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Research Article

## ***Nebria* Species (Subfamily *Nebriinae*, Family: *Carabidae*) from the Romanian Carpathians. Morphological and Molecular Data**

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### **Abstract**

Among the ten species of the *Nebria* genus, present in the Romanian Carpathians, seven are the object of morphological, geographical distribution and molecular biological studies: *Nebria* (*Eunebria*) *jockischi* hoepfneri Dejean, 1826, *Nebria* (*Boreonebria*) *heegeri* Dejean, 1826, *Nebria* (*Boreonebria*) *gyllenhali* Schönherr, 1806, *Nebria* (*Alpaeonebria*) *reichei* Dejean, 1826, *Nebria* (*Alpaeonebria*) *reitteri* Rybinsky, 1902, *Nebria* (*Alpaeonebria*) *bissenica* Bielz, 1887, *Nebria* (*Nebria*) *transsylvanica* Germar, 1824, *Nebria* (*Nebria*) *femoralis* alpigrada Csiki 1905), collected from the Maramureş Mt., Rodnei Mt., Parâng Mt., Rarău Mt., Făgăraş Mt., Cozia Mt., Bucegi Mt., Retezat Mt., Muntele Mic and Semenic Mt.

The morphological description exploits the body size, the elytral reflection, the colour of the appendages and the legs, the shape of the first antennary segment, its chaetotaxy and that of the submentum, the shape and size of the elytra and alae, the position of the bristles on the ventrites 4-5-6. The shape of the first antennary segment and its chaetotaxy appear as more discriminating criteria, but supposes the integrity of the bristles. The identification is sometimes malaise due to the fragility of the bristles (first antennomere, submentum). *Nebria* (*Alpaeonebria*) *reichei* Dejean, 1826 has a variable chaetotaxy of antenna, 1 to 3 bristles on the first antennomere. The individuals with yellow appendages and legs provided with 2 unequal length can be confused with *transsylvanica*. They are distinguished by the triangular shape of the aileron (S-shaped in *transsylvanica*). Molecular data are given for the first time on Carpathian *Nebria*. The mitochondrial markers (COI I, cyt b) clearly identify the species studied and confirm that *alpigrada* does not belong to *transsylvanica*. The results show an infraspecific variability of geographic and altitudinal origin in *jockischi* one of the most widespread species (*gyllenhali*, *jockischi*, *reichei*).

**Keywords:** *Nebria*, geographical distribution, morphology, molecular systematics.

### **I. Introduction**

#### **STUDY FRAMEWORK**


The Carpathians of Romania host about ten species of the genus *Nebria* above an altitude of 800-1000 m [1]. They include rare species like *N. (Boreonebria) heegeri* Dejean 1826, perhaps

underestimated because of identification difficulties and considered vulnerable (IUCN code) in Ukraine and Romania [2], and localized ones, such as *N. (Alpaeonebria) bissonica* Bielz 1887 [3, 4, 5] and *N. (Alpaeonebria) carpathica* Bielz 1850.

Various prospectings carried out in the mountains of Maramureş, Rodnei, Rarău, Muntele Mic, Făgăraş, Bucegi, Parâng, Cozia, and to their foothills provided 7 species of *Nebria*.

These are described below, based on morphological characteristics and molecular data

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(except *bissenica* and *heegeri* which were captured in small numbers).

## II. Material and Methods

The insects were captured under stones, in torrents or in the vicinity of snow creek streams and are placed immediately in 95<sup>0</sup> ethanol and then stored at -20<sup>0</sup> C. The provenance of the analyzed specimens is the Romanian Mountains: Rodna, Parâng, Rarău, Făgăraș, Cozia, Bucegi, Retezat, Muntele Mic and Semenic. The analysed species are those described above except *bissenica* which was found in too low numbers. *Nebria brevicollis* Fabricius 1792 was added from Greci TL and Cozia Top (Vâlcea County). The molecular analysis was conducted on 2 individuals (1 male and 1 female); in the event of a strong divergence of sequencing results, the number is doubled. The extraction of the DNA made on the femurs of the four hind legs of the insect. The methods for purification extraction, PCR, and DNA sequencing are those described in a previous study [6].

### ●Choice of markers:

- Nuclear marker: 29S 5F5R
- Mitochondrial markers: COI I: ICO 1490, HCO 2 198 [7], cyt b: CP1 (Harry et al 1998), CB2 [8].

### ●Sequence analysis

Sequencing results: nuclear marker; mitochondrial markers in concatenation COI I / cyt b were analysed using two matrices:

- The genetic distance (K2P) and the number of different base pairs. This one records all the substitutions whatever is their nature.
- The matrix K80 of Kimura, taking into account the transition-transversion processes.

Sequences were analysed using the Geneious software Version 10. Multiple alignments were performed with the algorithm MAFFT [9]; and the phylogenetic trees were constructed using the algorithm PHYML [10] with the substitution model IC69.

## III. Results and Discussions

### 1. MORPHOLOGICAL DESCRIPTION

The main distinctive characters of the ten subalpine and alpine *Nebria* species of the Romanian Carpathians were briefly presented earlier [1] in the form of tables. The following descriptions (for seven species) provide details and illustrate the most relevant identification criteria.

These include:

- the color of the appendages and the various parts of the legs,

- the form of the first antennary segment and the two following, their chaetotaxy and that of the submentum,

- the elytra and alae (membranous wings, hind wings), an important but rarely exploited character,

- the chaetotaxy of the ventrites 4-5-6 when it is useful to confirm identification (genus *Boronebria*).

The use of chaetotaxy (antenna, submentum, ventrites), an important element of identification, requires the integrity of bristles, that are very fragile elements, especially those of the submentum. Their partial or total absence makes identification impossible or uncertain; the importance of the forewings (elytra) or hind wings (ailerons) being of some help.

### 1.1. Ubiquitary *Nebria*

Two *Nebria* have a very wide geographical distribution, which occur in the mountains of Romania from the foothill to the summit [11, 12, 13, 14]:

- Nebria (Eunebria) jockischi hoepfneri* Dejean 1826 [15].

- Nebria (Boronebria) gyllenhali* Schönherr 1806 often referred to with its former name: *rufescens* Storm 1768.

Considered as glacial relics, they are found at high altitudes (2300-2400 m), for example in the Austrian (Oetzal) and Italian [16]. Alps and constitute pioneer riparian species colonizing areas liberated by the retreat of glaciers [17, 18, 19]. Although with highly developed membranous wings, folded under the elytra (Figure 1), their ability to fly is unknown. They occupy a wide altitudinal range, raising the question of the modalities of settlement (flight, drift by water to the low parts).

**1.1.1. *Nebria (Eunebria) jockischi hoepfneri* Dejean 1826 ; synonyms: *nigricornis* Villa, *atterima* Fiori 1896.** Described by Dejean, the type was not found in the collection 'Chaudoir' of the MNHH Paris [20], but the species is reported in several ancient documents of the mountains of Banat.

- Black species, shiny elytra with varying size according to the altitude: 13-14 mm at the base of the mountains; 7-8 mm at the summit.

- Third elytral interval without discal pore.

- Head with a red stain frequent at the vertex, sometimes absent (var. *nigriceps* Schifshy 1888, individual variation).

- Appendices of the extremities reddish brown, tarsi often brown fairly light (sometimes blacks), antennae brown from 4. antennal segment.

-The first antennomere subcylindrical, rather big compared with the following ones, with a rather long bristle upsides; slightly conical with 3-4 bristles implanted on the periphery.

-Submentum with 2 bristles, very rarely 3.

-Membranous wings are complete, very developed, folded under the elytra.

Very hygrophilous species, found near the streams, in the banks or under the stones and

immersed trunks. Captured in several localities, in medium and high altitude: Văliug 639 m., Muntele Mic 845 m., Latoritei Valley 300-400 m., Rarău Mountains 1200 m., Bucegi Mountains (Piatra Arsă 1950m.), Făgăraș Mountains along the Transfăgărășan Route 1410 m, 1613 m., 1844 m., 1944 m.

*Nebria (Eunebria) jockischi hoepfneri* Dejean 1826

*Nebria (Boreonebria) gyllenhali* Schönheer 1806



Bristles of the firsts three antennomeres



Aedeagus



Developed membranous wings



Red stain on vertex

**Figure 1.** Morphological aspects of *jockischi* and *gyllenhali*

**1.1.2. *Nebria (Boreonebria) gyllenhali* (Schönherr 1806) [21]; synonym *rufescens* Strom 1768**

-Black species with shiny elytra. Size varying according to the altitude: 13-14 mm at the foothills of the mountains, 9-10 mm at the top.

-Third elytral interval with typically 4-5 discal pores but in variable number for the individuals of higher altitude.

-Distal parts of the appendages and antennae, brightened, brown, slightly reddish, sometimes legs totally reddish yellow (form *balbi*).

-First antennomere long-ovoid, narrowed at the base with a long apical bristle; second antennomere very short with a short bristle underneath; third antennomere rather long with generally 2 bristle above, 2 bristle below.

-Submentum with 8-10 bristles.

-Ventriles 4-5-6 with one bristle on each side of the middle.

-Membranous wings are complete, well developed, folded under the elytra.

●Possible confusion with:

-*jockischi hoepfneri* (when red stain vertex is undetectable) species with 2-3 bristles to the ventrites 4-5-6,

-*heegeri*, a smaller species, with reduced membranous wings, the second antennomere with 2 bristles (1 above, 1 below).

●Species habitat and locations:

- very hygrophilous, encountered under stones, in torrents at medium altitude in streams or in the immediate vicinity of high-altitude areas,

- very frequent and present in a wide altitudinal range: Latorița Valley 210 m., Muntele Mic 845 m., Făgăraș to the various altitudes from 1800 m. to 2089 m, Rodnei Mountains (1845 m), Bucegi Mountains (Piatra Arsă 1950).

## 1.2. Carpathian species

### 1.2.1. *Nebria (Boronebria) heegeri* Dejean 1826

Endemic species of the Romanian and Ukrainian Carpathians, considered rare and threatened with extinction [2].

In Ukraine [22, 23, 24] is located at the Chornogora and Gorgany Mountains, on the mountain forest zone [25, 26, 27].

In Romania: the species formerly cited by [28, 29] in the mountains Retezat (Zanoaga Lake), Cindrel, Făgăraș (Balea Lake), Bucegi, Rastolita, Ciucas and rarely reported since in the recent faunistical studies, except Ciucas Mountains (Berk Patak 1160 m.) [30].

-Black shiny insect, 10-12 mm., the appendices low or not brilliant.

-Third elytral interval with 2-3 disc pores.

-First antennomere is conical, large and quite long with a long apical bristle. Second antennomere is shorter, conical with a bristle above and another below often implanted obliquely. Third antennomere is long, conical with 3 bristles on the perimeter.

-Submentum with 4 bristles.

-Ventriles 4-5-6 with 1 bristle.

-Reduced membranous wings, fairly large.

In partial or total absence of antenna and/or submentum bristles, the species can be confused with *gyllenhali*. It differs from *gyllenhali* by more oval shape of elytra, sloping shoulders and conical first antennomere

●Distinction from *Boronebria* species:

-Species with highly developed membranous wings:

-Head with red stain to vertex; ventrites 4-5-6 with 2-3 bristles.....*jockischi hoepfneri* Dejean 1826

-Head without vertex stain; ventrites 4-5-6 with 1 bristle.....*gyllenhali* Schönherr 1806

-Species with reduced membranous wings:

-Head without vertex stain; ventrites 4-5-6 with 1 bristle.....*heegeri* Dejean 1826

### 1.2.2. Group *transsylvanica*

A group comprising two species, morphologically similar and often confused: *Nebria (Nebria) transsylvanica* Germar 1824 and *Nebria (Nebria) femoralis* Chaudoir 1843 redescribed by [31], present in the Rodnei Mountains [20]. We did not find the nominate form but collected *Nebria (Nebria) femoralis alpigrada* Csiki 1906 [32, 12].

●Taxonomic note

In the literature [33] there is a *Nebria femoralis* Motshulsky 1859 (synonym *nivalis* Paykull 1798, Russian species.).

#### A. *Nebria (Nebria) transsylvanica* Germar 1824

Species mentioned by Germar from Transylvania [34].

*Nebria* with shiny black elytra, fairly short (10.5-12 mm), with variable elytral reflection in dark tanned, greenish or purplish.

Appendages (mandibular extremities, palps) antennae, tarsi reddish brown, rarely totally black. Ganglbauer [35,36] mentioned specimens from the top of Kuhorn (Rodnei 2200 m) with red legs and other *rufinos*.

Head with occasionally thinning stain on the vertex.

First antennomere large, short and thick with two bristles implanted close to each other (1 long, 1 short). Second antennomere conical, short with a bristle below; third antennomere of conical shape, long and with 2 bristles above and 1 below.

Submentum with 4 bristles. Alae reduced, S shaped.

This *Nebria* is present in the Ukrainian Carpathians (Gorgany, Chornagora, Svydovets, Marmarosh Mts) at altitudes above 1400 m, and in varied biotopes: alpine meadows, *Pinus mugho* settlement, rock outcrops [25]. Neculiseanu [37] quotes her from Moldova, based on the resources of the collection from Academy of Sciences of Moldova. This location deserves confirmation, unless the origin is located at the northern border with the Ukrainian Carpathians.

The species, according to the literature, seems to be common in Romania. Numerous citations of localities are of medium and high altitudes (1600-1800 m): in Southern Carpathians: Retezat Mountains, Bucegi Mountains, Făgăraș Mountains, Parâng Mountains; but also in the north: Călimani Mountains or recently cited Maramureș Mountains [38, 39], Rodnei Mountains [13, 40, 41, 42, 43]. We collected it only in the Făgăraș Mountains.

It is possible that the species has been confused in the north (notably Rodna Mountains) with *Nebria (Alpaeonebria) reitteri* Rybinsky, 1902 because of its similar habitus, distinct by the

antennary chaetotaxy (first antennomere with a long bristle, against two with unequal length for *transsylvanica*). (Figure 2.)

*Nebria (Nebria) transsylvanica*  
Germar 1824

*Nebria (Alpaeonebria) reitteri*  
Rybinsky 1902

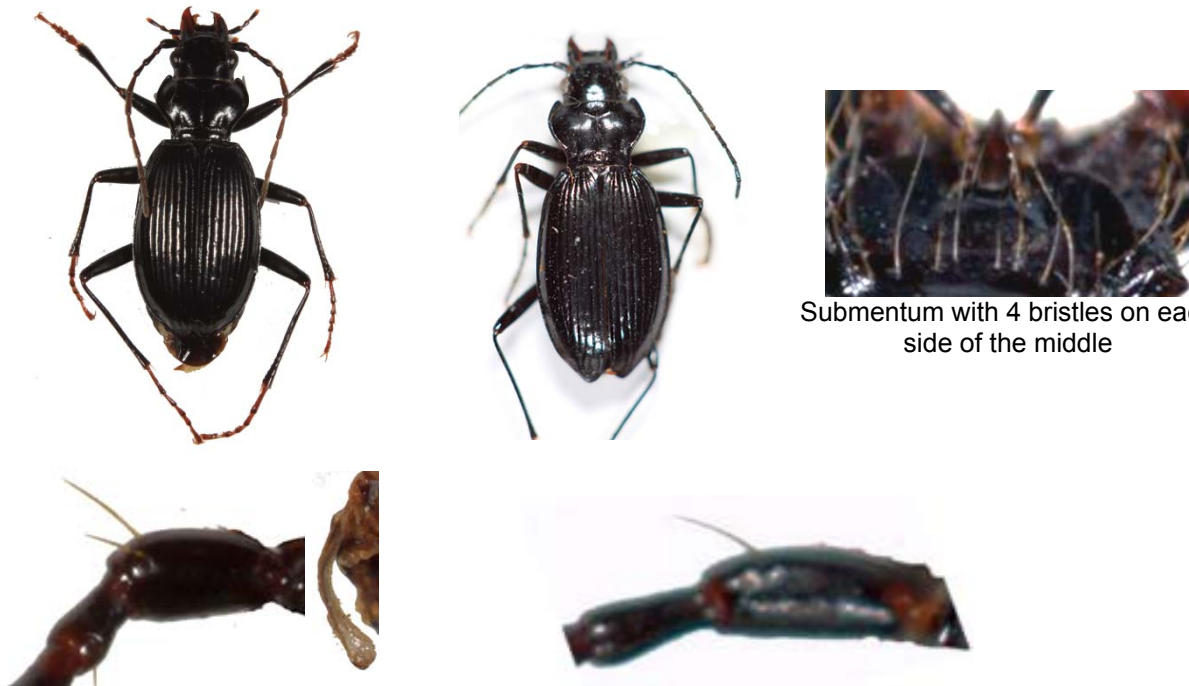


Figure 2. Morphological aspects of *transsylvanica* and *reitteri*

### **B. *Nebria femoralis alpigrada* Csiki 1906**

Initially *alpigrada* Csiki 1906, its synonym *ormayi* Ganglbauer 1892 was attached to *transsylvanica*. The attachment to *transsylvanica* is still adopted in the recent Romanian fauna [14]. Ledoux [20] considers it as a subspecies of *femoralis*. Its distribution seems to be quite broad: it is mentioned as *ormayi (alpigrada)* of the Calimani Mountains, Bucegi Mountains [44, 45], populations of Transylvania (attached to *transsylvanica*) by [29], Retezat Mountains [46, 47].

We captured from two populations morphologically related to *alpigrada* in the Parângului Mountains at two altitudes (840 m and 1848 m., near Pasul Urdele) and in the Retezat Mountains (Poiana Pelegii 1618 m.).

Both have the following characteristics of the species and subspecies:

-*femoralis*: size 11.0 to 12.5 mm, the first cylindrical or sub-cylindrical article with a long bristle, the other characteristics (alae and aedeagus shape) being those of the type.

-*alpigrada*: shiny black elytra, yellowish appendages and antennae (sometimes slightly reddish); legs altogether of flavescent colour, with darkening of the femur-tibia joint.

Certain forms of *reichei* with appendages, antennae and legs entirely of yellow colour, could be confused with *alpigrada* (figure 3,4). However, *reichei* often holds 2 bristles at the first article of the antenna and the triangular ala shape avoids any confusion (*alpigrada* having an S-shaped ala). Her geographical distribution and possibly her infraspecific variability (see data of molecular biology) remain to be specified. The prospecting should be carried out in the localities mentioned for *transsylvanica alpigrada* Csiki.

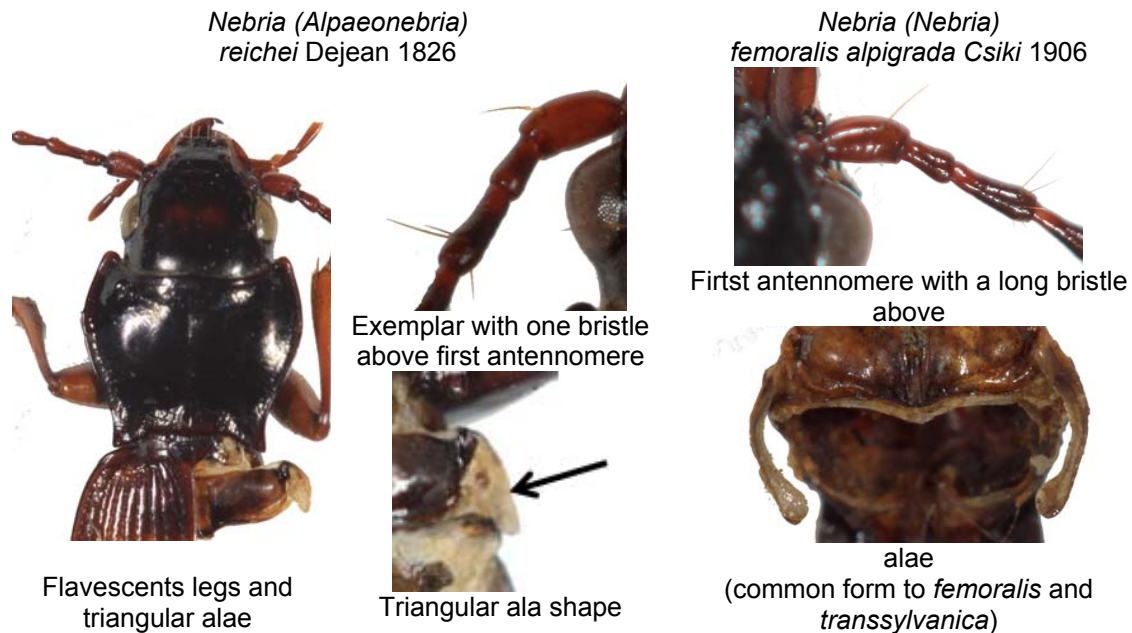


Figure 3. Morphological aspects of *reichei* and *femoralis alpigrada*

### 1.2.3. Group *Alpaeonebria*

Three studied species:

- Nebria (Alpaeonebria) reichei* Dejean 1826,
- Nebria (Alpaeonebria) bisсенica* Bielz 1887,
- Nebria (Alpaeonebria) reitteri* Rybinsky, 1902,

#### **A. *Nebria (Alpaeonebria) reichei* Dejean 1825**

Species mentioned in Banat by Dejean (1837), also cited from Ukraine [26, 22, 23, 24] and Austria in Carinthia Südsteiermark [47]. Its availability in Ukraine, is rather doubtful (PANIN Ruslan 2017 personal communication).

Size 10-12 mm. Shiny black elytra. Head normally without red stain to vertex, except immature individuals. Extremities (mandibles, palps, antennae, tarsi) are flavescent. Femurs usually (72% on average of the populations) are blackish brownish. The most of the populations studied (Făgăraș -Bălea Lake 2085 m., Rodnei Mountains - Iezer Lake 1825 m., Bucegi Mountains -Piatra Arsă 1950) have 28% of individuals with a red stain to the vertex and yellow femurs. This form could be confused with *femoralis alpigrada* Csiki. First antennomere is subcylindrical with a variable chaetotaxy of 1 to 3 bristles (1 long bristle and 1 to 2 short bristles implanted in the vicinity). The number of bristles varies according to the population, the most frequent being 2 bristles. For example, in Făgăraș (Balea Lac 2085 m.) 11% of individuals hold 1 bristle, 88% 2 bristle and 1% 3 bristle. Second antennomere with a bristle above, third antennomere longer, with 3-4 bristles. The literature [20] mentions only 1 bristle.

The alae are very atrophied with triangular shape. (figure 4).

Individuals with appendages and legs totally yellow (often referred as *ormayi alpigrada* Csiki 1905 [32]).

Other common features: first antennomere ovoid, more or less elongated; ventrites 4-5-6 with one bristle on each side of the middle.

Three cases considered:

-first antennomere with two bristles of equal length, triangular ala. The form of *Nebria (Alpaeonebria) reichei*, no doubt related to *transsylvanica* in the past.

-first antennomere with one long bristle:

- triangular ala: *Nebria (Alpaeonebria) reichei*
- ala in the form of S: *Nebria (Nebria) femoralis alpigrada*

Species widely quoted from the Southern Carpathians where it is found (Transfăgărășan road at various altitudes including the top 2200 m, Retezat 1800 m, Bucegi 1900-200 m) but also present in the Eastern Carpathians (M. Rodnei 1800-2000 m.)

#### **B. *Nebria (Alpaeonebria) bisсенica* Bielz 1887**

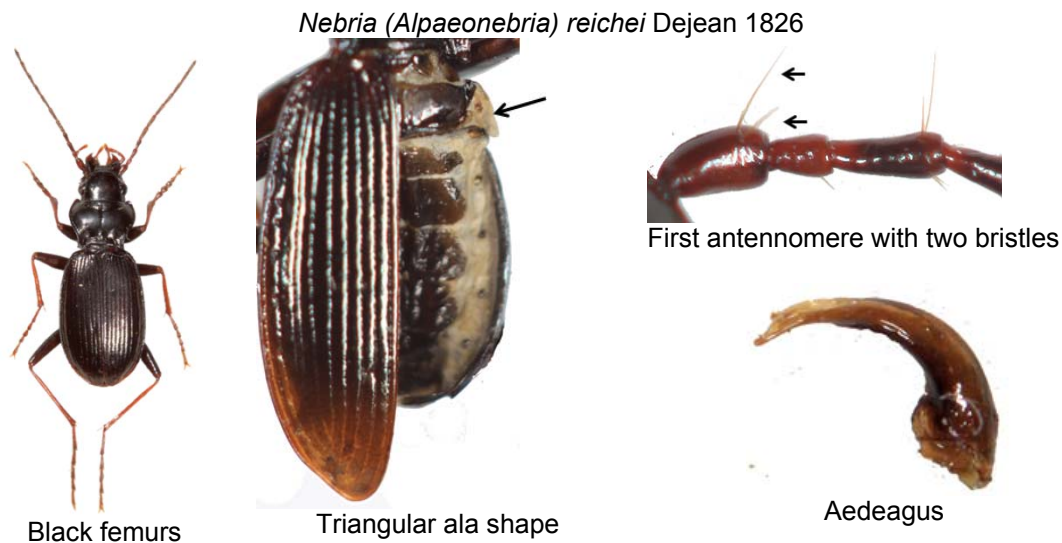
This species was considered as a variety of *Nebria reichei* Dejean 1826 for a long time and is still under this heading in several recent fauna classifications.

It is distinguished by (illustration in BARLOY et al., 2011):

- the difference in colour of the oral appendages, antennae and legs: flavescents at *reichei*, only lighten at *bisсенica*
- the shape of the first antennomere: short and subovoid at *reichei*, conic at *bisсенica* with 2 bristle of unequal length (as some populations

of *reichei*, (figure 4), but distinct by the flavescence of the appendages and legs).

-the number of the bristle of the submentum: 3 to 4 at *reichei*, 10 to 12 at *bissenica*.



**Figure 4.** Morphological aspects of *reichei*

Collected only in the Făgăraș Mountains, above the Transfăgărașan road tunnel, towards 2200 m., in the edge of streams of snowflake. Population mixed with *reichei*, sometimes *transsylvanica* and very localized.

**C. *Nebria (Alpaeonebria) reitteri* Rybinsky 1902**

Species also present in Ukraine: Chornagora (loco.typ), Gorgany, Marmarosh, (Panin R, personal com. 2017), Beskid [49], Transcappathia [25, 26, 27, 22, 23, 24] in subalpine zone 1300-1650 m.

Size 11-13 mm., black glossy concolored appendages or barely brightened. First antennomere ovoid, with a long bristle (like *femoralis*), second article with a bristle below. Submentum with 4-5 bristles on each side of the middle. Black legs. Ventrites 4, 5, 6 with 2 bristles on each side of the middle (only one in *femoralis*). Shape of the ala in S, as in *transsylvanica* and *femoralis*.

Horvatovich cited *rodnaensis* as a subspecies of the Rodnei Mountains [50], also formerly considered as a subspecies *reichei*, currently invalidated [20].

Widespread in the Maramureș Mountains (Borșa 1000 m.), Mount Rodna (Iezer Lake 1825 m., Mount Cisa 2000 m.), Cozia Mountains (Meteorological Station 1668 m) on the banks of streams or in forest areas at high altitudes (1000 to 2000 m.). Also cited by Merkl [38] of the Maramureș Mountains, Borșa Fântana Stanchii 1654 m, and by Csiki [13] and Hurka [40] of the Rodna Mountains

**2. MOLECULAR DATA**

Some molecular biology studies have been dedicated in Europe to:

- species with wide distribution: *jockischi*, *gyllenhali* with an identification of the most appropriate markers
- Nebria brevicollis* Fabricius 1792 with characterization of the entire mitochondrium [51]
- some other species (*helwigi* [52], *salina* [53], and GenBank).

To our knowledge, no studies have been carried out on the *Nebria* species from Carpathian Mountains.

**Nuclear marker**

This marker does not separate the species from each other but the subgenera with the exception of subgenus *Nebria (transsylvanica, femoralis)* and *Alpaeonebria reichii* that were confused with each other without significant variability. The distinction is clear between these two subgroups and *Eunebria jockischi* and *Boronebria (gyllenhali) heegeri* (Figure 5).

**Mitochondrial markers**

The analysis was done by concatenation of COI I and cyt b (1042 bp).

**2.1. Global analysis** (Figure 6) showed that all studied species are distinguished from one another with genetic differences (% K2P) ranging from 7.8 (*femoralis / alpigrada*) to 14.2 (*jockischi / gyllenhali*).

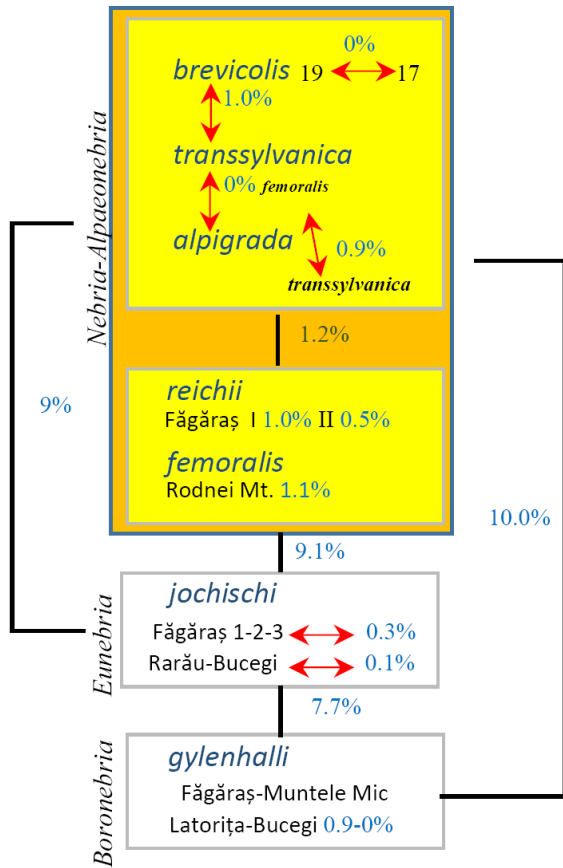


Figure 5. Marker 28S 5F5R

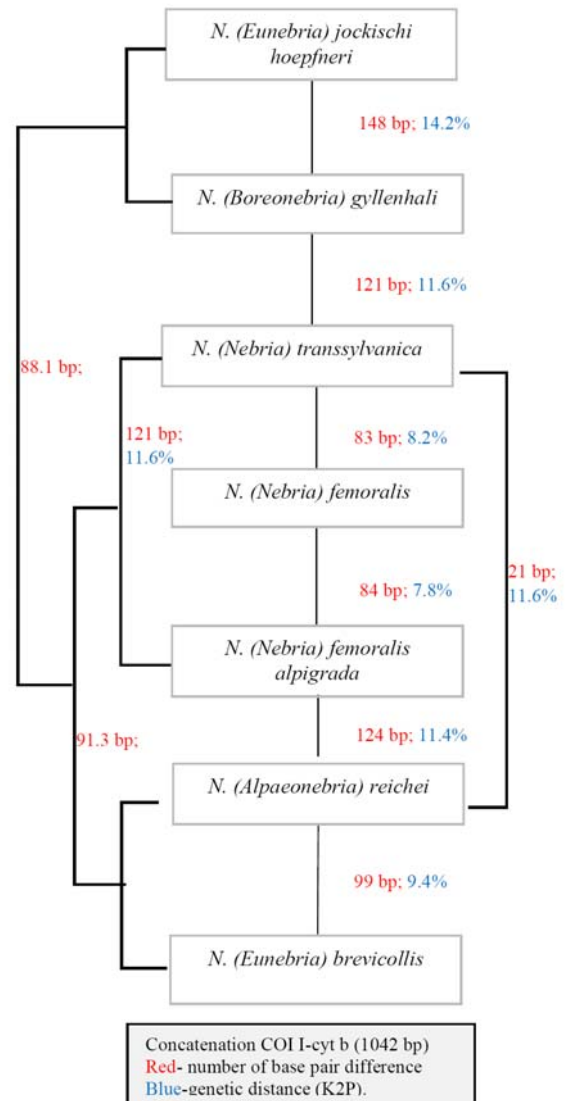


Figure 6. Interspecific distinction

## 2.2. Species analysis

### 2.2.1. *Nebria (Eunebria) jockischi hoepfneri* Dejean 1826

The widespread species, exhibits variability (Figure 7):

-geographical: case of Rarău beside Făgăraș-Bucegi-Muntele Mic

-altitudinal: the populations which are present at different altitudes (1550 to 2005 m) along the Transfăgărășan road vary slightly at medium altitudes (1550-1613 m.) but different towards the summit (1844 m.) and especially 2005 m. This suggests independent evolution.



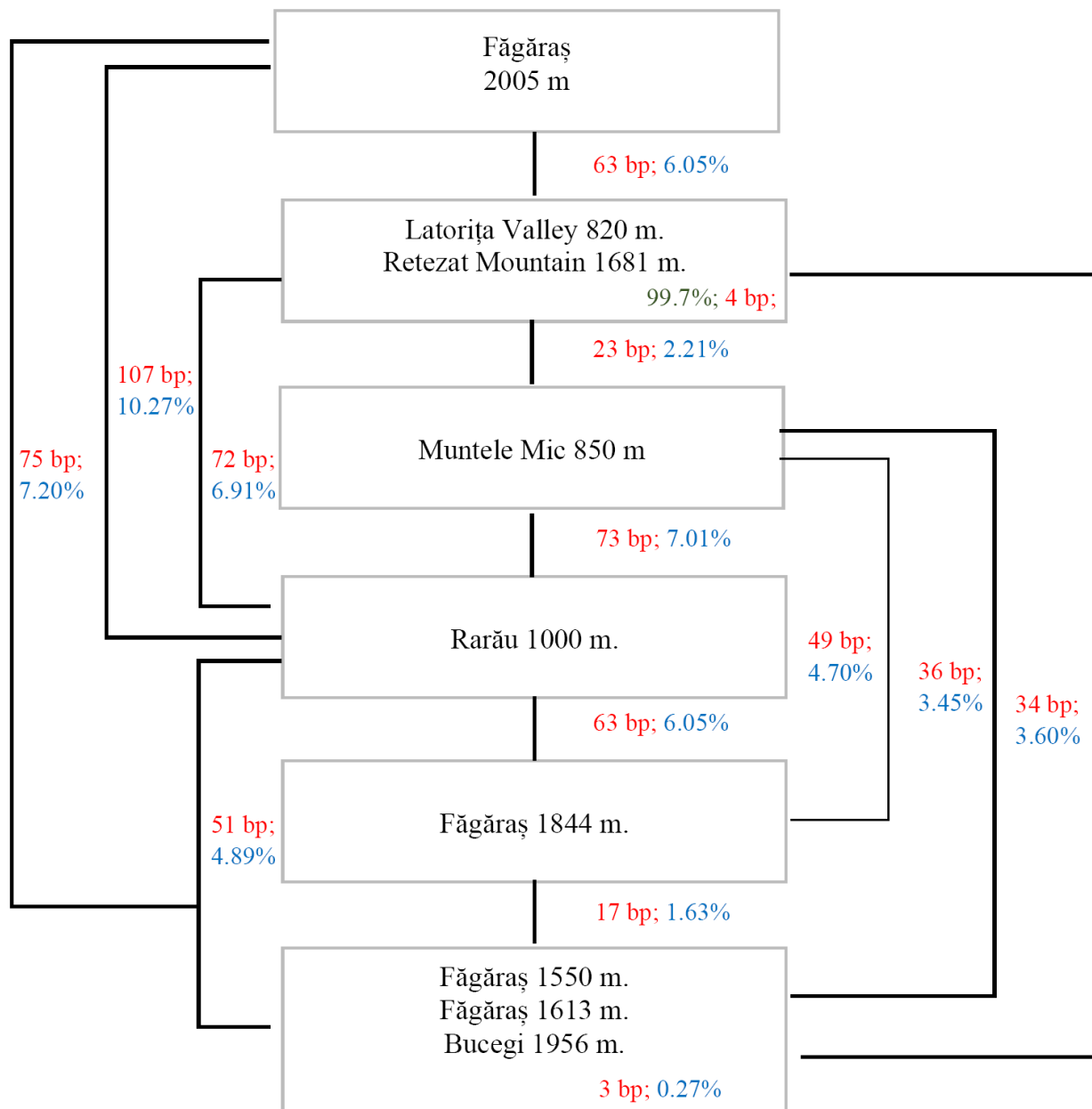


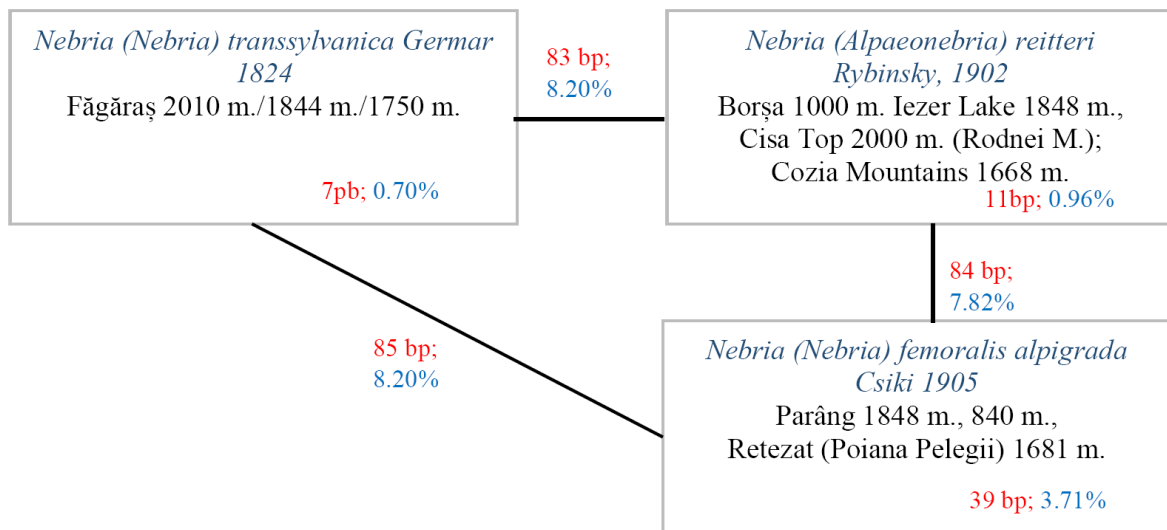
Figure 7. *Nebria (Eunebria) jockischi hoepfneri*

**2.2.2 *Nebria (Nebria) transsylvanica* Germar 1824, *Nebria (Alpaeonebria) reitteri* Rybinsky, 1902, *Nebria (Nebria) femoralis alpigrada* Csiki 1905**

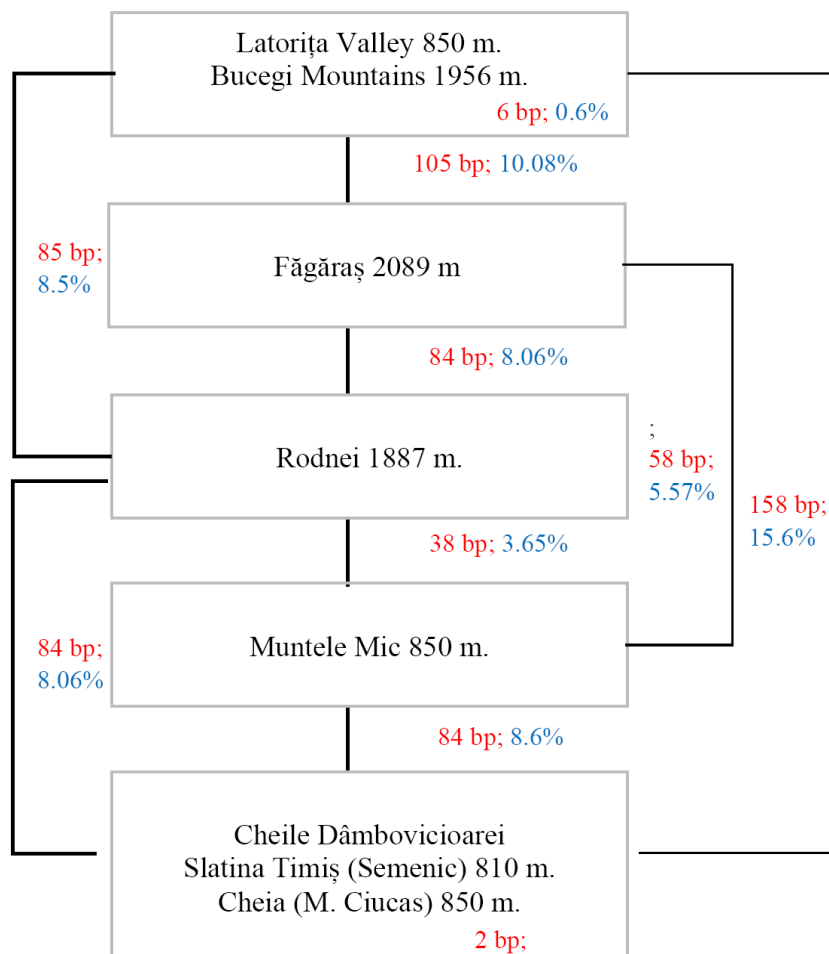
The intraspecific variability is lower for *femoralis* (0.9 % with 4 origins), strong (3.71%) for *alpigrada* (3 origins). *Nebria transsylvanica* differs from *femoralis alpigrada* and *reitteri* by the same genetic distances (8.2%) (Figure 8).

**2.2.3. *Nebria (Boreonebria) gyllenhali* Schönherr, 1806 (= *rufescens* Strom 1768)**

This very widespread species at the world level, partially studied from a genetic point of view, show a great infra-specific variability (K2P genomic distances between 3.6 and 15.2). These very important values require confirmation because they are not very compatible with an intraspecific status (Figure 9).



**Figure 8.** *Nebria (Nebria) transsylvanica* Germar 1824, *Nebria (Alpaeonebria) reitteri* Rybinsky, 1902, *Nebria (Nebria) femoralis alpigrada* Csiki 1905



**Figure 9.** *Nebria (Boreonebria) gyllenhalii* Schönherr

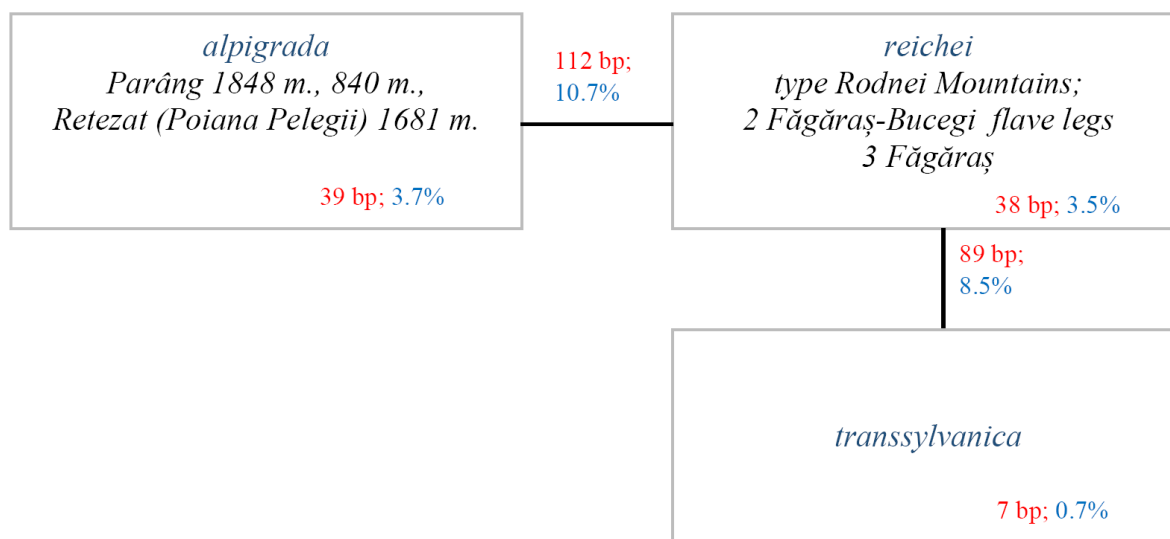


Figure 10. Group *reichei/alpigrada*

*Nebria femoralis alpigrada* differs from *reitteri* type by an average genetic distance of 7.8%; the two origins being different: Parâng Mountains 7.5%, Retezat Mountains 8.2%.

*Nebria femoralis alpigrada* is heterogeneous with an intraspecific variability of 3.7%, close to that found by RAUPACH et al (2010) for *Nebria helwigi*.

#### 2.2.4. Group of *Nebria* (*Alpaeonebria*) *reichei* Dejean, 1826, *Nebria* (*Nebria*) *femoralis alpigrada* Csiki 1905

The *reichei* specimens with yellow coloured legs can be confused with *alpigrada* (however *reichei* has a triangular atrophied aileron, *alpigrada* has an S-shaped aileron).

*Alpaeonebria reichei* presents on average a significant intraspecific variability (3.5% intrapopulation genetic distance) without distinction between the colour of the legs but with distinction of genetic nature:

-Bucegi/Făgăraș; Rodnei/Făgăraș: difference 5.3%

-Rodnei/Bucegi/Bucegi: difference 5.5%.

-*reichei*, which is genetically distinct from *transsylvanica* (8.5%), is strongly differentiated from *N. femoralis alpigrada* (genetic distance 10.7%) (figure 10).

#### 2.3. Nucleotide variations (study in COI I)

The majority of substitutions observed between *reitteri* and *femoralis alpigrada* are being transition (1.3%):

-a neutral effect at *reitteri* (all amino acids identical)

-a modification of the nature of the amino acids for *alpigrada*, compared to *reitteri*.

-provenance of Parâng Mountain: 2 amino acids of different biochemical class, methionine replacement by valine, valine by isoleucine  
-provenance of Retezat Mountain: alanine replacement by serine and asparagine by serine.

#### IV. Conclusions

##### A. From a morphological point of view

1. Two common species: *Nebria* (*Eunebria*) *jockischi hoepfneri* Dejean, 1826 and *Nebria* (*Boreonebria*) *gyllenhali* Schönherr, 1806 occur in many mountain regions from the foothill to the summit with geographical and altitudinal variability (see molecular biology). *Nebria* (*Eunebria*) *jockischi hoepfneri* is distinguished by a red stain on the vertex, with a very similar habitus (notably wings are very developed but without established functionality). In the stain absence, it differs from *Nebria* (*Boreonebria*) *gyllenhali* by 2-3 bristles on each side of the ventrites 4-5-6 in contrast to a single one of *gyllenhali*.

2. *Nebria* (*Nebria*) *transsylvanica*, captured only in the Făgăraș Mountains has a habitus characteristic for the type and the *alpigrada* form.

3. *Nebria* (*Alpaeonebria*) *reitteri* must be studied from several provenances (the captures are only from Rodnei, Mountains, Borșa, Cozia).

4. *Nebria* (*Alpaeonebria*) *reichei* has very variable antenna chaetotaxy (1 to 3 bristles) such as the colour of the femurs (black or yellow) and appears to have marked geographical variability (see molecular biology). It is distinguished from other species by its atrophied alae of triangular shape. Some populations of *reichei* have characteristics similar to *alpigrada* but are identified by their

triangular shape of the alae atrophy (alae S shape to alpigra and data of molecular biology).

5. *Nebria (Alpaeonebria) bisseica*, a species of high altitude and very localized, is distinguished from *reichei* by the lighter appendages and legs, two bristles of unequal length at the first antennomere of conical form (short, subvoid at *reichei*); the number of bristles on the submentum (10 to 12) being difficult to assess, given their fragility.

6. *Nebria (Boreonebria) heegeri*, a species of doubtful capture because it is difficult to separate from *gyllenhali*. Endowed with more reduced membranous wings and antennomeres with the same shape and dimension, *heegeri* has the second antennal segment provided with a bristle above and another below, unfortunately fragile. It maybe more widespread than is indicated in the literature. The first antennomere is conical to *Nebria (Boreonebria) heegeri* and more oval, cylindrical to *gyllenhali*.

#### B. In molecular biology

1. Mitochondrial markers exploited in concatenation COI I / cyt b distinctly separate the studied species, with genetic distances (coefficient K2P) varying from 7.8 (*reitteri* / *alpigra*) to 14.2 (*jockischi* / *gyllenhali*).

2. *Nebria (Eunebria) jockischi hoepfneri* has a great geographical and altitudinal variability (Făgăraş Mountains) like *N. (Boreonebria) gyllenhali*; the infraspecific variability is more moderate (3.5 to 3.7%) for *reichei* and *alpigra*.

3. *Nebria (Nebria) transsylvanica* is clearly identified by molecular data, which becomes a criterion for identification in case of rupture of the bristles of the first antennomere.

4. *Nebria (Nebria) femoralis alpigra* morphologically distinct from *reichei* (yellow colour of appendages and legs) is also separated by molecular data.

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