CARABUS (MORPHOCARABUS) HAMPEI TELEKII CSIKI 1937 AND *CARABUS (MORPHOCARABUS) ROTHI ROTHI* DEJEAN 1829 IN NORTHERN BANAT

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Abstract: *In the North Banat coexist in the same stations and biotopes two species of the genus Morphocarabus attributed to C. (Morphocarabus) hampei telekii Csikii 1937 and C. (Morphocarabus) rothi rothi Dejean 1829. On the left bank of the Mureș River, the restricted area of tthese species distribution is situated from Groși – Căpâlnaș in the East until Pojoga - Tisa in the West. Beyond this perimeter, the researches remained fruitless. The cohabitation of these two species seems to be a unique case, not found in Transylvania, region with habitat type of C. (Morphocarabus) rothi rothi Dejean 1829. For lack of relevant morphological characters, the distinction between both aforesaid species is difficult. The absence of the 5-th primary interval at hampei telekii and the presence of the quadricatenatus Kraatz 1878 which is a rothi form with sculpture near to hampei put in doubt the existence of two species. As telekii and rothi have elytral sculpture with four primary intervals, SZEL G. (2007) has attached hampei telekii to rothi. For the mixed population from Pojoga, the morphological and morphometric criteria are insufficient to distinguish telekii by rothi. The examination of an important effective of hampei telekii type individuals, point out that 4% of insects have an elytral sculpture with 5 primary intervals. The mitochondrial DNA comparison, NADPH gene, subunit 5, provides a clear distinction between the hampei and rothi types. The population of rothi from Pojoga also has a polymorphism which brings closer some individuals to those from Transylvania (origin Danes near Sighișoara). This study confirms the suspected presence of two species (C. (Morphocarabus) hampei telekii Csikii 1937 and C. (Morphocarabus) rothi rothi Dejean 1829) in the prospected area. Two problems remain posed = that of the relationships with rothi populations from Transylvania and that of the possible crossbreeding between hampei telekii and rothi, difficult to identify using the mtDNA at maternal heredity.*

Key words: *carabus, hampei telekii, rothi, taxonomy, morphology, inventory, mapping.*

INTRODUCTION

Several studies mention the presence in mixture, in the same stations and the biotopes (forest edges, light wood of deciduous bushes meadows) of the *C. (Morhocarabus) hampei telekii* Csiki 1937 and *C. (Morphocarabus) rothi rothi* Dejean 1829, in diverse localities of the North Banat. These are situated in a hilly area zone limited enough on the left bank of the Mureș River:

- Ciuta Hill near Groși 45 55N, 22 17 E 400 m, xerophytic forest of broadleaves (LIE 1988)
- Nemesesti 4 km SSE Groși 45 53 N 22 19 E 250 m, and Icuiul Costeiului Valley, mesohydrophytic forest of broadleaves (LIE 1991).
- Căprioara 6 km E Căpâlnaș 45 59 N 22 16 E, 210 m., xerophytic forest of broadleaves (BARLOY *et al.* 2008)

- Pojoga from the bridge Ilteu on river Mureș-Pojoga-Tisa 10 km W Căprioara 46 58 N 22 24 E, 220-250 m. Forest of broadleaves on calcareous and karstic mesohydrophytic formations (LIE 1991, BARLOY *et al.*2008)
- Bushes on meadow from the Ilteu railway station (LIE 1991)

The hill Brâznic 200 m, near Săcămaș, 45 55 N 22 41 E detains only *hampei telekii* (LIE 1997).

Researches pursuit on the left bank of the Mureș River, beyond Groși-Căpâlnaș in West and of Pojoga-Tisa in East, did not reveale new stations for both species aforesaid. Did not reveal new stations for both species aforesaid on the right bank LIE (1990) quote Pârnești situated at 5 km N Săvârșin, 46 04 N 22 13 E, 305 m.

The cohabitation of both species of *Morphocarabus* seems a unique case, not found in Transylvania, the region typifies of the *C. (Morphocarabus) rothi rothi* habitat.

C. (Morphocarabus) hampei telekii is the only *hampei* known on the left bank of the Mureș River, from Arad to Teiuș.

The species

C. (Morphocarabus) hampei telekii was described by Csiki in 1937, after individuals from NE of "Komitate Karasso-Szoreny", which at the time of the Austro-Hungarian Empire encompassed the localities quoted above. The fact that Csiki had dedicated this form to the Count Eugen von Teleki, in homage to this famous entomologist, can also to be interpreted as the supply by the latter of the described insects. The Count had a castle at Căpâlnaș, location very close to the area where figure the population of this *hampei*. (CSIKI quote as place type - thereabouts the cave Podsag the karst area).

C. (Morphocarabus) rothi rothi was described by Dejean after an individual originated from Transylvania. After BIRTHLER (1885), this exemplar would have been transmitted in 1824 by Kollars, having received it from his discoverer J.J. Roth, a priest from Sibiu. The species is found in the quadrangle Sibiu-Copșa Mica-Bunești-Fagaras (BARLOY et al 2012).

The problems

1. **Effective presence of two species**
   a. *C. (Morphocarabus) hampei telekii* is known to present a elytral sculpture characterized by 4 primary detectable intervals as *C. (Morphocarabus) rothi*. For this reason, SZEL *et al.* (2007) considered *telekii* as subsp. of *rothi*. This would put in doubt the cohabitation of two different species and the presence of a polymorphic population of *rothi*.
   b. *C. (Morphocarabus) rothi* presents a great sculpture variability, some aberrations as *vitiosus* Csiki or *ignoscus* Csiki getting closer of *hampei*. LAPOUGE (1916) already mentioned the difficulty in differentiating *rothi* of *teleki*.
2. **Links with the *rothi* populations of Transylvania.**
   The population of *rothi* from the zone Groși-Pojoga is very distant from the Transylvania, considered cradle of the species (approximately 300 km by Mediaș, the closest known station) what phylogenetic relations between populations?

RESULTS

The study attempts to answer two questions:

- Is it possible to differentiate C. *(Morphocarabus) hampei telekii* from *C. (Morphocarabus) rothi* by:
  - morphometric characters?
  - molecular biology criteria (using mtDNA)?
- What are phylogenetic relationships between the populations of the two distribution areas?

1. Comparative morphological study

a. Elytral sculpture

The determination carries on a certain individuals from Căprioara-Pojoga.

- ***C. (Morphocarabus) hampei telekii* Csiki 1937**

4% of insects present five well detectable primary intervals, spotted by the characteristic protuberances situated downstream to the dimple (figure 1)

All others have four primary intervals, with streaks of same importance, variously interrupted. For the upper half of the elytron can be identified two types of sculptures:

-Type I = only primary interrupted (figure 2)

-Type II= all ridges interrupted (figure 3)

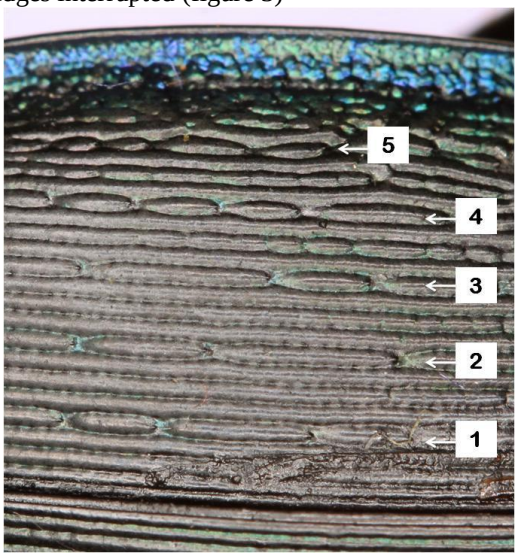

Figure 1. *C. (Morphocarabus) rothi hampei telekii* Pojoga with five primary intervals

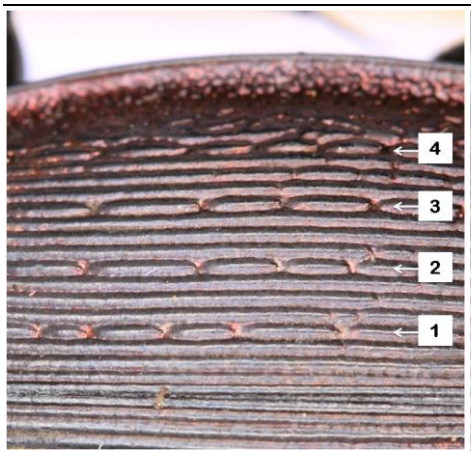

Figure 2. *C. (Morphocarabus) rothi hampei telekii* Pojoga, with four primary intervals Type I

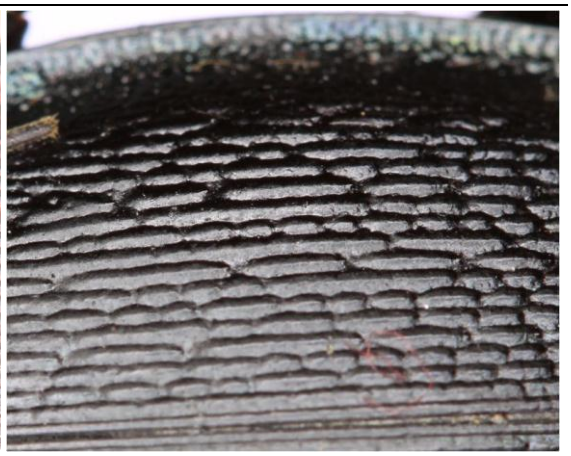

Figure 3. *C. (Morphocarabus) rothi hampei telekii* Pojoga, with four primary intervals Type II

It must be reminded that LAPOUGE (1916) reported the variability of the primary intervals number for *hampei* (the Maros - current Harghita- where are found together *hampei* with four and five primary ridges).

- ***Carabus (Morphocarabus) rothi rothi* Dejean 1829**

The population is divided, appreciably equally between two aberrations:

- *ab. latestriatus* Kraatz 1878 for *rothi* described by Dejean with the tertiary sketched by the small granules. (figure 4)
- ab. *quadricatenatus* Kraatz 1878 with tertiary more weaker (figure 5)

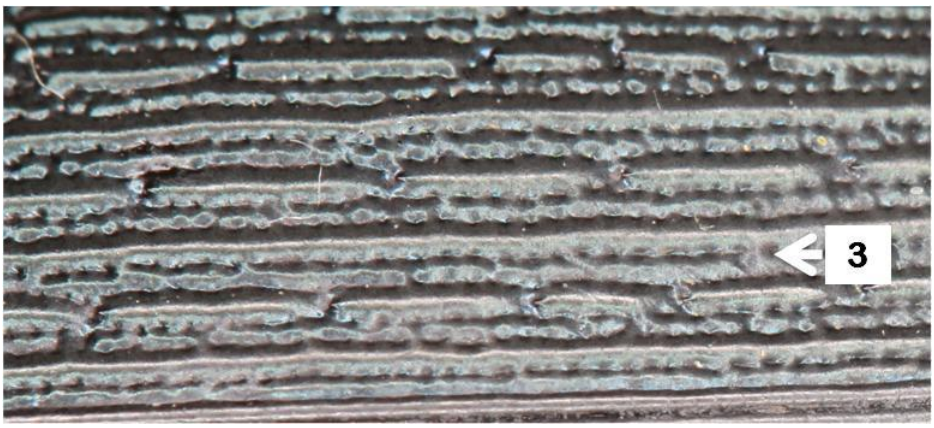

Figure 4. *C. (Morphocarabus) rothi rothi* Pojoga, *ab. latestriatus* Kraatz, tertiary granular intervals

Figure 5. *C. (Morphocarabus) rothi rothi* Pojoga, *ab. quadricatentus* Kraatz, tertiary thinner continuous intervals

b. Morphometric measures

Those relate to 30 male and female individuals, taken at random among a hundred insects.

Measurements performed (table 1): L = total length imago, l = maximum width, Le=elytral length measured at the suture; e = maximum width of pronotum, h = pronotum height at the level of the median line.

Table 1.

Morphometric measures

	L	**l**	**L/l**	**Le**	**Le/L**	**e**	**h**	**e/h**
♀*hampei*	32,11	12,00	2,67	21,64	67,39	9,35	6,27	1,49
♀*rothi*	32,34	11,96	2,70	20,84	64,46	9,28	6,35	1,46
♂*hampei*	30,63	11,57	2,65	19,46	63,53	8,94	6,18	1,45
♂*rothi*	30,44	11,39	2,67	19,53	64,16	8,84	6,22	1,42

Statistical analysis (Test t Student) indicates no significant difference between *hampei* and *rothi* from Pojoga.

c. Other morphometric criteria

The comparative examination of the aedeagus (general shape, size and apex curvature) and the endophalus, does not appear discriminatory to separate *hampei* and *rothi* from Pojoga. (figure 6)

Figure 6. Aedeagus of *rothi* et *hampei* from Pojoga

2. Molecular biology study

2.1 Material and method

a. Material

Insects captured in activity are preserved in ethanol 90^0 at -20^0 C and originate for:

-*C. (Morphocarabus) rothi hampei telekii* and *C. (Morphocarabus) rothi rothi* from Pojoga zone,

-*C. (Morphocarabus) rothi hampei hunyadensis* forestry edges near Săcărâmb 45 58 N, 23 02 E,

-C. *(Morphocarabus) rothi rothi* wooded hills near Danes (345 m) 46 13 N 24 42 E in Transylvania considered the cradle of the species (analysis 1 ♂).

The analyses of molecular biology carry for both species present at Pojoga for:

-*C. (Morphocarabus) rothi hampei telekii*= n 65 ♀ sculpture 1; n^0 66 ♂ sculpture 2;

-*C. (Morphocarabus) rothi rothi* n^0 72 ♀ sculpture 1; n^0 73 sculpture 2; n^0 75♂ sculpture 2; n^0 76 ♂ sculpture 1.

b. Method

Total DNA is extracted from the thoracic muscle by the method CTAB (DOYE J.J. and DOYLE J. 1987). PCR adapted by Zhi-Hui Su in Genes Genet Sys (2003).

Protocol of the sequences "classic": DNA 100ng (1 à 2 UI); Primer 3Umol (1UI); BD V3.1 Life technology 2 UI; H_2O qsp 10 UI.

Cycles of sequencing for Primer V04: 3 min à 95^0C (1 fois); 30 sec à 95^0C +; 45 sec à 95^0C+; 1 min at 60^0C this for 30 cycles; then 15 ^{0} C for ever.

Cycles of sequencing for Primer V06: 3 min à 95^0C (once); 30 sec à 95^0C +; 45 sec à 58^0C+; 1 min à 60^0C this for 30 cycles; then 15 ^{0} for ever.

Used sequencer is 3130XL Genetic Analyser (16 caps) Applied Biosystems. The polymer used is the Pop 7 with capillaries of 50 cm (Life Technology). The sequence parameters are all identical with the basic parameters. The primary analyses of the raw data (output of the sequencer) are made with Analysis Sequencing software. The V4 and V6 data

are connected by alignment via Smith-Waterman algorithm using for consensus over the quality criterion.

2.2 Results

a. Global interpretation of the molecular analysis data

The analyzed sequences concern two regions of the gene NADPH, subunit 5 region of the nucleotides 128 at 350 and the region V6 of the nucleotides 701 at 924. The overall analysis of the two regions are concatenated and aligned with the algorithm Clustal W (matrice Blosumb 62, GOP 12 and GEP 3). The phylogenetic tree was generated from this alignment by PHYML method (Jukes and Cantor model substitution 69 Bootstrap 10 optimize rate).

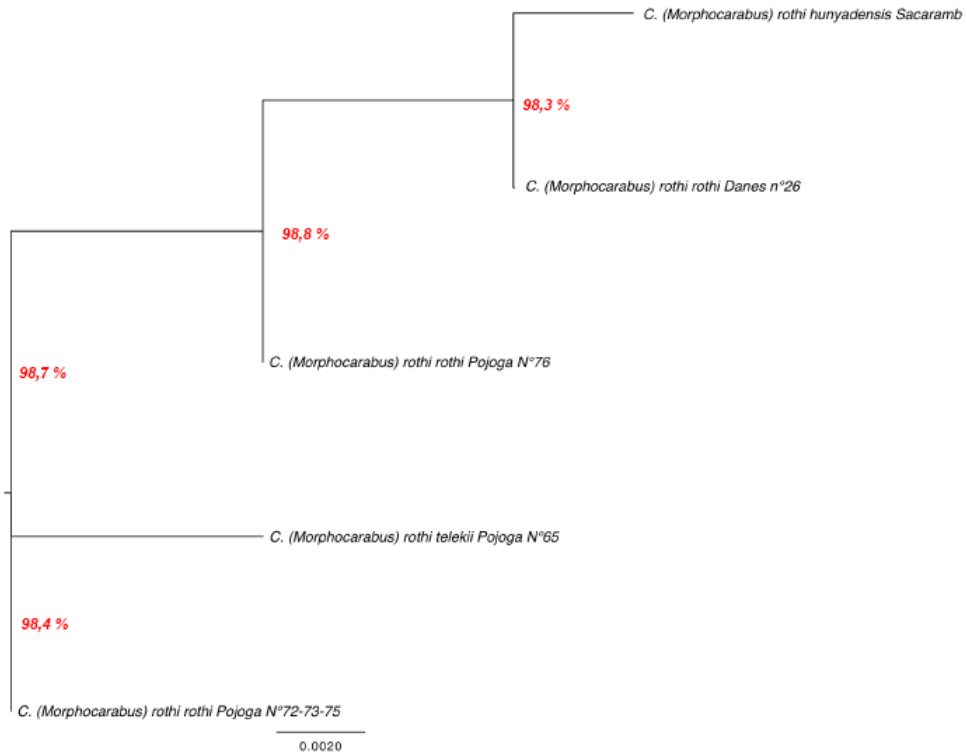

Figure 6. Dendrograme of *rothi* mitochondrial gene NADH sequence

This tree shows:

1. Three distinct populations for Pojoga station
   - *C. (Morphocarabus) rothi hampei telekii* (number 65-66)
   - *C. (Morphocarabus) rothi rothi* (number 72-73-75)
   - *C. (Morphocarabus) rothi rothi* (number 76)
2. The number 76 gets closer to the group *C. (Morphocarabus) rothi rothi* by Daneș, himself close enough to *C (Morphocarabus) rothi hampei hunyadensis.* This closeness must be nuanced for a more important group of *C. (Morphocarabus) rothi hampei* taxa, because of the variability specific intra (forthcoming).

b. Analysis of *rothi* group from Pojoga-Daneș

Sequence alignment of the mitochondrial gene NADH subunit 5 (ND5) shows for all origins (Pojoga-Danes) 10 polymorphic bases (98.4 for global identity)

For the origin Pojoga the 72-73-75 numbers are mutually identical, different from the number 76 by 8 polymorphic bases and of the origin Danes by 8 base pairs. Danes looks like the number 76 but has 5 pairs of different bases (see table 2).

Table 2.

Table of characteristics nucleotides

	44	**77**	**166**	**317**	**329**	**386**	**388**	**508**	**583**	**613**
Pojoga 72, 73, 75	T	T	T	G	A	T	T	A	A	T
Pojoga 76	C	T	C	T	G	A	C	T	A	C
Danes	C	A	C	G	G	A	T	T	G	C

The population *rothi* of Pojoga seems polymorphic; a portion (72, 73 and 75) differed from that of Danes, the number 76 being closer to.

This requires to be verified on a larger sampling.

CONCLUSIONS

Despite certain proximity (94.4% of identity), the molecular analysis confirms the presence of two species: *C. (Morphocarabus) rothi rothi* and *C. (Morphocarabus) rothi hampei telekii* to Pojoga. This was already suspected by the elytral sculpture examination (despite the low frequency 4 %) of a fifth primary. The case of *C. (Morphocarabus) rothi hampei* with 4 intervals outside of the zones where was reported *C. (Morphocarabus) rothi rothi* is indicated since a long time.

The polymorphism of the population *rothi* of Pojoga must be verified; a part being different from that of Danes, region typifies of *C. (Morphocarabus) rothi rothi* distribution.

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