY397810	KX671647	KX671679
Remyella hussoni Jeannel, 1934	IR6	Serbia, Pešter, Đerekare, Piskova livada	P. Lazarević	КҮЗ97793	KY397829	KX671639	KX671687
Remyella hussoni Jeannel, 1934	IR19	Serbia, Pešter, Đerekare, Sijera livada, Ponor Ledenice	D. Pavićević	KY397792	KY397828	KY397844	KY397865
Remyella hussoni Jeannel, 1934	IR20	Serbia, Pešter, Đerekare, Sijera jama	S. Ognjenović	KX755498	KX719972	KX671644	KX671683
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	MP07	Serbia, Sjenica, Ursule, Baždarska pećina	S. Ognjenović	KY397786	KY397822	1	KY397869
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	IR10	Serbia, Sjenica, Ursule, Baždarska pećina	S. Ognjenović	KY397789	KY397825	Ι	KY397867
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	IR8	Serbia, Sjenica, Ursule, Baždarska pećina	S. Ognjenović	KY397790	KY397826	KY397845	KY397866
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	IR3	Serbia, Sjenica, Ursule, Baždarska pećina	S. Ognjenović	KY397791	KY397827	KX671643	KX671684
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	IR21	Serbia, Sjenica, Donje Lopiže, Ušačka pećina	I. Njunjić	KY397787	KY397823	KY397846	KY397868
Remyella javorensis S. Ćurčić & B. Ćurčić, 2008	IR14	Serbia, Sjenica, Donje Lopiže, Ušačka pećina	I. Njunjić	KY397788	KY397824	KX671642	KX671685
Remyella propiformis Winkler, 1933	VRE1	Serbia, Pešter, Crvsko, Jagoševa pećina	M. Popović	KY397776	KY397812	KX671641	KY397873
Remyella propiformis Winkler, 1933	IR2	Serbia, Pešter, Crvsko, Jagoševa pećina	M. Popović	KY397783	KY397819	KY397848	I
Remyella propiformis Winkler, 1933	IR13	Serbia, Pešter, Dolići, Jama bez dna	S. Ognjenović	КҮЗ97778	KY397814	KX671638	KX671688
Remyella propiformis Winkler, 1933	IR7	Serbia, Pešter, Dolići, Jama bez dna	S. Ognjenović	KY397780	KY397816	KY397851	I
Remyella propiformis Winkler, 1933	IR1	Serbia, Pešter, Dolići, Jama bez dna	S. Ognjenović	KY397784	KY397820	KY397847	I
Remyella propiformis Winkler, 1933	IR18	Serbia, Pešter, Boljare, Kaćunova jama	S. Ognjenović	KY397777	KY397813	KX671645	KX671682
Remyella propiformis Winkler, 1933	IR5	Serbia, Pešter, Kaćunova pećina	M. Popović	KY397781	KY397817	KY397850	KY397871
Remyella propiformis Winkler, 1933	IR9	Serbia, Pešter, Lopužino brdo, Lopužina jama	M. Popović	КҮЗ97779	KY397815	KY397852	KY397872

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Species	Voucher	Locality	Source		GenBank	accession	
	code			185	28S	COla	COIb
Remyella propiformis Winkler, 1933	IR4	Serbia, Pešter, Lopužino brdo, Lopužina jama	M. Popović	KY397782	KY397818	KY397849	KY397870
Remyella propiformis Winkler, 1933	IE8	Serbia, Pešter, Lopužino brdo, Lopužina jama	M. Popović	KY397785	KY397821	KX671640	KX671686
<i>Remyella</i> sp. Jeannel, 1910	IR16	Serbia, Pešter, Đerekare, Vrelo Đerekarske reke	S. Ognjenović	KY397775	KY397811	KX671646	KX671681
Rozajella deelemani Perreau & Pavićević, 2008	IR07	Cma Gora, Berane, Petnjik, Građa pećina	I. Njunjić	KY397803	KY 397839	KY397853	KY397874
Rozajella deelemani Perreau & Pavićević, 2008	IR08	Crna Gora, Berane, Petnjik, Građa pećina	I. Njunjić	KY397802	KY 397838	KX671637	KX671689
Rozajella deelemani Perreau & Pavićević, 2008	IR09	Cma Gora, Berane, Petnjik, Građa pećina	I. Njunjić	KY397801	KY397837	KY397854	KY 397875
<i>Rozajella madzgalji</i> n.sp.	VR1	Crna Gora, Berane, Goražde, Mala pećina na Gareškom kršu	I. Njunjić	KY397795	KY397831	KY397858	KY397880
<i>Rozajella madzgalji</i> n.sp.	VR2	Crna Gora, Berane, Goražde, Mala pećina na Gareškom kršu	I. Njunjić	KY397794	KY397830	KY397859	KY397881
<i>Rozajella madzgalji</i> n.sp.	IR01	Crna Gora, Berane, Goražde, Mala pećina na Gareškom kršu	I. Njunjić	KY397800	KY397836	1	KY 397876
<i>Rozajella madzgalji</i> n.sp.	IR02	Crna Gora, Berane, Goražde, Velika pećina na Gareškom kršu	I. Njunjić	KY397799	KY 397835	KY397855	KY397877
<i>Rozajella madzgalji</i> n.sp.	IR03	Crna Gora, Berane, Goražde, Velika pećina na Gareškom kršu	I. Njunjić	KY397798	KY397834	KX671636	KX671690
<i>Rozajella madzgalji</i> n.sp.	IR04	Cma Gora, Berane, Goražde, Velika pećina na Gareškom kršu	I. Njunjić	KY397797	KY 397833	KY397856	KY 397878
<i>Rozajella madzgalji</i> n.sp.	IR05	Cma Gora, Berane, Goražde, Velika pećina na Gareškom kršu	I. Njunjić	KY397796	KY397832	KY397857	KY 397879
Speonesiotes sp. Jeannel, 1910	1039	BIH, Trebinje, Zupci, Vilina pećina	I. Njunjić	KU980170	1	KX121108	KX121134
Speonomus (Speonomus) diecki (Saulcy, 1872)	MNCN-AI536	Ariège, Moulis, Grotte d'Aubert	A. Faille	GU356938	GU 356986	GU356896	1
Stygiophyes akarsticus (Escolà, 1980)	NHM-IRC7	Lleida, Altron, Forat del Tincatinc	J. Fresneda	GU356940	GU 356990	GU356899	I

#### 2.2. Taxon sampling

A total of 48 specimens representing 21 taxa from 9 genera were collected for molecular analysis in caves of the Dinaric range, in Montenegro, Serbia, and Bosnia and Herzegovina as listed in Table 4. For amplification and sequencing we used seven specimens of R. madzgalji n.sp., three specimens of R. deelemani and one or two specimens per population of other genera. Eleven populations of Remyella were available for molecular investigations and 24 populations for morphological investigations, making a total of 290 specimens. For outgroup rooting we chose three species of the subtribe Pholeuina: Antrocharis querilhaci (Lespes, 1857), Stygiophyes akarsticus (Escolà, 1980), and Speonomus diecki (Saulcy, 1872), of which sequences are available from RIBERA et al. (2010). All populations examined (including molecular and morphological studies) of the genera Remyella, Rozajella, and Nonveilleriella are listed in Table 3.

#### 2.3. Morphological study

After extraction, male genitalia were cleared in clove oil, mounted in Euparal on glass slides, glued on cardboard and pinned beneath the specimens. The external morphology of specimens was examined using Leica MZ75 and Leica M10 stereomicroscopes. Microphotographs (Figs. 8-27) were taken on a Leica DIAPLAN or a Zeiss AXIOLAB microscope with a camera diagnostic instrument Spot INSIGHT IN1820. Photographs of habitus (Figs. 2, 3) were taken using a Nikon SMZ 1000 stereomicroscope with a Canon EOS 60D camera. Digital pictures were processed using the Helicon Focus software 5.3 (http://www.heliconsoft.com/heliconsoft-products/helicon-focus/). High resolution pictures of external morphology were taken with a microscope Keyence VHX5000 with lenses VH-Z20T (Figs. 28-35) and VH-Z250T (Figs. 4-7, 36-37).

#### 2.4. DNA extraction, PCR amplification, and sequencing

The specimens used for DNA extraction were collected alive in the field and preserved in 96% ethanol. DNA was extracted from whole specimens or from one leg with a standard phenol-chloroform extraction (BLIN & STAF-FORD 1976) or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Voucher specimens are stored in the MNHN (Paris, France) and DNA aliquots are kept in the tissue collections of Naturalis (Leiden, The Netherlands).

We amplified fragments of two nuclear genes: 5' end of the small ribosomal subunit, 18S rRNA (SSU), and an internal fragment of the large ribosomal subunit, 28S rRNA (LSU), and of two non-overlapping sections of mitochondrial gene fragments: the 5' and 3' halves of cytochrome c oxidase subunit 1 (which we here term COIa and COIb, respectively). Primers used are given in Table 1, and PCR protocols are given in Table 2. Sequences were assembled and edited using Geneious version 8.0.5 (http://www.geneious.com/, KEARSE et al. 2012). DNA sequences obtained for each genetic marker were aligned separately using MAFFT version 7 (KATOH & STANDLEY 2013). The alignments, including those of 18S and 28S, were unambiguous. Sequences have been deposited in GenBank with Accession Numbers given in Table 4. The alignment block as it went into the analyses is available in the Electronic Supplement File 1.

#### 2.5. Phylogenetic analyses

For each gene separately, we performed a MrBayes analysis in Geneious version 8.0.5. (http://www.geneious. com, KEARSE et al. 2012) using default settings and did not found any conflict between the topologies obtained (data not shown/available on request). With one minor exception (see: Results and discussion) single gene topologies were fully congruent. We therefore proceeded with a concatenated analysis rather than a species tree approach, because we were primarily interested in species- and genus-level phylogenetic structure, i.e. the deeper divergences for which we do not expect incomplete lineage sorting or horizontal gene transfer to be an issue, and also because both methods tend to give very similar results (TONINI et al. 2015). For each sequence alignment, the optimal model of nucleotide substitution was determined using jModelTest2 (DARRIBA et al. 2012; GUINDON & GASCUEL 2003), run on the CIPRES webportal (MILLER et al. 2010). Selection was based on the Akaike Information Criterion (AIC), and resulted in selection of SYM+I, GTR+G, HKY+I+G, and GTR+I+G substitution models for 18S, 28S, COIa and COIb, respectively. Phylogenetic analysis was performed using MrBayes 3.2.1 (RONQUIST & HUELSENBECK 2003) on CIPRES (MILLER et al. 2010), with settings for multiple markers unlinked, running two replicates of  $15 \times 10^6$ generations each. Sample frequency was set to 5000. After discarding a 25% burn-in, the resulting majority-rule consensus tree was visualized using FigTree version 1.4 (RAMBAUT 2012). The correlations between genetic and geographic distances were investigated using the Mantel test through the software XLSTAT.

#### 2.6. Map

The map of Fig. 39 was built using SRTM3 data (Shuttle Radar Topography Mission) of NASA with the software QGIS. GDAM (global administrative area: www.gdam. org) data were used for country frontiers and Natural Earth data (http://www.naturalearthdata.com/) were used for hydrographic features: rivers and lakes. Other marks (location of species and caves, geographical names, and delimitations of distributions areas) were inserted with Photoshop CS2.

### 3. Results and discussion

# 3.1. Phylogenetic relationships and systematic placement of *Remyella*, *Rozajella*, and *Nonveilleriella*

In the single gene analyses we found full congruence with one minor exception, namely one individual of Rozajella deelemani grouped with R. madzgalji n.sp. in the 18S tree (data not shown but available on request). This is due to a single nucleotide difference which could be a result of intraspecific polymorphism or experimental contamination. The molecular investigations based on concatenated 18S, 28S, and COI gene fragments reveal the close relationship of Remyella and Rozajella (posterior probability = 1.00) (Fig. 1). Three genera of the subtribe Anthroherponina (Leptomeson Jeannel, 1924, Anthroherpon Reitter, 1889, and Hadesia Müller, 1911) form a highly supported clade (posterior probability= 1.00). This clade appears as the sister group of the genus Speonesiotes (subtribe Bathysciotina). However, this relationship is weakly supported (posterior probability= 0.56) and is unlikely on morphological grounds.

*Rozajella* and *Remyella* were previously (and tentatively) placed in Leptodirina (PERREAU & PAVIĆEVIĆ 2008a,b). On the basis of our phylogenetic analysis, the polyphyly of Leptodirina as presently defined appears clearly, and the subtribal assignation of *Remyella*, *Rozajella* (and also *Nonveilleriella* Perreau & Pavićević, 2008) should be reconsidered. However, the clarification of this question requires considering the whole tribe Leptodirina and not only the small number of genera used in this work (*Apholeuonus* Reitter, 1889, *Charonites* Apfelbeck, 1907, and *Parapropus* Ganglbauer, 1899). That is, however, out of the scope of this paper.

Even though we do not have genetic data for the genus Nonveilleriella, based on its morphology, this genus is clearly closely related to the genus Rozajella. They share the following apomorphic character states, that are normally not included in any troglomorphic syndrome and thus are likely not the result of adaptation to life in caves: (i) strong and dense microsculpture of the pronotum; (ii) explanate surface of the pronotum near the posterior angles (Fig. 6); (iii) punctuation of elytra transversely aligned with transverse strigae (Fig. 7) (also sporadically aligned in some specimens of *Remyella*, but without strigae). Punctuation of elytra is never transversally aligned in other troglomorphic species of Leptodirina or Anthroherponina (except some traces of alignments in Anthroherpon udrzali Giachino & Vailati). With Remvella, these two genera share the "V"-shape of the metendosternite (Figs. 10, 12, 13) rather than the "Y"shape (with a longer and thinner basal part) that is found in most other troglomorphic species of Leptodirina, for instance Anthroherpon primitivum Absolon (Fig. 8) and Parapropus sericeus Schmidt (Fig. 9).

These three genera (*Remyella*, *Rozajella*, and *Non-veilleriella*) are distributed over Sandžak (a historical



Fig. 1. Bayesian inference of combined 18S, 28S, COIa, and COIb data sets for *Remyella* and *Rozajella*. Numbers above nodes are posterior probabilities.

geo-political region straddling the border between Serbia and Montenegro). *Remyella* lives in Pešter polje and the following surrounding massifs: Jadovnik (1734 m), Javor (1520 m), Giljeva (1617 m), Ninaja (1358 m), Žilindar (1616 m); *Rozajella* in the karstic area located south from Pešter between Berane and Rožaje; *Nonveilleriella* in the massif Žljeb located on the northern border of the Kosovo plain (Fig. 39). They can be distinguished with the following identification key:

- 2' Antennae short relative to the body length, shorter or at most as long as the body (Figs. 2, 3) ..... *Rozajella*

#### 3.2. Phylogeny of Remyella

The genus Remyella was described by JEANNEL in 1931 with the single species R. scaphoides. Two additional taxa were subsequently introduced by WINKLER (1933): R. propiformis and R. borensis. The genus was reinvestigated by JEANNEL (1934), adding two taxa: R. hussoni and R. puncticollis, and later revised by GIACHINO & ETONTI (1995) with one new taxon, R. droveniki, and the synonymy of R. puncticollis with R. borensis. Three new taxa were subsequently described by S. Ćurčić et al. (2008; 2013). Before this work, four species were recognized: R. javorensis, R. montenegrina, R. raskae, and R. scaphoides, with the latter one comprising five subspecies: R. s. borensis, R. s. droveniki, R. s. hussoni, R. s. propiformis, and R. s. scaphoides. Most caves in which Remyella were collected were described in REMY (1953) and NESIĆ (2015).

The distribution area of the genus *Remyella* is located on the border of the Pešter polje (Fig. 39). It is divided into three isolated areas that correspond to the three disconnected karstic areas (Fig. 39):

(1) In the surroundings of Sjenica (dark blue line), on the north-western side of Pešter polje, two caves hosting *R. javorensis*: Ušačka pećina (= Ušački pećinski sistem) in Gornje Lopiže and Baždarska pećina (the type locality) near Ursule.

(2) In the surroundings of Novi Pazar, on the northeastern side of Pešter polje, Pećina u vrelu Raške (the springs of the Raška river), the type locality and only known locality of *R. raskae* (yellow dot).

(3) The third area is a zone 25 km long and 10 km wide, oriented NW-SE on the southern border of the Pešter polje, along the frontier between Serbia and Montenegro. In this area, 20 caves host populations previously assigned to six taxa: *R. s. borensis, R. s. droveniki, R. montenegrina, R. s. propiformis, R. s. puncticollis*, and *R. s. scaphoides*. Velika pećina near Grgaje, the type locality of *R. scaphoides*, is the westernmost population of this area (pink dot). Then, from west to east we find a set of populations including two populations in Montenegro near Đalovići (Đalovića pećina, the type locality of *R. montenegrina* and Jagoševa pećina), the northernmost population on Giljeva mountain (Lopužina jama in

Lopužino brdo), three caves in the vicinity of Kapeš (Uleva pećina III, the type locality of R. droveniki, Pećina u Anin Kapeš, Pećina Ispod Gluare), three in the vicinity of Boljare and Doliće (Jama bez dna, Pećina kod Jagodina Dola, Kaćunova Jama), then four caves in the vicinity of Ugao (Špela Hajnet, the type locality of *R. scaphoides* propiformis, Špela Bores, the type locality of R. scaphoides borensis, Špela Vogel, and sinkhole of Boroštica river) (light blue line). After another gap of 4.5 km, five caves are located in the western vicinity of Derekare, on the Serbian side (Pećina u Hamidovoj vrtači, the type locality of R. scaphoides hussoni, Pećina u Hamidovoj vrtači, Sijera jama, Jama u Piskovoj livadi, Ponor Ledenice) and on the Montenegrian side (Ledenica pećina, the type locality of R. puncticollis, Pećina u vrh livade Radojeve) (green line). The easternmost population of this area is Đerekarsko vrelo: the spring of the Boroštica river (= Đerekarska reka river (NEŠIĆ 2015)) which hosts a likely new species, not described in this paper due to the small number of specimens presently available.

Except for the two geographically clearly remote (Fig. 39) and morphologically distinct species, R. javorensis and R. raskae, the populations of other taxa, especially the subspecies of R. scaphoides, but also R. montenegrina, are geographically and morphologically very close together. They are distributed in caves only a few kilometres apart from each other, on a single karstic plateau without any obvious geological barriers able to induce such a high level of endemism. Moreover, the distributions of some subspecies are overlapping. For example, GIACHINO & ETONTI (1995) reported two subspecies located in the village Ugao: R. scaphoides borensis from Špela Hajnit and R. scaphoides propiformis from Špela Maja Hajnet. During our field work, local people from this village explained to one of the authors (D. Pavićević) that these are different names for the same cave: Spela Hanjet, meaning that two subspecies are reported from a single cave. Even though this situation clearly comes from a confusion in the denomination of the cave, it casts a serious doubt on the validity of the morphological criteria used to distinguish these subspecies.

Morphological characters originally used to distinguish *Remyella* species (JEANNEL 1931, 1934; WINKLER 1933) have been further discussed by GIACHINO & ETONTI (1995). The measurements given by the latter authors show that the differences in the length/width ratios of the head, pronotum, and elytra between taxa are small and do not exceed intrapopulational variations. Giachino and Etonti based their taxonomical divisions on a single character of the relative location and relative distances of the three apical setae of the parameres. However, looking at their illustrations (GIACHINO & ETONTI 1995: 85), it is clear that these differences also do not exceed usual intrapopulational variability.

To clarify this intricate situation, we performed a molecular genetic analysis and reinvestigated the morphology. The populations of *Remyella* are listed in Table 3, including already known localities, as well as newly explored caves or pits. Table 3 also gives taxonomic names



Figs. 2–7. 2: *Rozajella madzgalji* n.sp. holotype, habitus. 3: *R. deelemani* male, habitus. 4: *Remyella javorensis*, profemur. 5: *Rozajella madzgalji* n.sp., profemur. 6: *Nonveilleriella ognjenovici*, explanate posterior angle of pronotum (arrow). 7: *Rozajella madzgalji* n.sp., elytral punctuation.

used by previous authors and the taxonomic assignations as resulting from our present work.

The results of the molecular phylogenetic analysis are shown in Fig. 1, and the genetic distances are given in Fig. 38. The populations are initially identified by their locality rather than by taxonomic names, as their final taxonomic assignation will be contingent on the results of the combined phylogenetic analysis and morphological characterization.

Our phylogenetic tree first confirms the monophyly of the genus *Remyella* and reveals the following nesting of populations. For simplicity, we start with the terminal branches containing the populations near Doliće (Jama bez dna) and those near Đalovići (Jagoševa pećina). These form the sister group of the populations near Boljare (genetic distances 0.6-1.5% from the population of Jagoševa pećina), and subsequently these three populations are (although with very low support) sister to the population from Lopužina jama (genetic distance 2.9% from populations of Doliće). The sister clade of the preceding group are the two populations of Sijera jama and Jama u Piskovoj livadi near Đerekare (genetic distances 3.6-4.4% from populations of Đalovići, Doliće and Boljare). More basal in the tree we find the separation of the populations from Baždarska pećina and Ušačka pećina, genetically divergent by 7.3% but morphologically identical (see section 3.3). The earliest separation within *Remyella* leads to the population from the spring of Boroštica (Đerekarsko vrelo), which shows a genetic distance of more than 9% from the other populations located also near Đerekare, despite their geographical proximity. This population of Đerekarsko vrelo is therefore enigmatic, and we exclude it presently from this study awaiting new material for further investigations.

We have no available specimens for molecular analysis of the following populations: the single known locality of *R. scaphoides* (Velika pećina); the single known locality of *R. raskae*; populations located near Ugao (Špela Hajnet, Špela Bores, Špela Vogel, and the Sinkhole of Boroštica), Pećina u Hamidovoj vrtači (the type locality of *R. hussoni*); and populations of Ledenica pećina and Pećina u vrh livade Radojeve (both assigned to *R. puncticollis* by Jeannel).

*R. raskae* and *R. scaphoides* are both known from a single and geographically isolated location are also morphologically well characterized. Populations of Ušačka



Figs. 8–27. Internal characters. 8: Anthroherpon primitivum, metendosternite. 9: Parapropus sericeus, metendosternite. 10: Remyella javorensis, metendosternite. 11: Remyella javorensis, metatergum. 12: Rozajella deelemani, metendosternite. 13: R. madzgalji n.sp., metendosternite. 14: Remyella scaphoides, aedeagus dorsal view. 15: R. propiformis (from Špela Bores), aedeagus dorsal view. 16: R. propiformis (from Špela Bores), male genital segment, dorsal part (cs = central setae). 17: R. propiformis (from Špela Bores), male genital segment, dorsal part. 19: Rozajella jovanvladimiri, aedeagus dorsal view. 20: R. jovanvladimiri, aedeagus dorsal view. 20: R. jovanvladimiri, aedeagus dorsal view. 20: R. madzgalji, aedeagus dorsal view.
24: R. madzgalji, apex of paramere. 25: R. madzgalji, longitudinal row of spines of the endophallus. 26: Remyella raskae, female abdominal ventrite VIII.

pećina and Baždarska pećina corresponding to *R. javorensis* are morphologically similar and also geographically isolated. The specific status of the three preceding populations is undisputable. Based on the morphological similarity and biogeographic coherence, the specific assignation of the other populations is done in the following way: Populations near Ugao are not morphologically distinct from the populations located near Doliće, Boljare, Đalovići and Lopužino brdo, so we consider them as conspecific, the senior names for them are *R. propiformis* or *R. borensis* (described in the same paper of WINKLER 1933), and we choose *R. propiformis*. The population from the surroundings of Đerekare, Pećina u Hamidovoj vrtači, Ledenica pećina, and Pećina u vrh livade Radojeve are morphologically similar, we consider them as conspecific, the senior names are *R. hussoni* and *R. puncticollis* 



Figs. 28–37. Pronotum, 28–31 dorsal view, 32–35 lateral view (arrow shows maximal extension of lateral rim), 36–37 detail of dorsal punctuation. 28: *Remyella raskae*. 29, 32: *R. javorensis*. 30, 36: *R. scaphoides*. 31: *R. hussoni* (from Ledenica pećina). 34: *Rozajella madz-galji*. 35: *R. jovanvladimiri*. 37: *R. hussoni* (from Pećina u Hamidovoj vrtači).

(described in the same paper of JEANNEL 1934), and we choose *R. hussoni*. We did not examine the type specimen of *R. hussoni* but instead we rely on topotypical material.

We should notice that an uncertainty remains regarding the exact type locality of *R. puncticollis*, namely Ledenica pećina. A cave with the same name is cited in NEŠIĆ (2015), located close, but slightly to the east of the location given by GIACHINO & ETONTI (1995). However, since the location of this cave in GIACHINO & ETONTI (1995) is given approximately (Giachino, pers. comm.), we assume that it is probably the same.

The taxonomical consequences of the preceding interpretation are given in the following section.

# 3.3. Morphology and taxonomy of *Remyella* species

**Diagnosis.** Genus with a scaphoid body shape, length: 3.8-4.9 mm (males), 4.0-5.4 mm (females). Anoph-

croreticulated. Antennae sexually dimorphic, approximately  $1.35 \times$  as long as the body in males,  $1.1 \times$  as long as the body in females. Punctuation of the dorsal parts always associated with setae. Punctuation of pronotum distributed in variable patterns according to species, but generally always present and/or denser along the posterior margin. Punctuation of the elytra always dense and with few specific variations, sometimes with sporadic traces of transversal alignments, but without transversal striae. Sutural stria absent. Apex of the elytra widely rounded and widely separated in males, shortly rounded and more closely joined in females (but yet with a small gap). Mesoventral process developed but not fused with the metasternum. Metatergum with a short posterior expansion (Fig. 11). Metendosternite "V"-shaped (Fig. 10). Profemur with a dense coating of tiny setae (Fig. 4). Protibiae without a lateral external row of spines. Protarsi five-segmented in males, four-segmented in females, not dilated in both sexes. Female genital segment

thalmic, depigmented. All external parts of the body mi-

Genetic distance (%)	lanoševa nećina	lama hez dna	Kaćunova nećina	l onužina iama	Ponor Ledenice	Siera iama	l lšačka nećina	Baždarska nećina
Geographic distance (km)	ic distance (km)		Nacunova pecina	Lopuzina jama	T ONOT Ledenice	Ojera jarria		Dazuarska peciria
Jagoševa pećina	0	0.6	1.5	3.5	3.9	4.2	9.15	8.35
Jama bez dna	9.24	0	1.2	2.9	3.6	3.9	8.65	8.45
Kaćunova pećina	6.55	2.67	0	3.8	4.25	4.4	8.8	8.575
Lopužina jama	8.95	8.45	7.2	0	5.3	5.6	9.75	8.8
Ponor Ledenice	18.25	11.87	11.55	20.2	0	0.3	9.95	9.1
Sjera jama	8.95	8.45	7.2	20.2	1.47	0	9.45	9.15
Ušačka pećina	29.51	30.55	29.6	22.35	42.45	41	0	7.3
Baždarska pećina	32.83	31.9	32.8	25.38	45.1	43.62	3.65	0

#### Correlations between genetic and geographic distances





**Fig. 38.** Table and plot of the genetic distances (barcoding region) versus geographic distances (Mantel test: R = 0.86, p-value < 0,0001) with a linear regression (rate of 0.26% genetic distance per km,  $R^2 = 0.68$ ). Squares correspond to pairs of populations *R. javorensis* – *R. hussoni*; triangles correspond to pairs of populations *R. javorensis* – *R. propiformis*; the diamond corresponds to the two known populations of *R. javorensis*; circles are other pairs of populations.

with distinct appendicular parts, spermatheca weakly sclerotized. Male genital segment much more developed than generally in other Leptodirini (Figs. 16-18), with setae of the posterior edge of the tergum either distributed along the entire width of the posterior edge (Fig. 18), or restricted to the sides (Fig. 17), according to species. Aedeagus with a median lobe parallel from base to two third of its length, then terminated in equilateral triangle either with a sharp or blunt apex according to species (Figs. 14, 15). Parameres with two apical and one subapical setae (Figs. 14, 15).

**Characters distinguishing species.** The morphological characters which appear to be relevant to distinguish the five species are the following: body size; length of the lateral pronotal marginal rim (Figs. 32, 33); shape of lateral sides of the pronotum (Figs. 28–31); density of punctuation of the dorsal pronotum surface (Figs. 36, 37); shape of the apex of the median lobe of the aedeagus; setation of the apical edge of the male genital segment.

Using these morphological characters we recognize five relevant species (without any subspecies) in agreement with the phylogenetic analysis of the previous



Fig. 39. Distribution map of Remyella and Rozajella. Shaded areas are karstic zones of the Pešter polje (after MOJSILOVIĆ et al. 1973).

section and coherent with the geographical distribution (Fig. 39). They are listed below, and in Table 3, with the appropriate synonymies. We give a short diagnosis, the list of caves where the species were recorded, and the numbers of specimens examined in addition to those used for DNA extraction. An identification key is also given.

#### Remyella javorensis S. Ćurčić & B. Ćurčić, 2008

Remyella javorensis S. Ćurčić & B. Ćurčić, 2008: 112 loc. typ.: Baždarska pećina Cave, village of Ursule, Mt. Javor near Sjenica, Southwest Serbia

**Diagnosis.** Large size, length of males 4.6-4.8 mm, of females 4.5-5.2 mm. The largest species of the genus. Pronotum long:  $1.25 \times$  as long as wide, the maximum width at the middle, the anterior half linearly convergent

from the middle to the anterior edge, abruptly narrowed at the middle, then parallel on the posterior half (Fig. 29). Lateral marginal rim of the pronotum limited to the basal half of the pronotum (Fig. 32). Punctuation sparse, denser along the posterior edge (Fig. 29). Dorsal part of the male genital segment with lateral and central setae. Apex of the median lobe of the aedeagus sharp, and slightly bent upward.

**Distribution.** Known only from two caves: Baždarska pećina near Ursule (type locality) [53, 132] and Ušačka pećina near Ušak (new locality) [13].

#### Remyella raskae S. Ćurčić & B. Ćurčić, 2008

Remyella raskae S. Ćurčić & B. Ćurčić, 2008: 110

loc. typ.: Pećina u Vrelu Raške cave near Novi Pazar, Pešter Polje, Southwest Serbia **Diagnosis.** Length of males 4.6-4.8 mm, of females: 4.5-5.2 mm. Pronotum long:  $1.25 \times$  as long as wide, widest at the posterior third, the anterior  $\frac{2}{3}$  linearly convergent towards the anterior edge, abruptly narrowed at the basal third, then parallel towards the hind angles (Fig. 28). Lateral marginal rim of the pronotum limited to the basal half of the pronotum. Punctuation sparse, denser along the posterior edge (Fig. 28). Dorsal part of the male genital segment with lateral setae only, central setae absent (Fig. 18). Apex of the median lobe of the aedeagus sharp.

**Distribution.** Known only from the type locality, the spring of river Raška (Pećina u Vrelu Raške) near Novi Pazar, Serbia  $[3 \circlearrowright, 6 \circlearrowright]$ .

#### Remyella scaphoides Jeannel, 1931

Remyella scaphoides Jeannel, 1931: 263.

- Remyella scaphoides scaphoides Jeannel, 1931: Giachino & Etonti, 1995: 88
- loc. typ.: Yougoslavie: Velika pećina près du hameau de Grgaje, opstina Bare

**Diagnosis.** Length of males 4.1-4.5 mm, of females 4.5-4.75 mm. Pronotum  $1.15 \times$  as long as wide, with the maximum width approximately in the middle. Pronotal lateral sides convex in the anterior half, convergent in the posterior half until the base, or shortly parallel just before the hind angles. Lateral rim of the pronotum extending from the base to  $\frac{2}{3}$  or  $\frac{3}{4}$  of the pronotum. Pronotal punctuation sparse (Figs. 30, 36). Apex of the aedeagus blunt (Fig. 14).

**Distribution.** Known only from the type locality: Serbia, Velika pećina near Grgaje [93, 82].

#### Remyella propiformis Winkler, 1933 n.stat.

Remyella propiformis propiformis Winkler, 1933: 78

- loc. typ.: Spela Maja Hejne, nordöstlich von Ugo im Sandjak Novipazar
- Remyella scaphoides propiformis Winkler, 1933: Giachino & Etonti, 1995: 88

Remyella propiformis borensis Winkler, 1933 n.syn.

loc. typ.: Spela Bor, nordwestlich von Ugo im Sandjak Novipazar Remyella scaphoides borensis Winkler, 1933: Giachino & Etonti,

1995: 90. (partim)

*Remyella scaphoides droveniki* Giachino & Etonti, 1995: 91 **n.syn.** loc. typ.: Serbia occ.: Dolić, Pešter, Kapeš, Uleva pećina III

- Remyella montenegrina S. Ćurčić, Antić, N. Ćurčić & B. Ćurčić, 2013: 1218 n.syn.
- loc. typ.: Đalovića Pećina Cave (= Pećina nad Vražjim Firovima Cave), Đalovića Gorge, village of Đalovići near Bijelo Polje, Pešter Polje, northeastern Montenegro

**Diagnosis.** Length of males 3.8-4.6 mm, of females 4.0-4.8 mm. Pronotum  $1.2 \times$  as long as wide, with the maximum width approximately in the middle. Pronotal lateral sides convex in the anterior half, convergent in the

posterior half until the base, or shortly parallel just before the hind angles. Lateral rim of the pronotum extending from the base to  $\frac{2}{3}$  or  $\frac{3}{4}$  of the pronotum length. Pronotal punctuation sparse. Apex of aedeagus sharp (Fig. 15).

**Distribution.** Serbia: caves located in the surroundings of Ugao: Špela Hajne [213, 92], Špela Vogel [13, 52], sinkhole of Boroštica river (GIACHINO & ETONTI 1995), Špela Bores [153, 162]. Caves on Giljeva: Lopužino brdo. Caves in the surroundings of Doliće, Boljare and Kapeš: pećina Uleva III [133, 112], Pećina u Anin Kapeš [133, 152], Pećina Ispod Gluare [133, 142], Jama bez dna, Pećina kod Jagodina Dola [43, 102], Kaćunova Jama [13, 42]. Montenegro: caves located in the surroundings of Đalovići: Jagoševa pećina, Đalovića pećina (= Pećina nad Vražjim Firovima) [123, 222].

#### Remyella hussoni Jeannel, 1934 n.stat.

Remyella hussoni Jeannel, 1934: 103

- loc. typ.: U Hamidova vrtaca pećina à Krnja Jela, Sandjak de Novi-Pazar
- Remyella scaphoides hussoni Jeannel, 1934: Giachino & Etonti, 1995: 91
- Remyella puncticollis Jeannel, 1934: 103. n. syn.
- loc. typ.: Sandjak de Novi Pazar, Ledenica pećina, opština de Korito, srez de Bijelopolje
- Remyella scaphoides borensis Winkler, 1933: Giachino & Etonti, 1995: 90 (partim)

**Diagnosis.** Length of males 3.8-4.4 mm, of females 4.2-5 mm. Pronotum  $1.1 \times$  as long as wide, with the maximum width approximately in the middle. Pronotal lateral sides arcuate in the anterior half, convergent in the posterior half until the base, or shortly parallel just before the hind angles (Fig. 31). Lateral rim of the pronotum extending from the base to  $\frac{2}{3}$  or  $\frac{3}{4}$  of the pronotum (Fig. 37). Pronotal punctuation dense and more or less uniformly distributed (Fig. 31). Apex of the aedeagus sharp (Fig. 15).

**Distribution.** Serbia: cave in Hamidova vrtača (type locality) [13, 42]; caves in the surroundings of Derekare, on the left side of the Boroštica river: Sijera jama, Jama u Piskovoj livadi, Ponor Ledenice. Montenegro: caves located on the Montenegrian side of the border: Ledenica pećina [13, 42], Pećina u vrh livade Radojeve (JEANNEL 1934).

#### Identification key for Remyella

- 1' Lateral sides of the pronotum convex in the anterior part (Figs. 30, 31). Lateral marginal rim of the prono-

- Larger body size: 4.6-4.9 mm (males); 4.5-5.2 mm (females). Pronotum widest at the middle (Fig. 29). Male genital segment with lateral and central setae (Fig. 16) ...... *javorensis*
- 2' Smaller body size: 4.1-4.4 mm (males); 4.1-4.5 mm (females). Pronotum widest at the basal third (Fig. 28). Male genital segment only with lateral setae (Fig. 18) ...... raskae
- 3 Pronotal punctuation dense and more or less uniformly distributed (Fig. 37) ...... hussoni
- 4 Median lobe of the aedeagus wide, and with a blunt apex (Fig. 14) .....scaphoides
- 4' Median lobe of the aedeagus narrow, and with a sharp apex (Fig. 15) ..... *propiformis*

#### 3.4. Biogeography of Remyella

The table included in Fig. 38 contains above the diagonal the COI genetic distances, and below the diagonal their geographical distances. Using a Mantel test, we find a high correlation (R = 0.86, p-value < 0.0001) between geographic and genetic distances (Fig. 38). The single pair of populations that do not conform are the two populations of R. javorensis from Baždarska pećina and Ušačka pećina, geographically distant by less than 4 km, but with a genetical distance of 7.3%. Actually, these two populations are separated by the deep valley of the Vapa river which may be a strong barrier to gene flow and could explain this high genetic divergence. However, we find no significant morphological differences between these two populations and we consider them as conspecific. Fig. 38 shows also a linear regression enforced to intercept axis in (0,0) (zero-genetic distance for zerogeographic distance). The slope is 0.26% of genetic divergence by km with a fairly high coefficient of determination:  $R^2 = 0.68$ . (A fit not enforced to intercept on (0,0) gives a slope of 0.20 genetic distance by km with the coefficient of determination  $R^2 = 0.76$  and the intercept at (0,1.56)). This rough proportionality between genetic and geographic distances and, at least for populations located along the Serbia-Montenegro border: the gradient of morphological characters and the lack of clear natural barriers, may suggest a processes of speciation by distance (BAPTESTINI et al. 2013).

According to the genetic divergences given in Fig. 38, which range from 3.5% to 10%, and using as a preliminary approach the divergence rate of 2% per million years obtained by RIBERA et al. (2010) for mitochondrial genes of the western Mediterranean Leptodirini, the speciation events in *Remyella* occurred approximately from 5 to 2 million years ago. This corresponds to the end of the Miocene and the Pliocene, periods when the Pešter field was periodically filled by shallow lakes forming sedimentary deposits (MOJSILOVIĆ et al. 1973). The distribution area of the genus, confined to the periphery of Pešter (Fig. 39), is probably contingent to the presence of these lakes, preventing species to spread more uniformly over the entire area of the field.

#### 3.5. The genus Rozajella

The genus *Rozajella* S. Ćurčić, Brajković & B. Ćurčić, 2007 comprises two obligate cave species: *R. jovan-wladimiri* S. Ćurčić, Brajković & B. Ćurčić, 2007 and *R. deelemani* Perreau & Pavićević, 2008, distributed in the eastern range of the Dinaric Mountains in Montenegro. Both species are short-range endemics, known only from a single cave, one near Rozaje and one near Berane in Montenegro. A third species, *Rozajella madzgalji* n. sp., is described below and the male of *R. deelemani* is described for the first time.

#### Rozajella madzgalji n.sp.

**Type material.** Holotype male labeled as follows: "MONTENE-GRO, Berane, Goražde, Mala pećina u Garaškom kršu, 860 m, 19.vii.2014, leg. I. Njunjić" (white label, printed)/"HOLOTYPUS *Rozajella madzgalji* sp.n. I. Njunjić, M. Perreau & D. Pavićević det. 2016" (red label, printed), (MNHN). — Paratypes:  $43^{\circ}$  6 $\bigcirc$ , 19.vii.2014 (MNHN, CINJ, CMPR, CDPV),  $53^{\circ}$  and  $12\bigcirc$ , same cave, 22.xi.2014, leg. I. Njunjić (MNHN, CINJ, CMPR, CDPV);  $23^{\circ}$  4 $\bigcirc$  Montenegro, Berane, Goražde, Velika pećina u Garaškom kršu, 860 m, 22.xi.2014, leg. I. Njunjić (MNHN, CINJ, CMPR, CDPV). All paratypes are labeled with white, printed locality labels and with red printed labels "PARATYPUS *Rozajella madzgalji* sp.n. I. Njunjić, M. Perreau & D. Pavićević det. 2016".

**Type locality.** Mala pećina u Garaškom kršu, Goražde, Berane, Montenegro; N42°52′15.7″ E19°55′10.5″.

**Diagnosis.** Habitus illustrated in Fig. 2. Distinct from the other species of the genus by the following combination of characters: medium size of the body, shape of pronotum, parameres with a preapical constriction, and a sharp and pointed apex (Fig. 24).

**Description.** Body length 3.29–4.32 mm (HT 4.09 mm). General morphology leptodiroid, eyeless, wingless, and depigmented.

*Head* narrower than pronotum. Dorsal surface rough with dense, erected hairs. Occiput with deep, random punctures. The base of the terminal maxillary palpomere with a dorsal comb of very short hairs. Antennae inserted slightly after the middle of the length of the head (measured from the posterior margin of clypeus) and shorter than the body in both sexes. Second antennomere slightly longer than the first; 8<sup>th</sup> antennomere is the shortest; last antennomere longer than the penultimate in both sexes, but slightly more so in males. All antennomeres covered with hairs of medium length, but only antennomeres 5-11 bear up to 8 long, erected setae arranged circularly and symmetrically in the apical part. Hairs

Antennomere	I	II	III	IV	V	VI	VII	VIII	IX	Х	XI
R. madzgalji											
Length [mm]	0.23	0.25	0.27	0.29	0.29	0.34	0.35	0.21	0.35	0.32	0.42
Length [%]	6.93	7.53	8.13	8.79	8.79	10.24	10.54	6.33	10.54	9.64	12.65
R. deelemani											
Length [mm]	0.27	0.26	0.28	0.29	0.36	0.36	0.36	0.23	0.38	0.36	0,48
Length [%]	7.44	7.16	7.71	7.99	9.92	9.92	9.64	6.34	10.47	9.92	13.22

Table 5. Antennal formula of the holotype of *Rozajella madzgalji* n.sp. and of the male of *Rozajella deelemani*, with antennomere lengths given both in mm and in %.

of medium length are particularly dense on the two last antennomeres. Lengths of antennomeres are given in Table 5.

**Pronotum** approximately  $1.31 \times as$  long as wide (HT 1.32). The maximal width approximately at the middle of the length. Lateral margins sinuous towards the head and towards the posterior angles, with a lateral rim interrupted in the middle (Fig. 33). Posterior lateral angles acute or right. Posterior edge with a marginal carina (Fig. 34), anterior edge with a thin marginal carina. Dorsal surface rough with dense, short hairs (Fig. 34). Ventral surface with a microsculptural mesh resembling a honeycomb pattern, hairless, except a few sparse hairs on the ventrolateral side. Metendosternite "V"-shaped (Fig. 13).

*Abdomen*: Ventral surface with microsculptural mesh resembling a honeycomb pattern. Mesosternum and mesepisternum hairless, other ventrites covered with dense hairs.

*Elytra* elliptic, approximately  $1.6 \times$  as long as wide (HT 1.75), without sutural striae (Fig. 2). Dorsal surface with punctures and dense hairs transversally aligned and transversal strigae on the anterior part of the elytra.

*Legs* long and slender, protibia weakly curved inwards, profemora thicker than meso- and metafemora.

*Aedeagus*: Median lobe thick, slightly curved ventrally in lateral view and with rounded apex in dorsal view (Fig. 23). Parameres slightly shorter than the median lobe, with a strong constriction in the apical part, bearing three setae (Fig. 24). Apex of the parameres pointed and concave on the interior side. Endophallus with armature in two lateral fields with several dozens of short and thin teeth (Fig. 25). For comparison, we figure the aedeagus and apex of parameres of *R. jovanvladimiri* (Figs. 19, 20) and *R. deelemani* (Figs. 21, 22).

*Female genitalia*: Spermatheca unsclerotised. Abdominal ventrite VIII with a short and narrow anterior expansion. Urite IX with appendicular parts. Gonostyles with 3 setae, one apical and 2 lateral.

**Distribution.** *R. madzgalji* n.sp. is known from two caves: Mala pećina u Garaškom kršu (210 m long) and Velika pećina u Garaškom kršu (188 m long), located only 150 meters from each other, close to the village Zagrađe near Berane in Montenegro (Fig. 39). During the second visit to Mala pećina u Garaškom kršu, a large number of individuals was found feeding on a pigeon

wing in the dark zone of the cave, approximately 100 m from the entrance.

**Etymology.** The new species is named after speleologist Zeljko Madžgalj from Bijelo Polje (Montenegro), in recognition for his contribution to speleological investigations of Montenegro and enthusiastic help in the field.

#### Rozajella deelemani Perreau & Pavićević, 2008

**Material examined.** 1 $\bigcirc$ : Holotype, Crna Gora, Građa pećina, Petnik, Ivangrad (= Berane) 5.viii.1970, leg. Deeleman (CMPR). – 1 $\bigcirc$ : Paratype, same data (CDPV). –  $3 \Im$ : Crna Gora, Berane, Petnik, Građa pećina, 22.xi.2014, leg. I. Njunjić (CINJ); 4 $\bigcirc$ : same data (CINJ).

**Type locality.** Crna Gora, Berane, Petnik, Građa pećina; N42°50'06.7" E19°54'26.1" (Fig. 39).

**Description of a male.** Habitus illustrated in Fig. 3. Body length 4.35 mm. General morphology leptodiroid, eyeless, wingless, and depigmented.

*Head* narrower than pronotum. Dorsal surface rough with dense, erected hairs. Penultimate maxillary palpomere short and thick. Terminal maxillary palpomere thin and with a dorsal comb of very short hairs at the base. Antennae inserted slightly after the middle of the length of the head (measured from the posterior margin of clypeus) and shorter than the body. Second antennomere almost the same length as the first one; 8<sup>th</sup> antennomere is the shortest; last antennomere is the longest and it is longer than the penultimate. Hairs on the antennae like in other species of the genus, as described above in *R. madzgalji*. Lengths of antennomeres are given in Table 5.

**Pronotum**  $1.34 \times$  as long as wide. The maximal width approximately at the middle of the length. Lateral margins sinuous towards the head and slightly sinuous towards the posterior angles. Posterior lateral angles right. Posterior edge with a thick marginal carina, anterior edge with a thin marginal carina. Dorsal surface rough with short hairs, which are very rare on the dorso-lateral side. Metendosternite "V"-shaped (Fig. 13).

*Elytra* elliptic,  $1.85 \times$  as long as wide, without sutural stria. Dorsal surface with punctures and short, dense hairs transversally aligned on the anterior part of the elytra.

*Aedeagus*: Median lobe thick, somewhat curved ventrally in lateral view and with lanceolate apex in dorsal view (Fig. 21). Parameres shorter than the median lobe and slightly wider in the apical part. Apex of the parameres pointed, bearing three setae (Fig. 22). Endophallus with armature in two lateral fields with several dozens of thick teeth (Fig. 21).

#### Identification key for Rozajella

(updating the key in PERREAU & PAVIĆEVIĆ 2008a)

- 2 Median lobe of aedeagus with rounded apex in dorsal view (Fig. 23). Parameres with a preapical constriction. Endophallus with armature in two lateral fields with several dozens of short and thin teeth (Fig. 25) *madgalji*

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