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Jean Barloy, Stephane Dréano, Frédérique Barloy-Hubler, Frédérique Hubler, Florin Prunar, et al..
Study of Carabus (MORPHOCARABUS) ROTHII COMPTUS Dejean 1831. Advanced Research in
Life Sciences, 2019, 3 (1), pp.11 - 16. 10.2478/arls-2019-0004 . hal-02382787

HAL Id: hal-02382787

<https://univ-rennes.hal.science/hal-02382787v1>

Submitted on 27 Nov 2019

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DOI: 10.2478/arls-2019-0004
Research Article

Study of *Carabus (MORPHOCARABUS) ROTHII COMPTUS* Dejean 1831

Jean Barloy¹, Stephane Dréano², Frederique Barloy-Hubler³, Florin Prunar⁴, Silvia Prunar⁴,
Aline Primot⁵

¹ Agrocampus Ouest Rennes, France,

^{2,5} UMR 6290, CNRS -Institute of Genetics and Development of Rennes (IGDR), Faculty of Medicine,
University Rennes I, France

³ Plateforme Amadeus-Biosit Rennes I, France,

⁴ Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from
Timisoara, Romania

Accepted March, 2019

Abstract

C. (Morphocarabus) rothii comptus Dejean 1831, is a endemic species of the Romania, appearing localized to some mountainous massifs of Romanian Banat and their piedmont areas (Poiana Rusca Mountains, Țarcu Mountains). Its presence in the Apuseni Mountains is doubtful, due to the lack of recent captures and to the confusion with *C. (Morphocarabus) hampei diffinis* Csiki 1905, morphologically like. In Banat the species occurs in two forms: *szorenyensis* Csiki 1908 at higher altitude (1300-2000 m) and *ulrichhoffmanni* Lie 1982 in hilly areas, which is distinguished by a larger size. Whatever the altitude and the ecological conditions, the period of activity is brief, spread over a month with a maximum of over two weeks. Molecular biology studies: confirm the belonging of *comptus* to *rothii* as a subspecies of her (28SRNA5 marker); suggest (concatenation COI I / cyt b) a low infra-specific variability between provenances of the *szorenyensis* (Rusca, Muntele Mic) and between the origins of the *ulrichhoffmanni*.

Key words: *rothii comptus*, distribution, taxonomy, molecular biology

Introduction

The species were briefly described by Dejean [1] using individuals from Banat, without indication of locality. That was provided by Emerich (Imre) Frivaldszky (Hungarian physician) curator of the Hungarian National Museum in Budapest. The Chaudoir collection of the MNHN Paris holds a male lectotype provided by Frivaldszky and listed "Hungaria-Bannat Mountains". According to Dejean, the typical type measure is L=21-24 mm/l=6-7 mm. The lectotype male of MNHN has a size of L/l=20.0/7.7 mm.

The populations fall into two size categories: the typical form and *szorenyensis* Csiki 1908,

encountered at high altitude that has small dimensions (Muntele Mic 1500 m., L/l=♂♂ 19.9/8.0, ♀♀ 20.5/8.6 mm.) and the form *ulrichhoffmanni* Lie 1982 from low regions (350-450 m) that has a larger size (Borlova 300 m) L/l = ♂♂ 25.3/9.0, ♀♀ 27.3/9.7 mm., with some females up to 30 mm (figure 1) according to [2].

Material and Methods

Studies of various origins are for *comptus* (origin Banat): subspecies *szorenyensis* Csiki 1908 from Rusca (meadow Padesu Plateau 1382 m. 45039 N, 22019 E) and Muntele Mic (meadow 1525 m 45022 N/22028 E); subspecies *ulrichhoffmanni* Lie 1982 from Căvâran (Constatin Daicoviciu forest 45033 N/22009 E) and Borlova (Ogasul lui Nicoara 45022 N/22021 E). Method of material preservation, extraction, purification and sequencing after [3]. Two molecular markers were used: -COI I (I Co 1490, HCO 2198 [4] and Cyt b

* Corresponding author: Prunar Florin,
florinprunar@usab-tm.ro



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(CP1 [5] CB1 CB2 [6]). Sequence analysis: manually analysed sequence with the help Geneious software version 7, multiple alignments performed with the algorithm MAFT [7], phylogenetic axes prepared with the algorithm Phy M1 [8], the substitution model IC69 and bootstrap of 10 replicates.

Results and Discussions

Taxonomy

Most of the authors [9-14] classify *comptus* as a subspecies of *rothi* [15] rank the species in the *schleideri* group [16] considered *comptus* as a separate species. The molecular biology analysis with the marker 28S rRNA 5 [17] places *comptus* within the *rothi* group together with *hampei*, *kollari*, *alutensis* (figure 2).

The membership of *comptus* as a subspecies of *rothi* seems well established. The literature mentions several taxa from which we retain two. Morphologically they present little differences (see size above), but they are distinct by their environment: mountain taxa, attached to the standard form Dejean 1831 type, the main being *szorenyensis* Csiki 1908, present at altitude (1200-2000 m.) and taxon of plains and mountain

foothills (300-500 mm) containing the form *ulrichhoffmanni* Lie 1982.

The 28S rRNA5 marker studies apportion the species belonging to *C. (Morphocarabus)* in 4 groups: group I: *rothi*, *kollari*, *comptus*, *hampei*, *alutensis*; group II: *scheidleri*, *seriatissimus*, *zawadzskii*, *incompsus*; group III: *excellens*; group IV: *monilis*.

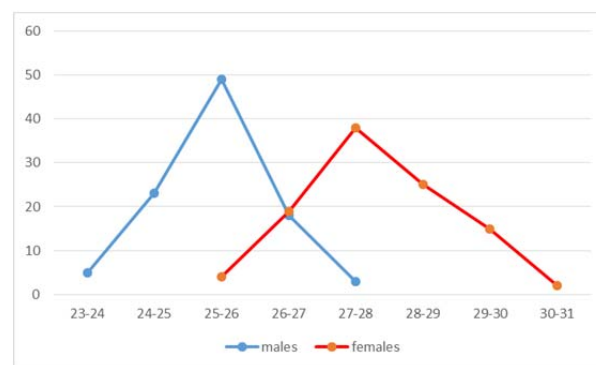


Fig. 1. Size variability of *C. (Morphocarabus) comptus ulrichhoffmanni* Lie 1982 (frequency in % /dimension in mm.) at Borlova (capture 2003)

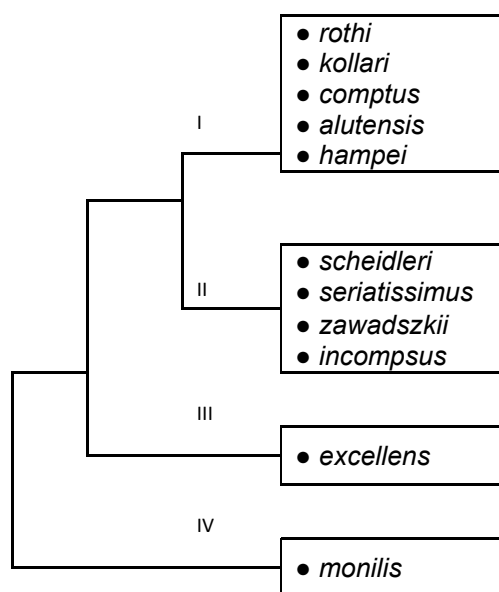


Fig. 2. Attachment to subgroup and specific identification (nuclear marker 28r RNA5)

The main features of the type form and szorenyensis

Narrow body, a little more bulbous at females. Bluish black individuals, rarely green or blackish brown. Above often bright, reflection is green or blue (especially at males). Elytral and pronotal borders coloured in blue or green. Elytra with 4 primary intervals, the external one sometimes difficult to identify. All the intervals (16 to 18)

salient in ribs shape, interrupted by small elytral foveoles often more frequent in the posterior part. The *ulrichhoffmanni* form is similar to the type, but differs by a larger size (see above), the margins and elytra being sometimes purple coloured, the taxon *micotensis* (Boutari) holding up to 20-22 primary intervals. *Comptus* is morphologically similar to *hampei*, the distinction being sometimes difficult although *hampei* should count 5 primary

Nucleotide	38	106	243
Group I	A	G	A
Group II	T	G	A
Group III	T	G	G
Group IV	T	A	A

558 nucleotides

intervals against 4 for *comptus*. Some small size forms of *hampei* can be confused with *comptus*. This is the case of *diffinis* Csiki 1905 (example Gârbău forest population 454 m. alt., near Cluj L/l=25.0-26.1/9.2-9.5 mm; blackish blue colour with elytral surface having in males often a greenish reflection and an elytral edge sometimes green). The position of this taxon is discussed: [18, 19, 14] place it under *comptus*; [20] attached it to *hampei* because of the presence of 5 primary intervals, [21-23] sees him as an intermediary form between *comptus* and *hampei*. In *diffinis* from Gârbău (see above) the fifth primary interval is hardly detectable, confounded in the multiple granulations of the elytral border. It is unlikely that *diffinis* is related to *comptus* for several reasons: inconsistency of the 5th primary at *hampei* of small

size forms and the location in plain (western half of Cluj County). The examination of the aedeagus and the endophallus (see below) shows that *diffinis* belongs certainly to *hampei*. The larger females of *comptus ulrichhoffmanni*, especially those from Bouțari (Micota Valley 600 m south Poiana Rusca Mountains), have large dimensions L / l = 30-31 / 11-12 mm and resemble *hampei*, but have 4 primary intervals.

Aedeagus and endophallus

There are small differences between curvatures of the aedeagus apex and the diversity of the endophallus shape comparatively *comptus* / *hampei* (*diffinis* taxon). In *comptus*, the aedeagus apex (figure 3) is more curved and the endophallus does not have a protuberance at the top of the agonoporus.

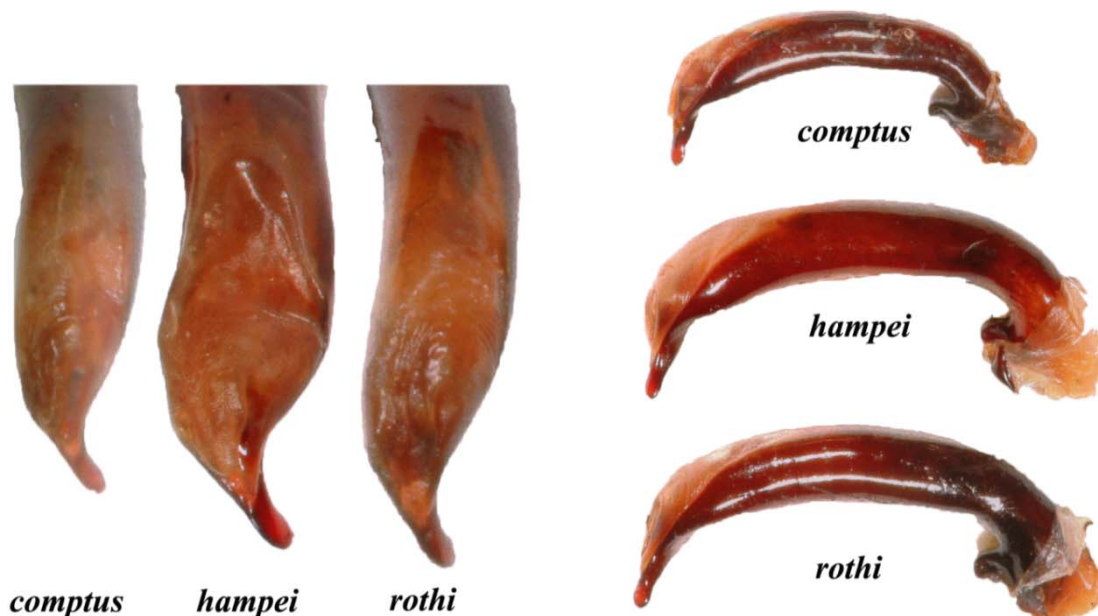


Fig. 3. Aedeagus of *comptus*, *hampei* and *rothi*

Geographical distribution

The species occurs in two regions.

a. Mountains and foothills of the mountain massifs of the Romanian Banat (Szorenyer Alpen=Poiana Rusca), Țarcu, Godeanu, Retezat [20, 24] with two forms: *szorenyensis* and *ulrichhoffmanni*. *Szorenyensis* Csiki 1908, is an altitude (1300-2000 m.) form. Past and recent captures are from: Țarcu, Muntele Mic, Poiana Rusca Mountains. Țarcu is quoted at Poarta Tarcului 2000 m., alpine meadow: L 20-21 mm [25]; Varful Nedeia, Varful Matania 2000 mm. [24, 26]. Muntele Mic is quoted by [20, 23, 27-29] where individuals are variously coloured (brown tanned, golden red, green, purple, some brown). We found it in summit meadow 1500 m. Size L/l ♂♂ 19.9-8.0 mm., ♀♀ 20.5-8.6 mm, but also as a small colony at the

foot of the Muntele Mic Massif near the Sebes River at lower altitude (400 m); the colony is supposed to be formed from specimens trapped by the water courses [2]. The specimens in this colony have the colours of *szorenyensis* and not of *ulrichhoffmanni*, which is found nearby at Borlova. In the Poiana Rusca Mountains the taxon is spread in the summit area of the Padesu Peak between 1200-1300 m under *Vaccinium myrtillus* and *Juniperus communis* in the *Nardus stricta* meadows of altitude with *Festuca rubra*, *Festuca supina* and *Bruckenthalia spiculifolia*. Size L/l ♂♂ 21.7-7.9 mm, ♀♀ 22.2-8.2 mm [2, 30]. Retezat is quoted by [24, 31, 32], but the species was not recently found [33]. *Ulrichhoffmanni* Lie 1982 is spread at the foothills of the previous mountains in hilly areas of Poiana Rusca Mountains. It was

found in 3 localities [28, 34]: place Gosta, Nadrag Valley 350-400 m., beech forest L/I = ♂♂25-27 mm./8-9 mm., ♀♀26-28/9-10 mm. Lie [28] (collect 1981); place Bouțari, Micota Valley 600 m., hardwood L/I = 30 mm./10-11 mm (collect 1995-1999); place Căvăran Forest (Constantin Daicoviciu) forest edge, oak wood (*Quercus petraea*), 250 m L/I= ♂♂24.5-26.5 mm. / ♀♀24.5-28.3 mm, [28]; ♂♂25.2-8.4 mm / ♀♀27.0-8.8 mm., [2]. In the hilly area of Muntele Mic, the taxon is found in the place named Ogasul lui Nicoara (Borlova), which is a wet valley at 380 m with the trees layer fairly rare (*Alnus glutinosa*, *Crataegus monogyna*, *Prunus spinosa*, *Rubus fruticosus*) on the side slopes [33, 34] with *Pteridium aquilinum*. Size L/I= ♂♂25.3-27.2/9.0-10.3 mm., ♀♀27.3-28.5/9.7-10.5 mm., [30]. These populations of *ulrichhoffmanni* presented in very different biotopes are morphologically very similar and large-sized.

b. Apuseni Mountains (Bihor Mountains) with mostly old references not confirmed by recent captures. The quotes are: [20] mentions it sporadically in the Bihor Mountains; Padiș in superior basin of Crișul Pietros [35], upper course of the Someșu Rece River 900-1300 m., Someșu Cald [19, 20, 31]; Colection Csiki: Ponor (MHN Budapest); Natura 2000 mentions Top Buteasa 1792 m., without specific identification. The presence of *comptus* in the Bihor Mountains is doubtful in the absence of recent captures and it is possible a confusion with *C. (Morphocarabus) hampei diffinis* of small size (populations of altitude).

Phenology. Activity.

In the mountains, the onset of adult activity begins after the snow melts. The grassland populations of the *comptus szorenyensis* species at Muntele Mic have a brief period of activity (2 to 3 weeks) usually in May. In the altitude pasture of Mount Țarcu (Poarta Țarcului) the maximal activity is situated (year 1985) in the second decade of August [33]. In the Rusca Mountains (1200-1300 m), the taxon activity is quite brief. In these localities, insects are active in full sun. In the mountainous foothills, *comptus ulrichhoffmanni* has an activity usually ranging from the end of May to the end of June with a maximum level from the last week of May to the first decade of June. This are the cases of the Căvăran station (at the rather dry forest edge 350 m.) and of the Borlova station (humid valley Ogasul lui Nicoara 300 m.). For this later locality, activity monitoring (figure 4 year 2003) shows a spreading of the imago exits from the end of May to the end of June. The male

insects are appearing before the females and their maximum activity is over 2 to 3 weeks. This progress is the same in Căvăran station. Whatever the altitude and the ecological conditions, the species seems to have a relatively brief period of activity of 3 to 4 weeks, even in humid environment in Borlova station.

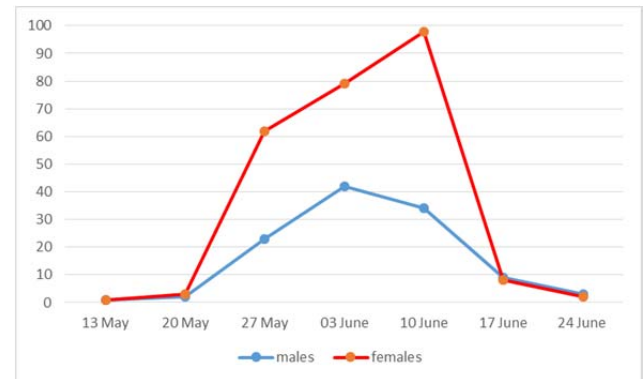


Fig. 4. Example of seasonal activity of *C. (Morphocarabus) comptus ulrichhoffmanni* Lie at Borlova (2003)

Intraspecific variability in molecular biology studies

The COI I / cyt b concatenation assay (1083 bp) provides the results below.

a. Distinction between the *C. (Morphocarabus) hampei diffinis* and *C. (Morphocarabus) comptus* (figure 5.)

b. Intraspecific variability of *C. (Morphocarabus) comptus* (all the origins from Banat). The local variability (figure 6) is great for Muntele Mic and Borlova because populations occupy a large area. Therefore, given the rates of genetic divergence, the population of *C. (Morphocarabus) comptus szorenyensis* from Rusca (Poiana Rusca Mountains) differs significantly from that at Muntele Mic (Țarcu Mountains) located 30 km away. For the markers used, the two origins of *C. (Morphocarabus) ulrichhoffmanni* are not different. The origin Borlova differs from Muntele Mic and does not seem to come from it, unlike Căvăran that is similar to Rusca. It should be noted, however, that the genetic difference values are not small. Intraspecific variability was studied in 2003 [36] with the mitochondrial marker ND5 for two forms of *M. rothi ulrichhoffmanni*, form *cavarani* Lie 1982 (foothills Poiana Rusca) and form *borlovensis* Lie 1994 (foothills Muntele Mic) and it was found an insignificant difference (single base 1083 in position 21 in transversion).

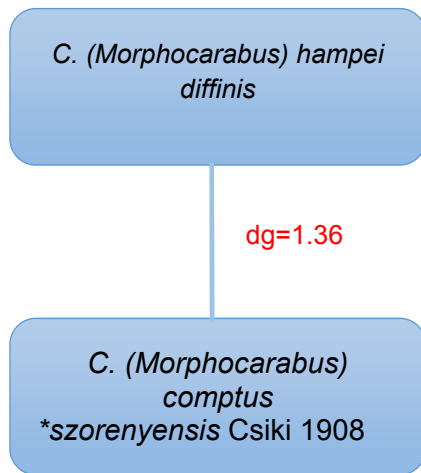


Fig. 5. Concatenation COI I/cyt b 1031 pb dg=genetic divergence

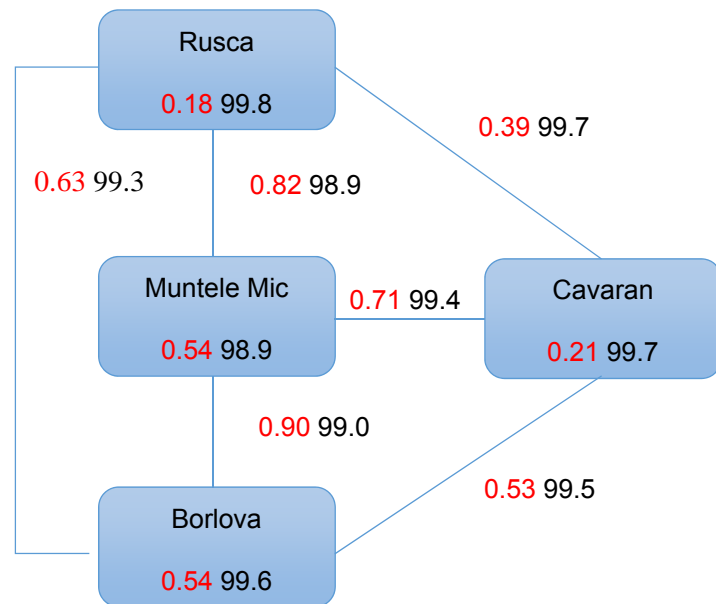


Fig. 6. Intraspecific variability of *C. (Morphocarabus) comptus* in Banat. Analyse in concatenation COI I/cyt b (1083 bp), genetic difference in percentage K2P Imura (red), parsimony percentage

Conclusions

C. (Morphocarabus) rothi comptus Dejean 1831 an endemic species of Romania, seems to be localized to the mountainous Massifs of Banat and to their foothills (Poiana Rusca Mountains, Tarcu Mountains, maybe also Retezat). Its presence in the Apuseni Mountains (Bihar Mountains) is doubtful, due to the lack of recent captures and the morphological resemblance to *C. Morphocarabus hampei diffinis* Csiki 1906 (absence of the primary interval). The *comptus-hampeii diffinis* distinction is related to the curvature of the apex of the aedeagus and the form of the endophallus; the classical molecular markers (ND 5, COI I, cyt b) are ensuring a low distinction. In Banat the species appears under two forms: *szorenyensis* Csiki 1908 at higher altitude (1300-2000 m) and *ulrichhoffmanni* Lie 1982 in the hilly areas (250-600 m) of bigger size and often more coloured (violaceous colour). Whatever the altitude and the ecological conditions, the period of activity is brief, spread over a month with a maximum peak.

Molecular biology studies with the marker 28S RNA 5, confirm the belonging of *comptus* to the supergroup of *Morphocarabus rothi* as subspecies (with *hampei*, *kollari*, *alutensis*). In concatenation COI I/cyt b the results show low rates of genetic divergence between forms and localities, but greater than in the local variability. With this reserve: *szorenyensis* Muntele Mic differs from Rusca (distance 30 km, 6 to 12 bp) and from

Borlova *ulrichhoffmanni* (7 to 11 bp). *Ulrichhoffmanni* from Căvărán (Poiana Rusca Hills) is close to Rusca *szorenyensis* (3 to 5 bp). This infra-specific variability appears weak.

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