# The belonging of *C. (Morphocarabus) rothi hampei telekii* Csiki, 1937 to *C. (Morphocarabus) rothi rothi* Dejean, 1829, confirmed in molecular biology

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***Abstract****: C. (Morphocarabus) rothi rothi Dejean is a Romanian endemic species spread in two areas at a distance of 250 km apart. In North of Banat, one of these areas, rothi is mixed with hampei, teleki form. The framing made by Csiki for telekii form, at Carabus (Morphocarabus) hampei Kuster, 1846, encountered in the hills of Fragulea (North of Banat) is questioned, due to the presence of 4 primary intervals which are theoretical characteristic for C. (Morphocarabus) rothi Dejean 1829. The other conventional morphological criteria (elytra sculpture, forms of the aedeagus and endophallus, body color) are not sufficient to ensure a clear separation between this two species. The inconstant number of primary intervals or the close shape of the aedeagus and endophallus make it impossible the distinction. The appeal to the mitochondrial markers, cytochrome c oxidase subunit I (COI) and cytochrome B (cyt b), applies to all the origins of rothi (North Banat and Transylvanian quadrilateral delimited by Sibiu, Copşa Mică, Sighişoara, Rupea, Făgăraş) and to the taxa of hampei by reference (Buzeşti from Maramureş County and Firminiş from Sălaj County), proves that telekii belong to C. (Morphocarabus) rothi like the aberration vitiosus Csiki, 1906. For the rothi rothi were taken in study the aberrations: latestriatus, quadricatenatus, aequistriatus and vitiosus. Therefore it is appropriate to remove the telekii Csiki 1937 form from the list of C. (Morphocarabus) hampei taxa. However, in North of Banat, the sculptural appearance rothi are heterogeneous in molecular biology: about 21% of individuals are belong to hampei with a very nearby genomic structure of hampei from Buzeşti but a rothi rothi ab. quadristriatus appearance and 71% rothi rothi including the forms considered hampei telekii. The genomic polymorphism of the populations of appearance rothi telekii of the North Banat and the attachment of some individuals to hampei had already been mentioned in a previous study with the marker NADRH 5, but with a reduced number of samples studied.*

***Key words:*** *Carabus (Morphocarabus) rothi* Dej., *Carabus (Morphocarabus) hampei* Kust., *telekii* Csiki, taxonomy, molecular biology, COI, cyt b.

## 1. INTRODUCTION

### 1.1. The relationship between *rothi rothi* and *hampei telekii*

- *C. (Morphocarabus) rothi rothi* Dejean, 1829, endemic species of Romania, can be found in two distinct regions (figure 1):
  - -Transylvanian quadrilateral (Sibiu, Copşa Mică, Sighişoara, Rupea, Făgăraş); being the only present species of the genus *Morphocarabus*.
  - -Northern Banat, mainly in the Fragulea Hill and some stations of the right bank of the Mureş River (localities in LIE, 1991, BARLOY et al., 2013) where is in mixture in with individuals assigned to *telekii* which are present in important proportion (50-55%).
- Problems posed by the populating of the North Banat:
  - that of the origin of the populating compared with the populations *rothi* of the main site of Southern Transylvania located at 250 km in direct line. It is about an

independent genetic centre or about a transport of insects by the way of rivers from Transylvania using the network Târnava Mare-Mureş.

-that of the taxonomic position of the form *hampei telekii* Csiki, 1937

●Problem of the taxonomic position of the form *telekii*:

-basing itself on the presence of 4 primary intervals, the theoretical characteristic of the *rothi*, Szel et al., 2007 consider *telekii* as belonging to *rothi*.

-inadequacies of the morphological criteria to ensure the distinction between *C. (Morphocarabus) rothi rothi* Dej., 1829 and *C. (Morphocarabus) rothi hampei* Kust., 1846.

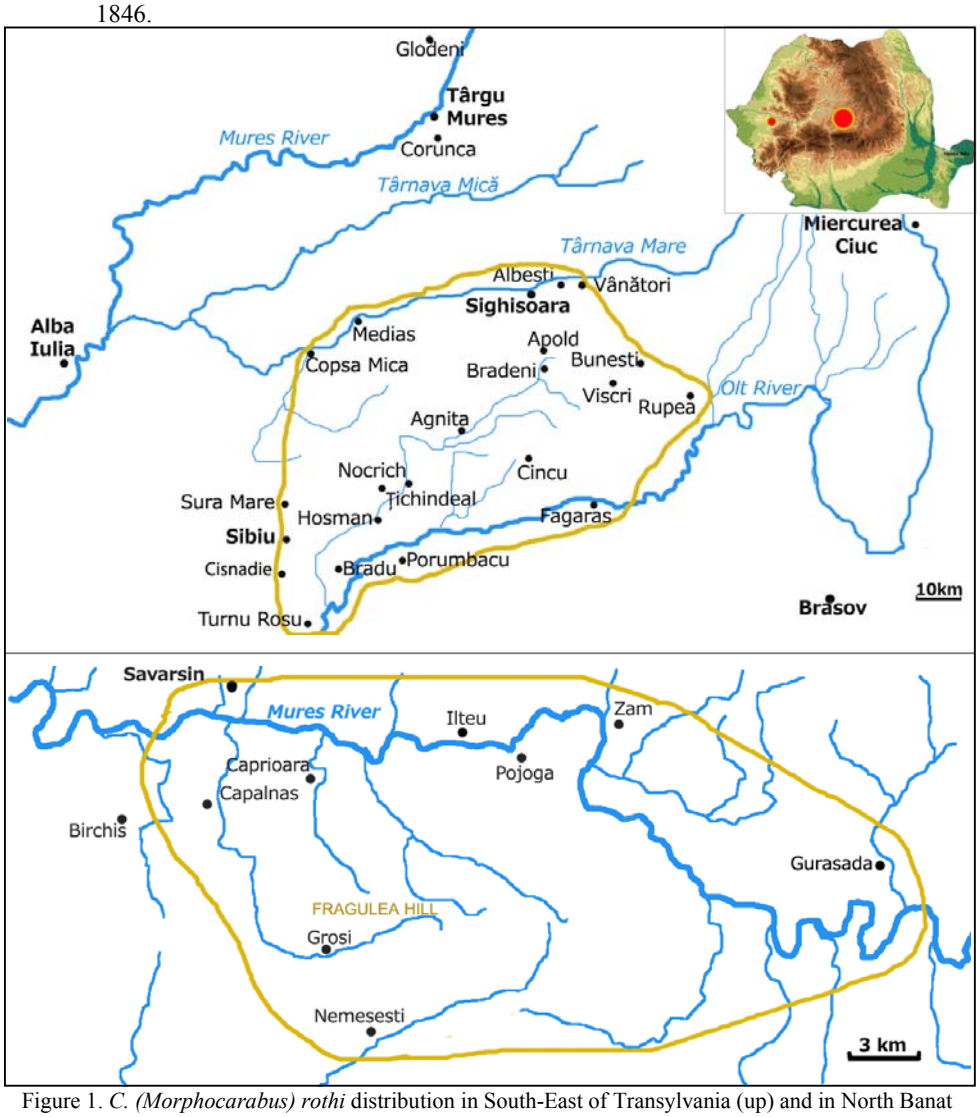


Figure 1. *C. (Morphocarabus) rothi* distribution in South-East of Transylvania (up) and in North Banat (down)

**1.2. Morphological criteria used to distinguish *C. (Morphocarabus) rothi rothi* Dej., 1829 by *C. (Morphocarabus) rothi hampei* Kust., 1846**

1.2.1. Elytra sculpture variability:

●The number of primary intervals

Conventionally the distinction concerns the number of primary intervals: 4 for *rothi* and 5 for *hampei*. The reality is more complex:

-the fifth primary interval may be undetectable among the multiple granulations of the elytra edge, frequent case also underlined by Breuning 1932, for small forms of *hampei* as *marusii* Ormay, 1896, *bokori* Csiki, 1927, *eximius* Csiki, 1906;

-the aberration *vitiosus* Csiki, 1906 of *rothi* Csiki, 1946; to the complete intervals and of identical thickness is hardly distinguishable of some *hampei* taxa with the same characteristics and the fifth primary interval not visible; fact mentioned by Lapouge 1916;

- among the females of large-size of *C. (Morphocarabus) rothi hampei telekii* Csiki 1937, 4 % have 5 detectable primary intervals (BARLOY et al., 2013), the rest of the population having 4 primary intervals.

● The sculptural aberrations presence to the *C. (Morphocarabus) rothi rothi* Dej.

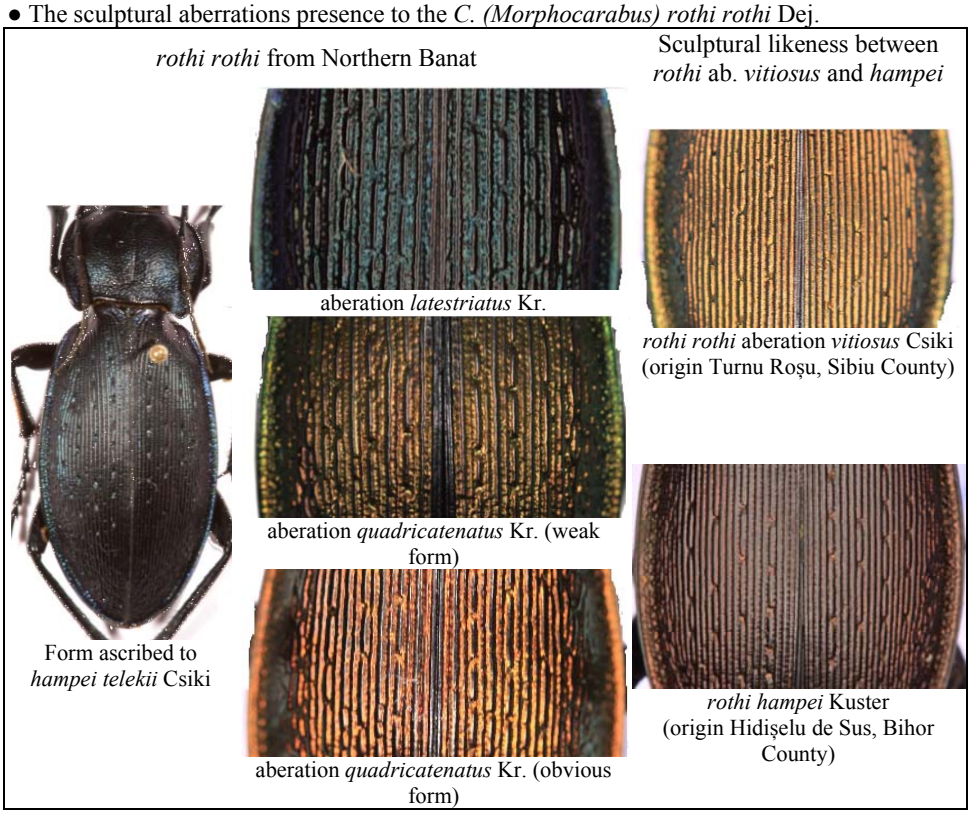


Figure 2. *C. (Morphocarabus) rothi* Dejean, 1829 elytra sculpture diversity

This species presents numerous sculptural aberrations (CSIKI, 1946, PRUNAR et al., 2015). They are found in different percentage in the two aforesaid regions.

The table 1 provides information's on the distribution of the aberrations:

- the Southern Transylvanian quadrilateral (Sibiu, Copşa Mică, Sighişoara, Rupea, Făgăraş); the aberration *vitiosus* being dominant in the south zone,
-the North Banat (left bank of the Mureş River).

*Table 1*

Percentage of main aberrations in diverse populations of *C. (Morphocarabus) rothi rothi* Dej. 1829

| Region / Aberration | Transylvanian quadrilateral | | | | Nord Banat Pojoga |
|---|---|---|---|---|---|
| | Daneş [(1)] | Hoşman [(2)] | Rodbav [(3)] | Turnu Roşu [(4)] | |
| *latestriatus* Kr. | 61 | 65 | 62 | - | 10 |
| *quadricatenatus* Kr. | 37 | 30 | 36 | - | 29 |
| *vitiosus* Csiki | 2 | 3 | 2 | 98 | 53* |
| *aequistriatus* Kr. | - | 2 | - | 2 | 8 |

* considered as *hampei telekii* Csiki 1937

[(1)] Nord, [(2)] Centre West, [(3)] Centre East, [(4)] South

The aberration *vitiosus* case:

The aberration *vitiosus* of *rothi* (4 primary intervals), with entire intervals, of identical thickness is indistinguishable of some forms of *hampei* having the same characteristics with the fifth not detectable interval; fact mentioned by Lapouge 1916.

1.2.2. The different forms of the aedeagus and endophallus:

The appeal to the shape of the aedeagus and the endophallus does not ensure an infallible detection, the characteristics being alike. (figure 3); the dimension and the curvature of the aggonoporus being inconstant pour *hampei*.

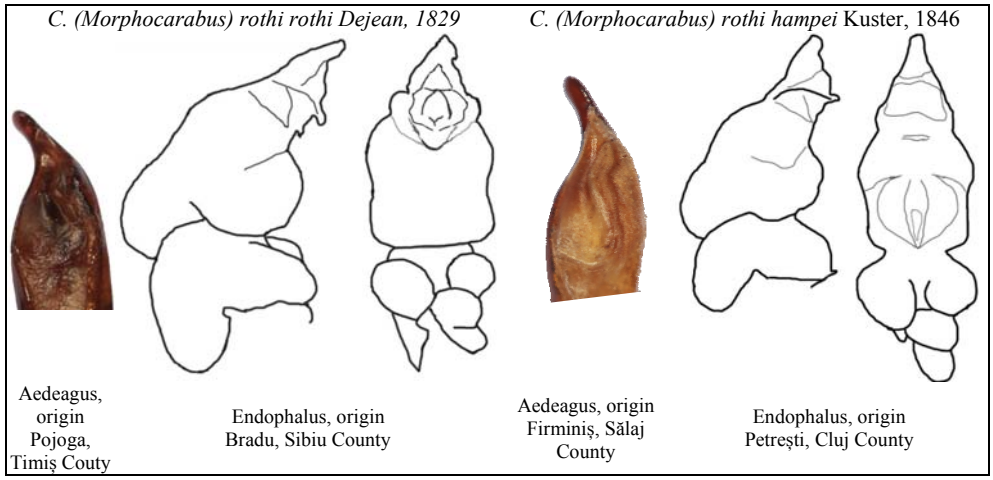


Figure 3. Resemblance of endophallus and aedeagus of *rothi* and *hampei*

1.2.3. The colours could argue the belonging of *telekii* to *rothi*. The *telekii* population is multichromatic (Barloy et al. 2008) like the individuals belonging to *rothi*. This characteristic is exceptional for the population's *hampei* and even for the *rothi* of the Transylvanian quadrilateral.

**1.3. Proposed solution**

The morphological criteria don't ensure the distinction between the two species, the study being oriented to the molecular biology with the hypothesis that *telekii* is an *vitiosus* aberration of *rothi*.

This involved to compare:

-the *telekii* form (4 and 5 primaries intervals) with others *rothi* aberrations from Northern Banat;

-the origins from North Banat with those of the Transylvanian quadrilateral for different identified aberrations;

-all the *rothi* origins for the reference *hampei* taxa

**2. MATERIAL AND METHODS**

**2.1. Studied material**

Among the very numerous collected insects (approximately 200 exemplars from the North Banat, 120 m. alt., South Transylvania) a reduced number was submitted to the molecular biology analyses, for some localities, the insects being preserved in the ethanol $95^0$ at $-20^0$ C.

● North Banat in two locations to the left bank of Mureş (Pojoga-Ilteu bridge) and one location to the right bank (Gurasada):

◦beetles appearance *telekii*: 4 primary intervals (7♂♂, 7♀♀); 5 primary intervals (6♀♀); (total 20 exemplars)

◦beetles appearance *rothi*: ab. *latestriatus* (18♂♂, 18♀♀); ab. *quadricatenatus* (18♂♂, 18♀♀); ab. *aequistriatus* (4♂♂); (total 76 exemplars)

◦ beetle from Gurasada, uncertain identification *hampei* or *rothi* ab. *vitiosus* (1♀)

●Transylvanian quadrilateral: *rothi* 4 geographical groups (table 2).

*Table 2.*

Samples of aberrations studied from Transylvanian quadrilateral

| Aberration | Nord (1) | Centre Est (2) | Centre Ouest (3) | Sud (4) |
|---|---|---|---|---|
| *latestriatus* Kr. | 6♂♂, 6♀♀ | 6♂♂, 6♀♀ | 6♂♂, 5♀♀ | 0 |
| *quadricatenatus* Kr. | 2♂♂, 2♀♀ | 2♂♂, 2♀♀ | 2♂♂, 2♀♀ | 0 |
| *vitiosus* Csiki | 1♂, 1♀ | 1♂, 1♀ | 2♂♂, 1♀ | 8♂♂, 8♀♀ |
| *aequistriatus* Kr. | 0 | 0 | 1♂ | 1♂, 1♀ |

(1) Sighisoara-Apold-Daneș-Broșteni
(2) Rodbav-Mureni-Viscri-Rupea (Homorod)
(3) Hoşman-Ţichindeal-Dealul Frumos
(4) Bradu-Turnu Roșu-Cisnădioara

●*C. (Morphocarabus) hampei*: 3 exemplars Buzeşti (Maramureş County), 3 exemplars Firminiş (Sălaj County).

**2.2. Used markers and molecular biology method:**

A preliminary study having shown that the following markers ensure no distinction: 12 S ; 16 S ; 28 $S_1$ $R_1$ ; 28 S 5F-5R ; Wg, Pepck. We retained the mitochondrial markers COI and cyt b.

-COI : LCO 1490 HCO - 2198 (Hebert *et al.*, 2003)

-cyt b: CB1, CB2 (Jermiin & Crozier, 1994)

Method of material conservation, extraction, purification and sequencing after BARLOY et al,. 2014.

Sequence analysis

- manually analysed sequences with the help of Geneious software Version 7;

- multiple alignments performed with the algorithm MAFFT (KATOH&STANDLEY, 2013);

- phylogenetic axes prepared with the algorithm PhyML (GUINDON&GASCUEL 2003), the substitution model IC69 and a bootstrap of 10 replicates.

## 3. RESULTS AND DISCUSSIONS

In a preliminary analysis (Prunar et al., 2015) it was shown that the used mitochondrial markers do not differentiate aberrations but characterize four major regions in the Transylvanian quadrilateral; identifying four haplotypes (North, Centre East, Centre West and South).

### 3.1. Presentation of the results in concatenation

The obtained results with the described material above is presented in the table 3 and the figure 4 by concatenation of COI and cyt b markers.

*Table 3*

Regional variability of *C. (Morphocarabus) rothi* in concatenation analysis (sequences 1 to 1014)

| Origins \ Position | 6 | 33 | 48 | 70 | 100 | 112 | 156 | 169 | 226 | 229 | 253 | 265 | 289 | 327 | 376 | 388 | 390 | 424 | 438 | 466 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *rothi* N [1] | T | C | G | A | A | T | T | T | T | T | T | A | C | A | C | T | A | C | G | T |
| *rothi* CW [2] | • | T | A | • | • | C | • | • | • | • | • | • | T | G | T | • | G | • | T | C |
| *rothi* CE [3] | • | • | A | G | • | C | • | C | • | • | C | G | T | G | T | C | G | • | T | • |
| *rothi* S [4] | C | • | A | • | • | C | A | • | C | • | • | • | T | G | T | • | G | T | T | • |
| Pojoga 1a [5] | • | • | A | • | • | C | • | • | • | • | • | • | T | G | T | • | G | T | T | • |
| Pojoga 1b [6] | • | • | A | • | • | C | • | • | • | • | • | • | T | G | T | • | G | T | T | • |
| Pojoga 2 [7] | • | • | A | • | G | C | • | • | • | C | • | • | T | G | T | • | G | T | G | C |
| *hampei* Bz [8] | • | • | A | • | G | C | • | • | • | C | • | • | T | G | C | • | G | T | G | C |
| *hampei* Fr [9] | • | • | A | • | • | C | • | • | • | • | • | • | T | G | T | • | G | T | T | • |

| Origins \ Position | 487 | 514 | 557 | 599 | 636 | 684 | 692 | 698 | 743 | 744 | 753 | 765 | 887 | 894 | 899 | 918 | 921 | 966 | 1011 | 1014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *rothi* N [1] | T | A | A | G | A | T | A | G | C | G | T | C | C | T | C | C | A | A | C | A |
| *rothi* CW [3] | • | • | • | • | G | • | G | A | • | • | • | • | T | C | T | • | • | • | • | • |
| *rothi* CE [2] | C | G | • | A | • | C | • | • | • | • | G | • | • | G | • | • | • | • | • | • |
| *rothi* S [4] | • | • | • | • | • | C | • | A | T | • | • | T | T | C | T | T | • | G | • | • |
| Pojoga 1a [5] | • | • | • | • | G | • | G | A | • | • | • | • | T | C | T | • | • | • | • | • |
| Pojoga 1b [6] | • | • | • | • | G | • | G | A | • | • | • | • | T | C | T | • | • | • | • | • |
| Pojoga 2 [7] | • | • | G | • | • | • | • | • | • | A | • | • | • | • | • | • | G | • | T | G |
| *hampei* Bz [8] | • | • | • | • | • | • | • | • | • | A | • | • | • | • | • | • | G | • | • | G |
| *hampei* Fr [9] | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | T | • |

• represent the base from the first line (*rothi* N)
[1]*rothi* North: Sighişoara, Apold, Daneş, Bunești
[2]*rothi* Centre West: Hosman, Cincu Sus
[3]*rothi* Centre East: Dealul Frumos, Cincu Jos
[4]*rothi* South: Turnu Roşu, Cisnădioara, Bradu
[5]Pojoga 1a: *rothi* sculpture, including Gurasada (62 exemplars)
[6]Pojoga 1b: *rothi* sculpture *telekii* (20 exemplars)
[7]Pojoga 2: *rothi* sculpture but *hampei* after molecular analysis (15 exemplars)
[8]*hampei* Bz: Buzești (Maramureş County)
[9]*hampei* Fr: Firminiş (Sălaj County)

### 3.2. Results analysis

The *rothi* group from Transylvanian quadrilateral it split in 4 haplotypes, confirming previous results (Prunar et al., 2015).

The origins of the North Banat divide into two groups:

●Pojoga 1 - gathering all the individuals morphologically *telekii* (with 4 and 5 primary intervals), Gurasada origin and 79 % of the population by *rothi* appearance, all confused aberrations.

●Pojoga 2 -including 21 % of insects morphologically *rothi* from the North Banat and belonging to the *quadricatenatus* aberration (the tertiary intervals into narrow lines or into joints granulations). This group, distinct of Pojoga 1 has a very nearby genomic structure of *hampei* from Buzești (difference 2 base pair).

The entire *rothi* group (origin Pojoga 1 and Transylvanian perimeter) differ from *hampei* group and Pojoga 2 (95.4% homology).

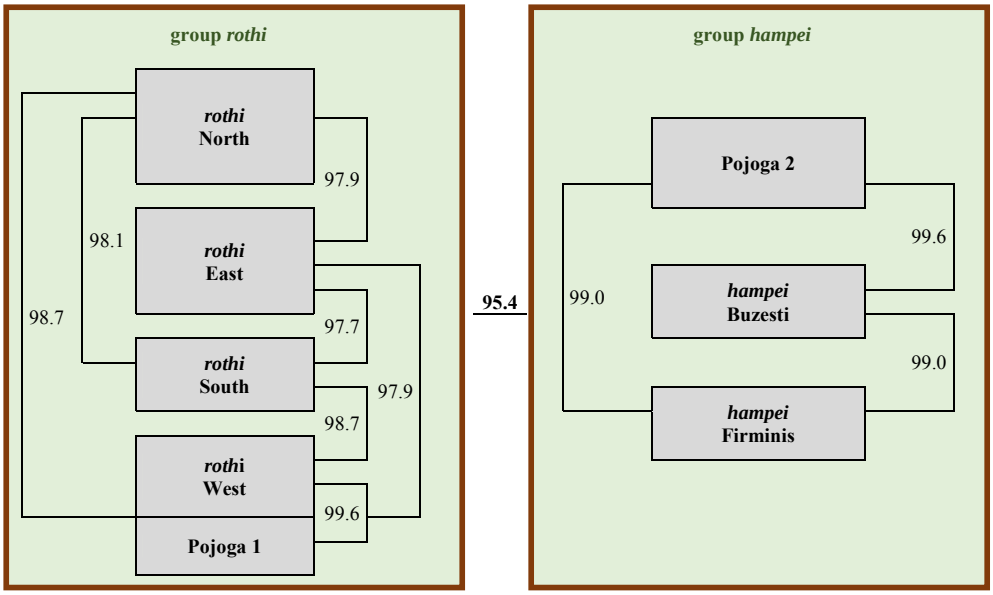


Figure 4. Homology percent in concatenation COI and cyt b

### 3.3. Discussion

3.3.1. The *telekii* form do not different from some *rothi* of the same populations in northern Banat.

This form does not linked to *C. (Morphocarabus) hampei,* Kust. and constitutes an aberration *vitiosus* of *C. (Morphocarabus) rothi,* Dej.. This result confirms the assumption of *telekii* belonging to *rothi*, formulated by Szel and al. 2007 by basing on the number of primary intervals. The mitochondrial markers COI and cytochrome b prove irrefutably this belonging.

The *telekii* form must be eliminated as taxon of *C. (Morphocarabus) hampei* Kust.

3.3.2. The group Pojoga 1a-Gursada (*rothi* Banat) has a nearby genomic structure to *rothi* Centre-West, despite the distance between the two sites (250 km in a direct line). The origin of this populating is unknown: independent genetic origin or river transport?

The last hypothesis seems unlikely since there are no connections between the rivers of the West *rothi* area and the Mureș River.

3.3.3. The group Pojoga 2 which holds *rothi* individuals belonging to the ab. *quadristriatus* dominant (tertiary intervals marked by a line of granulations or the fragments of line) has a genetic structure close to *hampei,* form (Buzești origin).

The genomic polymorphism of the populations of appearance *rothi telekii* of the North Banat and the attachment of some individuals to *hampei* had already been mentioned with the marker NADRH 5 (BARLOY et al 2013), but with a reduced number of samples studied.

This confirms the incapacity to distinguishing *rothi* of *hampei* based on the elytral sculpture and raise the problem of the links between this two species.

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