

## STATUS OF *C. (MORPHOCARABUS) INCOMPSUS* KRAATZ 1880 IN ROMANIA

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**Abstract:** The study of literature and the review of the national collections of the Hungarian Natural History Museum Budapest, the collection of K. Petri from Brukenthal National Museum Sibiu, the collections of the National Museum of Natural History “Grigore Antipa” from Bucharest show that *C. (Morphocarabus) incompsus* Kr. 1880, is mainly endemic in Romania in Eastern Carpathians (an enclave exists in Ukraine, in Chornogora Mountains). The identification was based on little discriminating morphological criteria having a confusion risk with some taxons of *C. Morphocarabus hampei* Küst. 1846. *C. (Morphocarabus) incompsus* Kr. 1880 is a species morphologically difficult to distinguish from certain taxa of *hampei*, having intervals of the same width and little interrupted by superficial foveolae. This difficulty lies in the origin of the still recent ranking (2007) of some taxa of *incompsus* as forms of *hampei*, and of the attachment of this species to the *rothi* group. Up to recently, several sources of *C. (Morphocarabus) incompsus* Kr. 1880 were considered as *hampei* taxons. Currently the use of the shape of the aedeagus end, and the aspect of the endophallus allow us to identify males individuals but not the female specimens, often more frequent than males. The 28S rRNA5 nuclear marker ensuring the separation. The study with this marker of several species of *G. Morphocarabus* identifies *incompsus* and suggests taxonomic changes. The attachment of *C. (Morphocarabus) kollari* Pall. at *Sg. rothi*, and not at *Sg. scheidleri*, *C. (Morphocarabus) incompsus* Kr. 1880 which has been until then ranked in the *Sg. rothi* is located in the *Sg. scheidleri*. The analyse also shows that some populations until then assigned to *incompsus* belong to *hampei*, changing the initial distribution map. The marker 28S rRNA5 indicates that certain origins, morphologically the *incompsus* kind, belong to *hampei* (Bichigiu, Varghis, Corongis medium altitude), unlike the others (Hășmaș for the form of *mehelyanus* Csiki 1906), *Bogata-Poiana Brașov* for the type. This encourages further investigation in order to determine the exact location of species.

**Key words:** *C. (Morphocarabus) incompsus* Kr. 1880, 28S rRNA5 nuclear marker, phylogenetics.

### INTRODUCTION

In external morphology *C. (Morphocarabus) incompsus* Kr. 1880 is very difficult to distinguish from *C. (Morphocarabus) hampei* Küst. 1846, especially the shapes with equal length and little interrupted intervals by superficial foveae. This difficulty lies at the origin of the rank made by CSIKI (1906-1946) as *hampei* taxons, of *incompsus* type and of most shapes he has described (*mendax*, *spectabilis*, *mehelyanus* 1906).

This classification has been recognized for a long time by many authors, until recently (KLEINFELD et al. 1999); LIE et al. (2001) SZEL et al. (2007) for *spectabilis* Csiki 1906 and *mehelyanus* Csiki 1906. However, SZEL et al. 2007, based on the shape of the inner bag follows ISHIKAWA (1973) considers *C. (Morphocarabus) incompsus* Kr. as a valid species.

Based on different criteria, other authors, after 1999: BREZINA (2000), LOBL et al. (2003), DEUVE (2004) rank *incompsus* (at least its shapes of altitude) as ssp. or subsp. of *rothi*; subgeneric positions generally accepted without many arguments.

Using the shape of endophallus, different between *C. (Morphocarabus) hampei* Küst. and *C. (Morphocarabus) incompsus* Kr. ensure the identification of male individuals. This allows to Panin:

- to identify, in 2011, *mendax* Csiki 1906 as belonging to *C. (Morphocarabus) incompsus* and to invalidate this taxon from the list of *hampei* shapes,
- to consider, in 2013, as *hampei* a native population originating from Tibles Mountains (Bichigiu from Bistrita Nasaud County) morphologically mimetic of *incompsus* for elytra sculpture.

Apart from an enclave in Ukraine (see below) mentioned by POVIGALO (1994) and PANIN (2006) in Chornogora Mountains at the limit of subalpine floor (1800-2020 m). *C. (Morphocarabus) incompsus* Kr. is an endemic species of the Eastern Carpathians in Romania (see geographic distribution map) established from the bibliographic references and the collections consultation.

No recent study has been dedicated to *C. (Morphocarabus) incompsus* exploiting the new criteria of identification (endophallus molecular biology) and aiming at:

- establish the taxonomic relationship with other species of the *Morphocarabus* genus,
- check the belonging of the populations quoted in the literature,
- specify the geographical distribution.

#### **GEOGRAPHICAL DISTRIBUTION**

*C. (Morphocarabus) incompsus* Kr. does not exist in the Czech Republic (NAKLADAL et al. 2012), nor in Hungary (RETEZAR 1997), but appears in Ukraine in the Chornogora Mountains (PANIN 2011), not being signaled in Maramures Mountains by NITU (2008), MERCK (2008). The species seems subservient to Romania, especially to the Eastern Carpathians. (fig 1)

#### **Known localities in Romania:**

The list below results from the literature data (including *Carabidae* of the World) and from collections' consultation (HNHM Budapest, collection K. Petri of Brukenthal NM Sibiu, collection NMNH Grigore Antipa Bucharest). The locations mentioned below are presented from north to south of the Carpathian arc:

- + Oaş Mountains - Camârzana-Călinești Oaş (Satu Mare County), RODICA SERAFIM 1987("Grigore Antipa" National Museum of Natural History Bucharest), citation under reserve,
- + Tibles Mountains: Bichigiu (Bistrița Năsăud County) 390 m. initially considered as *incompsus*, disabled (2014) by PANIN by basing itself on the shape of the aedeagus and attributed to *hampei* (transitus *incompsus*),
- + Rodnei Mountains (Bistrița Năsăud County) taxon *spectabilis* Csiki 1906:
  - Ineu, between 1500-1800 m. (HOLDHAUS et al. 1910),
  - Corongis (CSIKI 1906-1946), PETRI (1900), MÜLLER (1930):
    - between 1300-2000 m., SZEL et al. (2007),
    - 1950 m. LIE et al. (2001),
  - Saca Mountain 1600 m. Holdhaus et al. (1910),
- + Suhard Mountains: Vatra Dornei (Suceava County): 1500 m. LIE (1982),
- + Calimani Mountains: Colibița (Bistrița Năsăud County) exemplary female; not validated species,
- + Hășmaș Mountains (Harghita and Neamț Counties) taxon *mehelyanus* Csiki 1906:

-Hășmașu Mare 1783 m. and Tarcău 1589 in KARL PETRI collections: MEHELY (1891), DEUBEL (1894), CSIKI (1906, 1942, and 1946), BREUNING (1932), Piatra Tarcaului 1963m. (LIE *et al.* 2001), SZEL *et al.* (2007),

-Munticelu 1000 m. (LIE *et al.* 2001),

+ Harghita Mountains (Harghita County) :

-Vlahita-Varghis-Selters 720-820 m., LIE *et al.* (2000), MATHE *et al.* (2002),

-Odorheiu Secuiesc, CSIKI 1906,

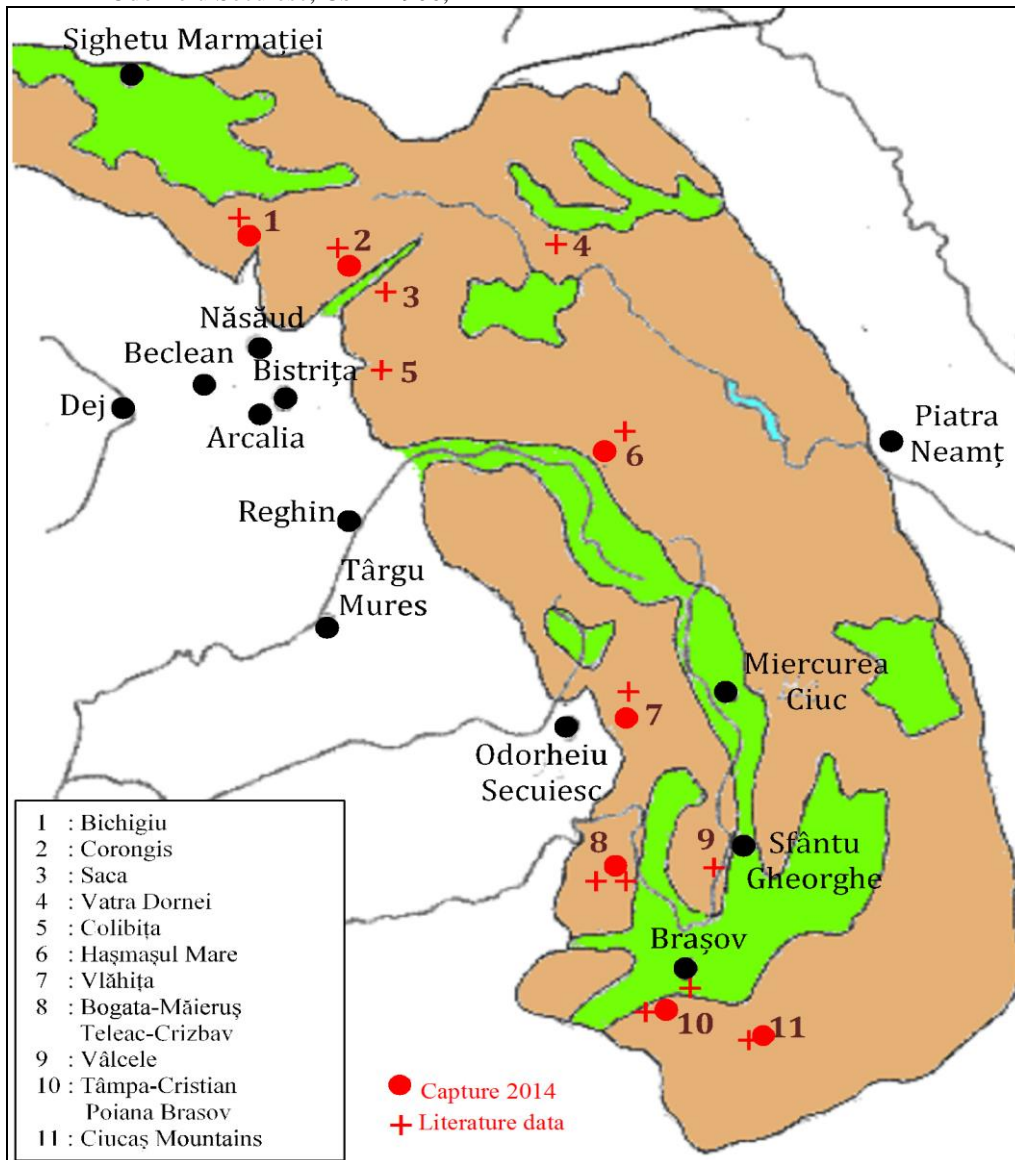


Figure 1. Geographical distribution of *C. (Morphocarabus) incompus* Kr. 1880

- + Persani Mountains (Braşov County) :
  - Maieruş-Teleac : PETRI (1912) ; Crizbav: BIELZ (1912),
- + Baraolt Mountains (Covasna County):
  - Valcele: PETRI (1912), BREUNING (1932),
- + Braşov Mountains (Braşov and Prahova Counties):
  - around of Brasov: KRAATZ (1880), in PETRI: BIRTHLER-DEUBEL (1895),
  - Postăvarul Massif (near Poiana Braşov) 1789 m. in Petri: KIMAKAVEZ (1895), Timiş-Tâmpa: LIE et al. (2001),
  - Cristian village (Braşov County) 380 m.,
- + Braşov Mountains (Braşov County):
  - Râşnov in PETRI: ALBRECHT (1895),
  - Azuga-Sinaia-Buşteni FLECK (1904), MONTANDON (1906),
- + Ciucaş Mountains (Braşov and Prahova Counties):
  - Muntele Roşu 1397m., Pasul Bratocea 1263 m., MEHELY (1890), PETRI (1912), KUTASI et al. (2011).

Except the fact that the true discoverers are the first to be quoted, the most recent authors have taken these references, without always having conducted an audit "in situ".

Most of the references are old and the assignation of populations to *C. (Morphocarabus) incompsus* Kr. 1880 is based on morphological criteria, available only at those times.

## STUDIES OF SOME ORIGINS OF *C. (MORPHOCARABUS)* SPECIES

### Material and methods

**Biological material:** *C. (Morphocarabus) incompsus* Kr. 1880 (c. localization, distribution map).

- Bichigiu-Valea Bichigiului (Bistriţa Năsăud County) 47°41' N, 24°35'E ; 435 m., ♂♂ 36.0/13.5 mm, ♀♀ 37.8/13.7 mm.
- Călineşti Oaş forest, 47°52' N, 23°14'E ; 170 m., 47°53' N, 23°16'E ; 180 m., ♂♂ 30.0/11.5 mm, ♀♀ 31.5/11.5 mm., blue, green, deep enough punctuations.
- Corongiş (Bistriţa Năsăud County) 48°76' N, 21°81'E ; forest 1087 m, and alpine meadow 1312 m, ♂♂ 30.6/11.8 mm, ♀♀ 31.0/12.0 mm. supposed *spectabilis* Csiki 1906. Sometimes the brightly colored blue or purple. In the open area unto the top of Corongisu Mare it was not found a population which to provide specimens.
- Hăşmaşu Mare 1783 m, 46°68' N, 25°82'E ; ♂♂ 22.0/8.5 mm, ♀♀ 25.0/9.4 mm. supposed *mehelyanus* (Csiki 1906). Margins often blue darkened.
- Vârghiş-Selters 789-806 m. 46°31' N, 26°54'E ; ♂♂ 28.7/11.5 mm, ♀♀ 29.7/11.6 mm.
- Bogata Forest 700 m. 45°54' N, 25°26'E ♂♂ 28.2/10.8 mm, ♀♀ 29.2/11.3 mm.
- Poiana Braşov Forest 987 m. 45°36' N, 25°33'E ♂♂ 27.9/10.0 mm, ♀♀ 28.8/10.7 mm.
- Tâmpa Braşov Forest 800 m. 45°38' N, 25°35'E;
- Ciucaş (forest from vicinity of Bratocea Pass at 1174 m.) 45°28' N, 25°54'E ♂♂ 28.0/11.0 mm., no visible punctuations

### Other *Morphocarabus* biological material

a. Romania: 2 *kollari*, 19 *rothi*, 3 *comptus*, 6 *alutensis*, 15 *hampei*, 6 *seriatissimus*, 2 *excellens*

b. France: 5 *monilis*,

c. Moldova: 2 *excellens*

For a, b, c, detailed references in Barloy et al. 2014.

d. Romania: *C. (Morphocarabus) hampei* Kust 1846. The closest morphologically form from *incompsus* Kr.

-Beclenut : 47°42' N, 26°19'E ; 275 m. ♂♂ 31.5/11.8 mm, ♀♀ 34.3/12.9 mm.

-Arcalia : 47°05' N, 24°20'E ; 296 m. ♂♂ 31.3/12.0 mm, ♀♀ 33.4/12.2 mm.

-Micula 47°53' N, 22°56'E 100 m. ♂ 30.0/11.7 mm

e. Hungary:

*C. Morphocarabus) scheidleri styriacus* Kraatz, 1887

-Bakonybél 47°15' N, 17°43'E; 344 m., ♂♂ 27.1/11.5 mm, ♀♀ 29.5/11.2 mm.

*C. (Morphocarabus) zawadzki dissimilis* Csiki, 1906

Village Óhuta (Háromhuta Borsod-Abaúj-Zemplén) 48°30' N, 21°30'E; 375 m., ♀♀ 31.5/12.0 mm,

#### **Methods**

**a. Morphometric studies:** external morphology, aedeagus, endophallus

**b. Molecular biology studies**

Attachment at subgroups and specific identification. Use of nuclear marker 28S rRNA<sub>5</sub>

*Primers:*

28SO1 and 28 SRO1 (Kergoat et al. 2005) ;

28S D45F (Belshaw and Quicke 2002)

*Methods* cf Dreano et al. (2015).

Comparison with *C. (Morphocarabus) hampei dacicus* Csiki, 1906 and infraspecific variability. The use of cytoplasmic markers COI I and cyt b.

COI I : CO 1490-HCO 2198 (Hébert et al. 2003)

cyt b CP1 (Harry et al. 1998) CB1-CB2 Jermin and Crozier (1994)

Methods cf. Barloy et al. 2014.

*Sequences analysis*

Sequences analyzed manually on using the software Geneious version 7,

Multiple alignment performed with the algorithm MAFT (Kalloh 2013)

Phylogenetic trees made with the algorithm PHYML (Guindon 2003), the substitution model JC 69 and bootstrap of 10 replicata.

## **RESULTS AND DISCUSSIONS**

### **Morphological studies**

#### **External morphology**

The external morphology comparison between the populations individuals of Poiana Braşov-Bogata, of substantially similar size and those of *C. (Morphocarabus) hampei dacicus* Csiki 1906, the most similar to *incompsus* the taxa of *hampei*, often shows some differences.



Bichigiu

Poiana Braşov

Beclean

Figure 2. Populations appearance *incompsus* (Bichigiu, Poiana Braşov); populations appearance *hampei* (Beclean)

The distinguishing criteria, very few ones, vary within populations, morphologically heterogeneous, involving the observation of a number of insects.

- a. Elytra - wider on the coasts, in the rear third, apex elytra more strongly rounded (pointed at in *dacicus*); feature especially valuable for ♂♂.
- b. Foveae of primary intervals, in small number, sometimes absent, shallower than *dacicus*.

As indicated, these criteria are sometimes insufficient because they are fickle to provide certain identification with the shape of *C. (Morphocarabus) hampei* Küster 1846 to equal width and slightly interrupted intervals. (fig. 2)

#### **Aedeagus and endophallus**

The comparison between aedeagus and endophallus *C. (Morphocarabus) incompsus* Kraatz, 1880 (population Poiana Brasov) and *C. (Morphocarabus) hampei dacicus* Csiki, 1906 shows (fig. 3))

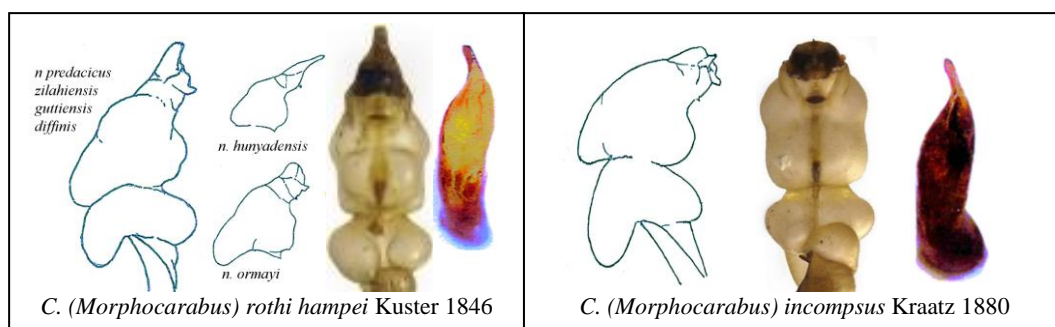


Figure 3. Length of the aedeagus apex and endophallus shape (endophallus after Panin)

- a similar shape of aedeagus, except the apex, longer in *incompsus*,
- a more curve endophallus with a very short to nonexistent aggonoporus.

#### **Molecular biology studies. Results**

##### **Attachment to subgroup and specific identification (nuclear marker 28r RNA5)**

The marker 28S rRNA5 apportions the studies species belonging to *C. (Morphocarabus)* in 4 groups (fig. 4):

- group I: *rothi*, *kollari*, *comptus*, *hampei*, *alutensis*,
- group II: *scheidleri*, *seriatissimus*, *zawadskii*, *incompsus*,
- group III: *excellens*,
- group IV: *monilis*,

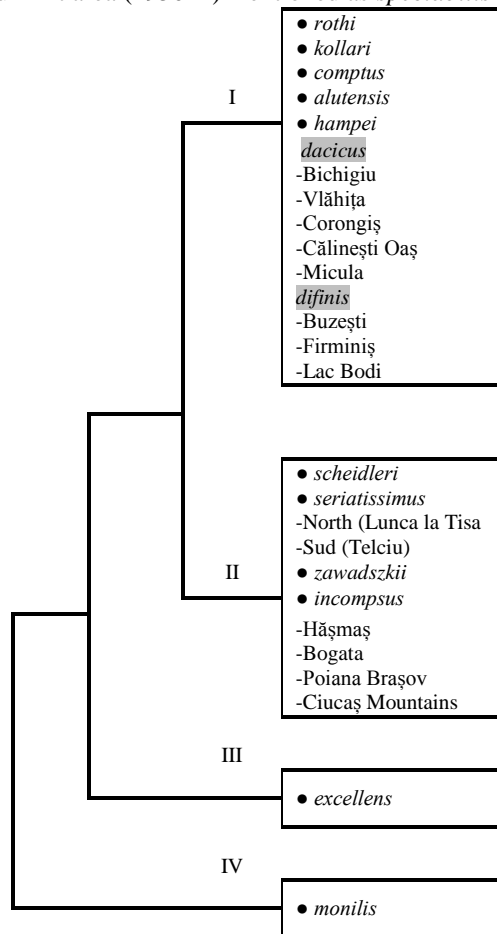
- a. Insofar as this marker groups the species by Subgenus (Sg)

- comptus-hampei-alutensis* can be grouped under the Sg *rothi*, classification accepted by many authors,
- kollari* should be placed under the Sg *rothi* and not under Sg *scheidleri*,
- seriatissimus-zawadzki dissimilis* is attachment to Sg *scheidleri*,
- incompsus* did not belong to *rothi*, or to *hampei* but to *scheidleri*,
- excellens* and *monilis* form different Sg.

These nomenclature changes are proposed on the basis of genetic criteria and not only morphological.

- b. The marker 28S r RNA5 divided the populations apparently „*incompsus*” in two groups:

b.1. Those related to the group *rothi-hampeii*, having a elytral mimetic sculpture of *incompsus* and which are *hampeii* (Bichigiu, Vlăhița, Corongiș). At Bichigiu, basing on the shape of aedeagus Panin (2014) had identified as belonging to *hampeii* a population considered *incompsus*, that il named *hampeii* transitus *incompsus*. Without being certain that our capture come from the same location, the analysis in molecular biology of insects apparently „*incompsus*” led to the same report. A Vlăhița in his doctoral thesis Mathé (2007) quotes *hampeii*, becoming *incompsus* in the publication of 2008 (Mathé et al.). For Corongiș, the catches were performed at half height (1087-1312 m) and belong to *hampeii*. One should study the populations in the summit area (1950 m) mentioned as *spectabilis* (Csiki 1906).

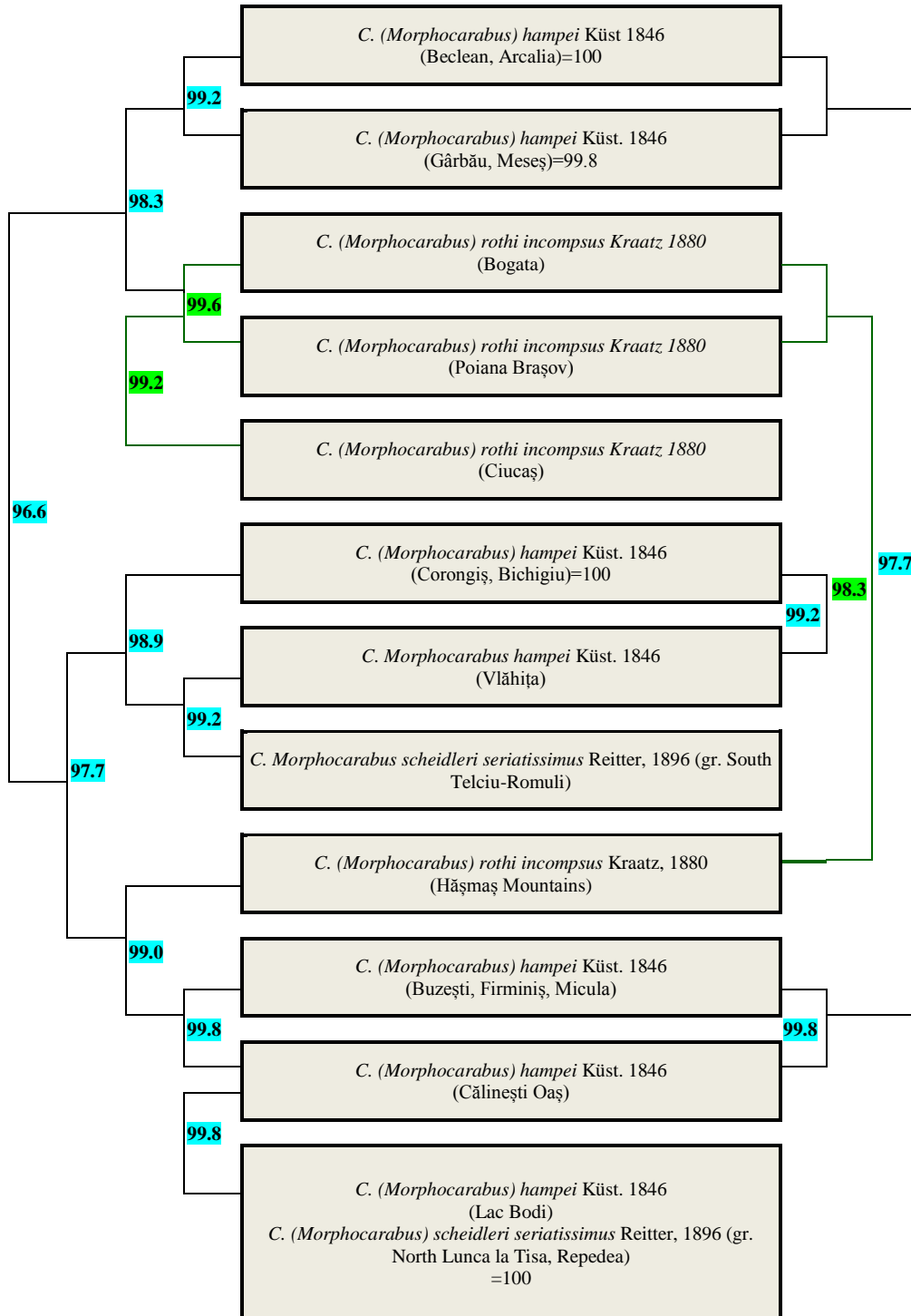


Nucleotide	38	106	243
Group I	A	G	A
Group II	T	G	A
Group III	T	G	G
Group IV	T	A	A

558 nucleotides

Group I: sg. *rothi*  
Group II: sg. *incompsus*

Figure 4. Attachment and identification using nuclear marker 28r RNA5





- Relations between populations *incompsus*
- Relations between populations *hampei*

Figure 5. Results using cytoplasmic marker COI I

Lie et al. 2001 signals the ambiguous status of this shape compared to *mendax*. Furthermore *C. (Morphocarabus) hampei* Küster, 1846 is mentioned in Rodnei Mountains -Telciu towards Telcisoara (pers. com. Nitu) and Natura 2000 (PM PN Rodna Mountains 2008).

b.2. Those related to the *scheidleri* group show the *incompsus* based on elytra sculpture by difference with that of *seriatissimus* attached to the same sg. (*scheidleri* do not exist in in Romania). The case of: Hășmaș, Bogata, Poiana Brașov.

#### **Conclusion - Discussion on the use of the 28S rRNA5 nuclear marker**

This marker, operational, regardless of sex:

- it is added to the shape of the endophallus as a criterion for distinguishing between *C. (Morphocarabus) incompsus* Kraatz, 1880 and shapes of *C. (Morphocarabus) hampei* Küster, 1846 has similar elytra costulation.
- suggest among the species of subgenus *Morphocarabus*, changes to the taxonomic connection for *kollari* and *incompsus*.
- shows that various populations considered as *incompsus* belong to *hampei* and encourages the study of all those mentioned by the discoverers which often are experienced entomologists (Csiki, Deubel, Petri and the members of Sibiu School).

#### **Intraspecific variability of *C. (Morphocarabus) incompsus***

##### **Results with the cytoplasmic marker COI I**

These results also cover *C. (Morphocarabus) scheidleri seriatissimus* Reitter, 1896 studied previously (Barloy et al. 2014) and *C. (Morphocarabus) hampei* Küst 1846. (fig 5)

#### **CONCLUSIONS**

Cause of the lack of relevant criteria in external morphology the distinction between *C. (Morphocarabus) hampei* Küst. 1846 and *C. (Morphocarabus) incompsus* Kraatz 1880 it turns out random and risks of confusion is high. The safety of identification is obtained by recourse to the shape of the aedeagus for the male insects and has the molecular biology for both sexes (28S rRNA5).

In the current state of the prospecting, four geographical zones hold *C. (Morphocarabus) incompsus* Kr.: Hășmaș Mountain and Tarcăului Mountain / Perșani Mountains / Postăvarul Mountain and Brașov / Ciucaș Mountains. The presence in other places (Rodnei Mountains; Călimani Mountains, Suhard Mountains) require confirmation. In the southern part of Rodnei Mountains in Saca Mountain (1600 m) and Corongisu Mic, in the forest area is only *C. (Morphocarabus) hampei* Küst., 1846.

These results:

Confirm the ancestral origin proximity between *C. (Morphocarabus) seriatissimus* Reitter, 1896 (northern group) and *C. (Morphocarabus) incompsus* Kraatz, 1880 (Hășmaș) already found in previous work.

Show that the populations of Bichigiu, Corongis, Vlahita are very close, do not belong to *C. (Morphocarabus) incompsus* Kraatz, 1880 but to *C. (Morphocarabus) hampei* Küst., 1846 (cf. results 28S rRNA5). The populations from Oasului Mountains also belong to *C. (Morphocarabus) hampei* Kraatz, 1880.

Show two homes for *C. (Morphocarabus) incompsus* Kraatz, 1880 with genetically distinct populations.

- a northern center of Hasmaș Mountains (and maybe close to Tarcaului Munticelului)

-a fairly large southern area, including Persani Mountains (Bogata), Postavarul Brasov and Ciucaş.

Among other potential sites one should study the Suhardului Mountains (Vatra Dornei mentioned by LIE *et al* (2001) and the Calimani Mountains evoked by Panin (Molbiol. Ru. 2008).

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