MOLECULAR PRELIMINARY STUDY ON THE *C. (MESOCARABUS) PROBLEMATICUS* HERBST 1786 IN AIM TO ESTABLISH THE POSITION OF ROMANIAN SSP. *HOLDHAUSI* BORN 1911 COMPARED TO THE OCCIDENTAL SUBSPECIES

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Abstract. *C. (Mesocarabus) problematicus Herbst 1786 is a fairly widespread European endemic species, especially on the Atlantic coast from the northern of Spain until Scandinavia. A recent map, based on the most recent data establishes the geographical distribution. From the eight European subspecies, the most isolated are the island subspecies, those from Faroe Islands (ssp. feroensis Lapouge, 1910) and Iceland (islandicus Lindroth, 1968) but also one continental subspecies from Romania (holdhausi Born 1911). Well represented in Western Europe, particularly in France (3 subsp.), the C. (Mesocarabus) problematicus Herbst 1786 species becomes very localized by going towards the East where is represented only by some relicts stations in Hungary and in Romania. In the last one the represented taxon holdhausi Born 1911, is quoted from eight localities among which two recent confirmed, all situated at high altitude (on 1500-1700 m). This fragmentation of the distribution and the localization in the mountainous zone pose two major questions: causes and anteriority of this residual localization in the refuges zones; genetic origin of this taxon and its link with the other forms of the species. The study wants to establish the position of the endemic taxon holdhausi (collected in the Mountains Hăşmaş, Bucegi) among 71 populations of C. (Mesocarabus) problematicus from Western provenances, mainly French, basing on molecular biology data (mitochondrial marker primarily COI I, cyt b; secondarily ND_1, ND_4, ND_5). The results with cyt b, the most selective marker, indicate: a big difference of maternal origin in Spain for the region pre-Pyrenees where appears ssp. planiusculus Haury in Gehin, 1885 and in France for the mountainous regions of the Massif Central (Sidobre, Montagne Noire), the Alps of Queyras of old forest from Centre-West, Italy in the Dolomites. The Romanians provenances from Bucegi and Hasmas are genetivaly close and neighbours of the majority of Western origins. These preliminary results incite to intensify the prospecting in Romania and nearby countries (HR, CZ, SK).*

Key-words: *C. (Mesocarabus) problematicus, geographical distribution, molecular biology, biodiversity*

INTRODUCTION

C. (Mesocarabus) problematicus, described by Herbst in 1786, from Austria provenance, is a pretty diversified European endemic species. Among the numerous subspecies described, 8 - 9 are currently used (LÖBL *et al*. 2003; TURIN *et al*. 2003). Four of them have a notable distribution: *problematicus* type Herbst 1786, subsp. *planiusculus* Gehin 1885; subsp. *inflatus* Kraatz 1878; subsp. *harcyniae* Sturm 1815. The species has generally the color blue, purple-blackish, rarely green (f. i *trapeti* Bleuse, 1885) of the French and Spanish Pyrenees. (figure 2)

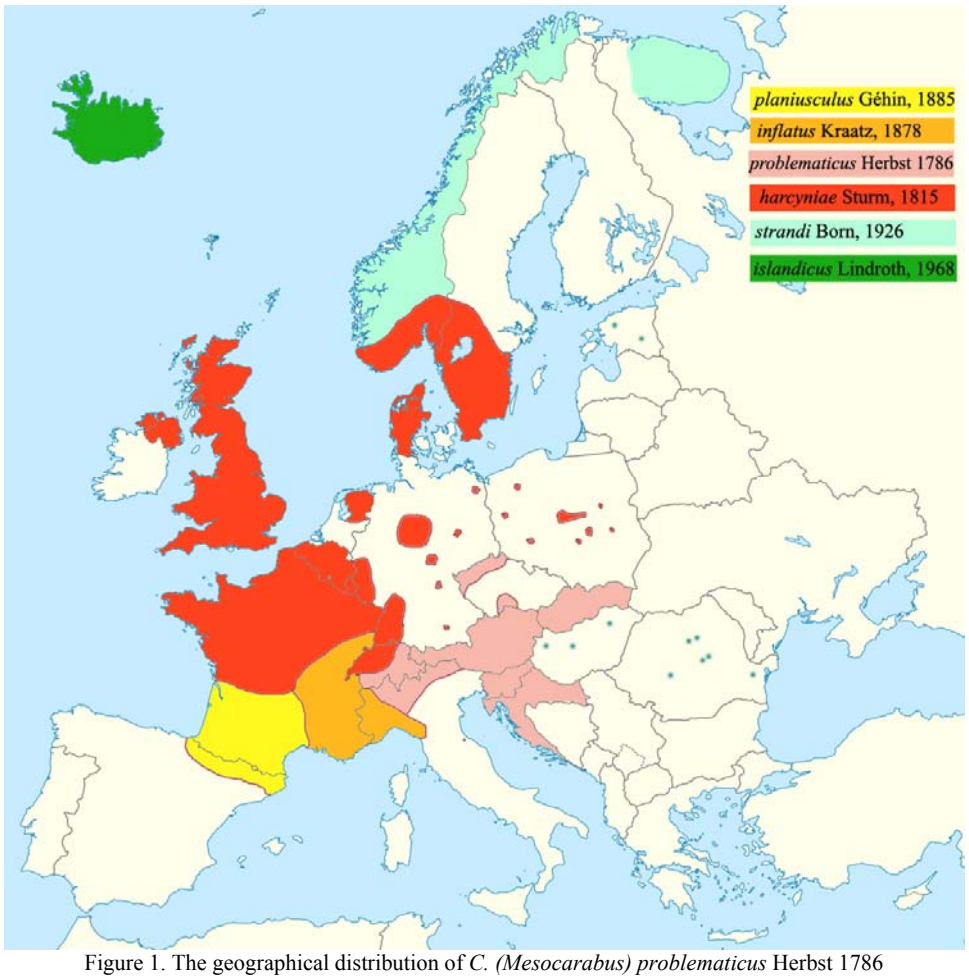

Figure 1. The geographical distribution of *C. (Mesocarabus) problematicus* Herbst 1786

1. OVERVIEW OF THE GEOGRAPHICAL DISTRIBUTION

The figure 1 establishes the geographical dispersal of these main *subsp.* based on the latest available data (see bibliography geographical distribution). Its review shows (Annexe 1):

-a wide presence on the Atlantic facade of the North of Spain, in the Scandinavian countries and in the islands of the Atlantic Ocean. Some countries like France and Britain have the highest density of implantation and in other (Spain, Italy, Germany, Denmark, Scandinavian countries) the species is localized.

-a more variable distribution in the East of Europe: frequent in Austria, Slovenia, Slovakia, localized in Germany, Czech Republic, Poland and some relict stations in Hungary and Romania (details below).

2. SITUATION IN FRANCE, HUNGARY, ROMANIA

2.1. France

France is the only country where are present three subspecies (FOREL *et al*. 1995), fig. 1:

-subsp. *harcyniae* Sturm 1815 account 3 nations. Occupies the northern half of France with individuals measuring: ♂ 20-24 mm., ♀ 22-25 mm.

-subsp. *planiusculus* Géhin, 1885 with 9 nations including 2 very localized in the eastern Pyrenees. In the Pyrenees, this *subsp.* is found in high altitudes alpine meadows: 2300 m. (Col du Tourmalet, Port de Gavarnie); 2500-2600 m. (Pic du Midi de Bigorre, Pic du Midi d'Ossau…) with a more reduced size: ♂♂ : 20-21 mm., ♀♀ 21-23 mm., certain individuals having the green margins (fig. 2.)

-subsp. *inflatus* Kraatz, 1878, with five nations, present on the left bank of the Rhône; extending in Italy and characterized by the wider elytra to females (size up to 27-28 mm.). Some nations are in alpine areas: for France up to 1800-2000 m. (Massif Pelvoux, Massif Authion, Tenda); Italy for up to 2200 m (Passo di Pennes) and even 2436 m. Col Sapin (Val d`Aoste, CASALE *et al*. 2007). At these altitudes, the size is smaller: ♂♂ 22-24 mm. ; ♀♀ 23-25 mm.

2.2. Hungary and Romania

2.2.1. Taxon *holdhausi* Born 1911

In both countries appears the *holdhausi* taxon, cited by Born 1911. In 1895, Born described *angustior* (attached to *C. catenulatus*[1]) the altitude form of Monte Generoso (1700 m. Lepontine Alps). This description is renewed in 1911 for the exemplars provided by Holdhaus and Deubel and named *holdhausi* (Ceahlău, Hăşmaş) with the description: small race of mountain with particularly narrow shoulders to the males, elytra slightly angular, primary tubers very short and very big (Figure 2).

This double naming seems useless for the insects to the same morphological characteristics; *angustior* Born 1895 having normally priority.

2.2.2. Hungary

According RETEZAR (1997), the species present 2 *subsp.* very localized:

- form type *problematicus* Herbst, known to 4 zones: Massif of Sopron 600 m.; Massif of Kőszeg 882m.; Massif of Keszhely 440m., at South-West of Lac Balaton; Massif Bakony (709 m.) in northern edge of the Lake Balaton. Size 23-27 mm. GABOR *et al*. 2002 added Trottko and Velem (near Bozsok), at north east of Szombathely, near the Austrian border.

-form *holdhausi* Born, Massif of Bükk (959 m) ; North-East of Hongrie. GABOR *et al*. 2002 specify Felsőtárkány and Szentléleki (Bükki National Park).

2.2.3. Romania

The form *holdhausi*, only represented, seems very localized; most of the stations being identified for a long time (HOLDHAUS *et al*. 1910); before the description of BORN (1911). Ten locations are mentioned in the literature, often with old references; some of them having recently been verified.

- Hăşmaş Mountains. Station cited by HOLDHAUS *et al*. 1910; CSIKI 1946 and confirmed by MATHÉ 2007, BARLOY *et al.* 2012: Piatra Singuratica, Hăşmaşu Mare, in the alpine uppermost zone (1600-1700 m.), in the junipers and under the stones to the Plateau Poiana Albă. Size : ♂♂ 21.0 x 10.5 mm, ♀♀ 21.5 x 10.5 mm.
- Ceahlău Mountains. Locality cited by the same authors as previously and confirmed by VARVARA *et al*. 2006: Plateau Dochia, above the Dochia hut 1750m., alpine meadow

[1] In this period, *problematicus* was attached to *catenulatus* Duftschmid 1812, nec. Scopoli 1763. Holdhaus et al. 1910 use this appellation.

and junipers; also subalpine to the site Potosi (Biological stations) in the coniferous forest.

- Bucegi Mountains. Cited by PANIN 1955, confirmed by LIE 1996, BARLOY *et al*. 2012 at the station over the tourist hotel Cota 1500 in the sparse forest (*Picea excelsa*) and above, in the *Ericacee-Vaccinium* moor until 1800 m. Size ♂ 21-22 mm, ♀ 23-24 mm. Cited by LIE 1996, from Vârful cu Dor (2006 m.) and Piatra Arsă (2007 m.).
- Ciuc Mountains. Exemplars of Gombas Top in the collection of the Hungarian Museum of Natural History, Budapest.
- Retezat Mountains. Not cited by HOLDHAUS *et al*. 1910 ; mentioned by PANIN 1955 towards 1700 m. in the *Pinus mungo* ; 1 exemplar (Csiki) at Hungarian Museum of Natural History, Budapest. Not found by LIE 1997.
- Făgăraș Mountains. Cited at Negoi by PANIN 1955.
- Piatra Craiului Mountains. Cited by HOLDHAUS *et al*. 1910 above the Plaiul Foii hut and also meet in the alpine zone cited by CSIKI 1946 without any recent confirmation.
- Greci Mountains. The only station of low altitude: Greci (Tutuianu Top, 467 m.) under the stones, SKOLKA *et al*. 2005 (a few exemplars).
- Roşu Lake. Forest station (resinous trees) cited by TAKACS *et al*. 2003; *angustior* Born 1885 form, that it must be considered as synonym of de *holdhausi*[2], size 24-26 mm. Captures in July on approximately 1100 m.
- Massif Postăvaru mentions by CSIKI (1946) without more details

It is possible that the species is more answered than suggest the few localities quoted above in particular in forest zone, having doubtless been little sought for because the activity period is late (in July).

This fragmentation of the distribution and its main location in rather mountainous zone (on 1100-1700 m.) pose two major questions:

-causes and anteriority of this residual localization in the refuges zones;

-genetic origin of these relicts populations with the other forms of the species.

The answer to the second question cannot be based on the diversity of elytral sculpture, exploited to differentiate the *subsp.* since this criterion presents a wide inter and intraspecific variability (STELMANT *et al*. 1992).

The study is oriented to the molecular biology, aimed to place the taxon *holdhausi* Born among diverse western most provenance of *C. (Mesocarabus) problematicus*, by using cytoplasmic marker to finding the maternal origin.

[2] This double naming seems unnecessary for insects having the same morphological characteristics, *angustior* having normally the priority (described 1895). We preserved however *holdhausi*, enshrined by use.

***C.* (*Mesocarabus*) *problematicus holdhausi* Born 1911**

21,5 X 10 mm

Origin: Hăşmaşul Mare
1763 m

21,5 X 10,5 mm

C. (Mesocarabus) problematicus

inflatus n. iserensis
Breunnig 1932

28,5 X 11,4 mm
Forest of Saou FR 26
416 m

planiusculus n. mulsanti
Gehin 1885
f.i. *trapeti*
Bleuse 1885 (green)

22,8 X 9,3 mm
Port de Boucharo FR 65
2270 m

Figure 2. Morphological characteristics

3. MOLECULAR BIOLOGY STUDY

3.1. Biological material

C. (Mesocarabus) problematicus was less studied in molecular biology (Data bank):

- SU *et al*. 1996-2001 : provenances 1FR, 1DE (ND5, 28Sr RNA)
- GAMBLOMME *et al*. 2003 : provenances BE (microsatelites)
- ANDUJAR *et al*. 2012 : provenances 6ES, 3 FR, 2 GB, 1BE (COI I, cyt b)
- DEUVE *et al*. 2012 : provenances 2 FR (COI I, cyt b)
- CRANAUD *et al*. 2014 : provenances 1 FR (COI I, cyt b)
- HEINDRICH *et al*. 2015 : provenances 6 DE, 1 BE, 1FR (COI I)

These data were exploited with 45 French origins, 2 Romanian (Bucegi, Hăşmaş Mountains) and Italian, Czech and Slovak provenances (Annex 2). The geographical distribution of the studied populations can be found in Annex 3.

The studied material is either from collections (dry samples) or from the exemplars preserved in ethanol 96^0 to -20^0 C.

3.2. Method

The extraction of the DNA carries on the backlog legs, the modalities of extraction, purification of PCR, sequencing for COI I and cyt b have been described before for COI I et cyt b in BARLOY *et al.* 2014.

Selected markers:

- COI I : LCO 1490 ; HCO 2198 Hébert *et al.* 2003
- Cyt b : CB1 ; CB2 Jermin and Crozier 1994
- ND_1 : F- TTGTGTTTTAGTAGGGGTTG ; R- CCTAATCTAGTACAAGCTAAAAA
- ND_4 : F- GGGTTATGGATTATTACGA ; R- ATCCTACATATTTGACACCA
- ND_5 : F- TAGTAACTGCTGGGGTTTAT ; R- CCAAAATATTCATTTCAACC
- PCR and sequencing primers for ND_1 ; ND_4 ; ND_5 :

  95^0 5 min.,
  95^0 1 min. / 51^0 1 min. /72^0 1 min. …40 cycles
  72^0 10 min
  Sequencing
  95^0 5 min.,
  95^0 1 min. / 51^0 1 min. /62^0 1 min. …32 cycles

3.3 Results

A. Marker COI I (Figure 3). Study concerning 46 provenances.

This marker turns out less selective to distinguish between the diverse populations but has isolated 3 groups.

Group A which comprises the majority of the populations studied with a intrapopulation variability of the order by 1%. It can distinguish 5 moderately different subgroups:

a. The East and the extreme oriental, including N/NE of France the Palatinat and the Westphalie (Germany); the Metalliferous Mountains in Czech Republic; the SW of Slovakia and two Romanian stations (Bucegi-Hasmas). These populations are very nearby between them (variability of the order by 1%).

b. The form *iserensis* Breuning 1932, The Vercors Massif, Alpes-de-Haute-Provence (Montagne de Lure); the attachment Bohemia remains to be verified.

c. Vosges Mountains

d. Large group including provenances United Kingdom, Belgium and many French stations geographically scattered

e. natio. *inflatus* Kraatz 1878

Group B-Spanish Pyrenees, clearly different from other provenances (variability 4 % with regard to the group A) but heterogeneous. The provenances, *xaxarsi* Born, 1917 and *andorranus* Lapouge, 1902 are practically identical between them (98.8 %) but distinguish themselves from the natio *navarrensis* Breuning 1932 which is more western.

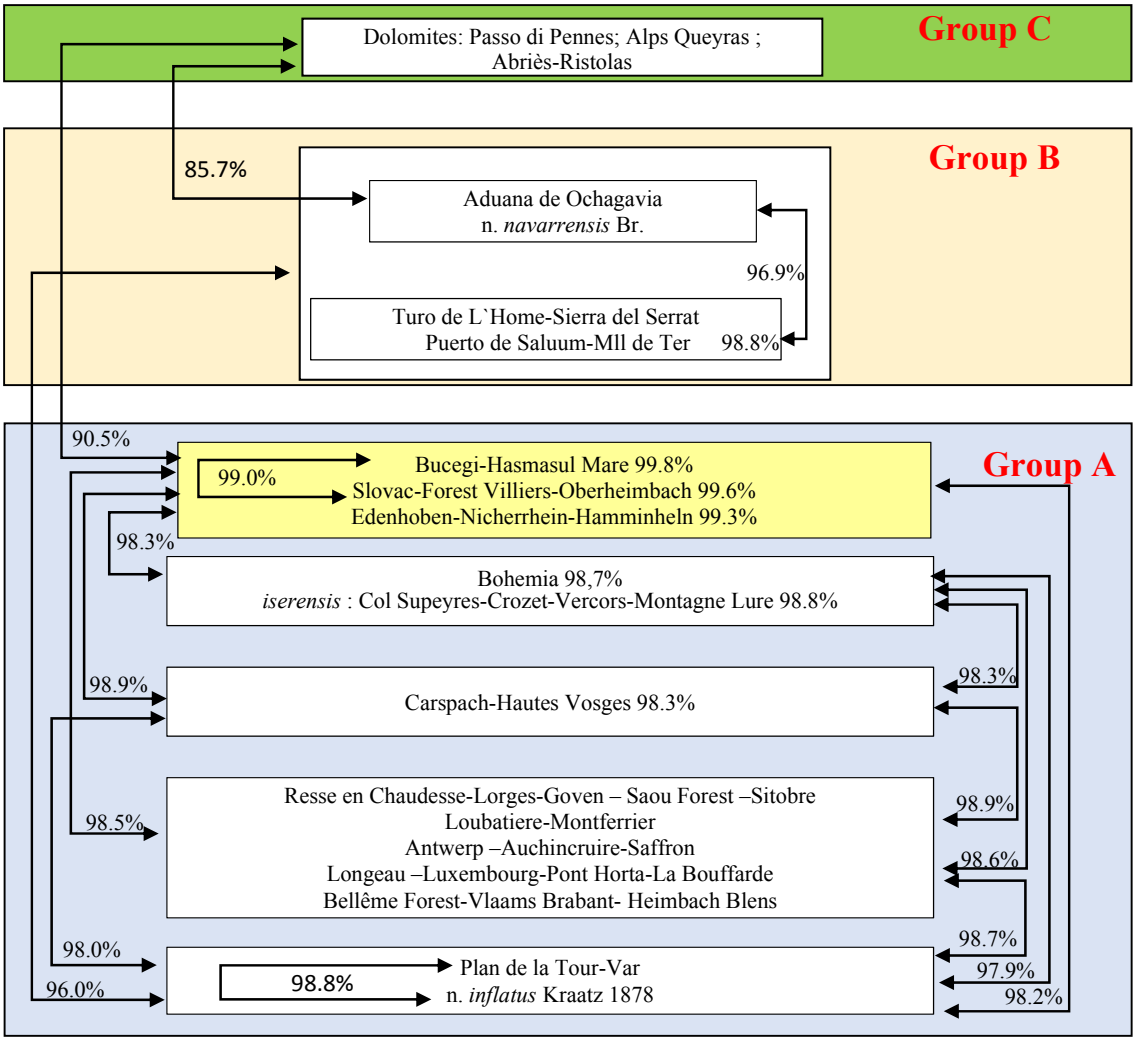

Figure 3. Analyse with COI I

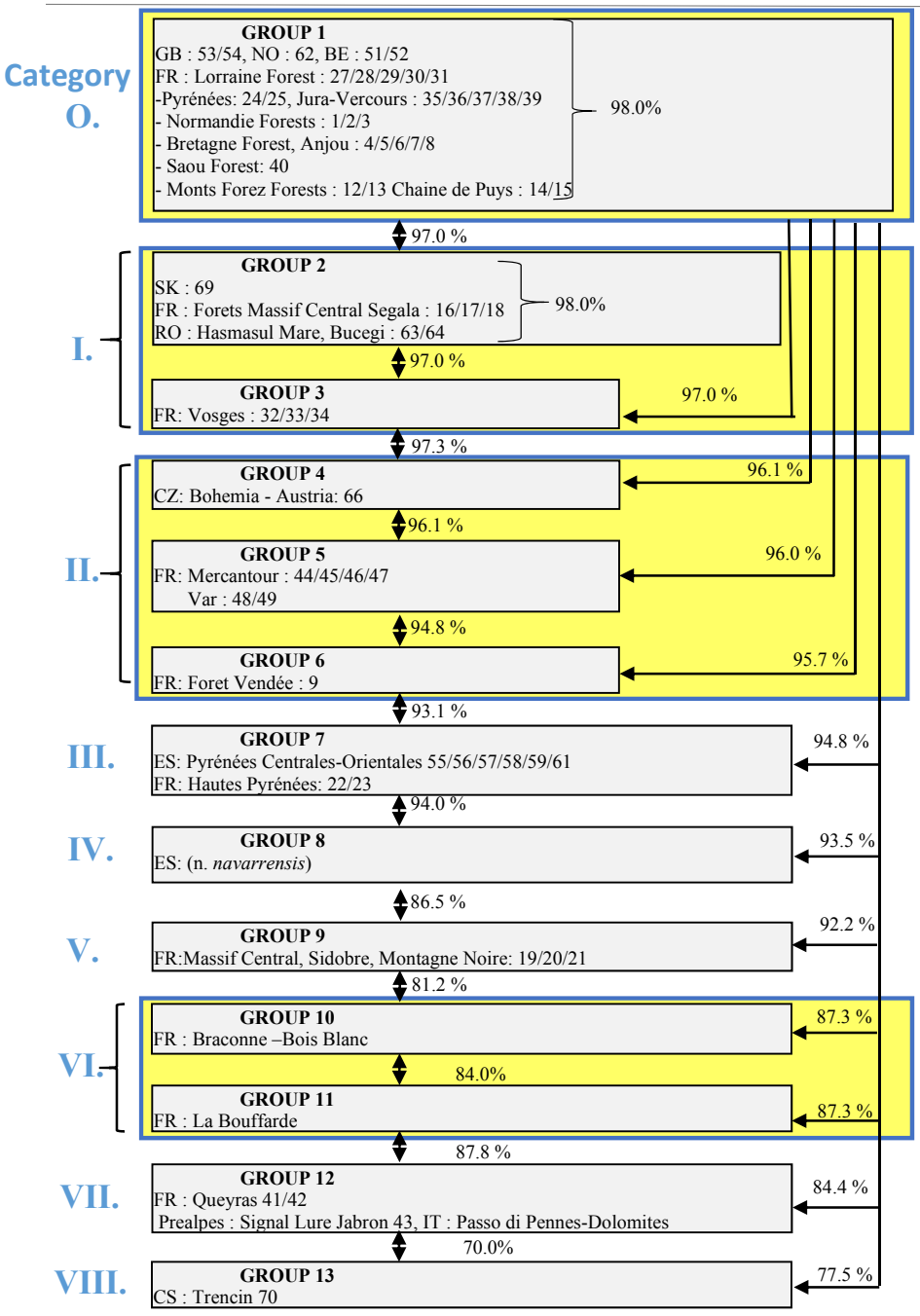

Figure 4. Marker cyt b. Classification according to the percentage of similarity

Group C-gathers altitude forms from the French Southern Alps (Massif of Queyras) and Italy (Dolomites).

B. Cytochrome b marker

The study concerns 70 provenances (list of the origins, the numbering, the geographical distribution) in Annex 2.

B.1. Data analysis

Data exploitation with analysis of parsimony is the following one:

- The distinct provenances between them of less than 2 % of dissimilarity form one group considered as identical and identified from 1 to 12. This value corresponds in most to the average of intrapopulation dissimilarity (analysis of 4 individuals) or 4-5 pairs base, the half corresponding to individual mutations. This criterion does not apply to literature data (ANDUJAR *et al*. 2012, HEINDRICH *et al*. 2015, DEUVE *et al*. 2001…).

- The matrix of identity in nucleic acids allows to compare two by two the groups and to classify them after the degree of dissimilarity in categories noted from 0 to VII.

B.2. General results

The results are presented by category of dissimilarity (table 1) expressed in percentage in reference to the category 0, considered as representing the type of *problematicus*.

Table 1.

Table of percentage of dissimilarity

Category	I	II	III	IV	V	VI	VII	VII (1)
Dissimilarity (%)	3,0	4,0	5,2	6,5	7,8	12,7	15,6	+ +

(1) Analysis of one single exemplar

- Category 0 (of reference) gathers 48 % of the studied populations, native of different countries (BE, GB, NO) and for France Forests of Anjou, Bretagne, Jura, Lorraine, Normandy, Saou, Vercors, Monts Forez, Chaîne des Puys. We consider this category of provenances as the *problematicus* Herbst type;
-Category I (3% dissimilarity inter IA/IB/0) includes 2 subgroups: a Slovak (Trstin-Male Karpaty), the two Romanian origins and for FR on the one hand the Massif Central, (Segala) forests (group 2); Massif des Vosges (group 3);
-Category II (4% dissimilarity inter IIA/IIB/IIC/0) gathers three subgroups, Czech origin Bohemia, on the Austrian border (group 4), FR the localities of the Massif of Mercantour (the Alps of the South) of the Var (group 5) and the forests of Vendée (group 6);
-Category III (5% dissimilarity) corresponds to Pyrenean origins of Spain (Oriental and central Pre-Alps) and of France (Hautes-Pyrénées) (group 7);
-Category IV (6.5% dissimilarity) characterizes *navarrensis* form (Breuning 1932) from western Spanish Pyrenees (group 8);
-Category V (7.8% dissimilarity) corresponds to the populations of the Southern part of Massif Central (Sidobre, Montagne Noire) attached to the natio *occitanus* (Lapouge 1910) (group 9);
-Category VI (12.7 % dissimilarity) encompasses despite differences, currently isolated forests of the Central West of France (Braconne, Bois Blanc, La Bouffarde) has belonging to primitive forests with dry valleys (groups 10 and 11);
-Category VII (15.6 % dissimilarity) associate the populations from French Pre-Alps of altitude, alpine origins of the Queyras (the Alps of the South close to the Mount Viso) and Italy Dolomites at Passo di Penne 2211 m., (group 12).

In summary, the recourse to the marker cytochrome b reveals a strong intraspecific variability; some results (category VIII) asking for confirmation because is based on a single

individual. Apart from some old French forests, the greatest diversity is found in mountainous areas (French and Spanish Pyrenees, Southern Massif Central, French and Italian Alps); the data for the Central Europe being too fragmentary.

Despite of the few studies on intraspecific variability within *Carabidae*, values of dissimilarity above 4% could mean the belonging to another species. In our study of morphological characteristics (labial palp chetae, aedeagus, endophallus) and the molecular biology (nuclear marker 28S RNA) show that insects studied belong to *C. (Mesocarabus) problematicus*.

C. Case of Romanian provenances, Munții Hășmașul Mare and Bucegi: studies in concatenation

These Romanian provenances, distant of 220 km in direct line were compared with two populations from the west of France (Breton forests Goven-Lorges 100 km) considered like of the type (cf. cyt b) and to the origins of Alps Queyras.

C.1. Concatenation COI I + cyt b (1051 pb)

The figure 5 indicates in concatenation COI I + cyt b the infraspecific variability between the provenances of Breton forests (Goven-Lorges), close to the category 0; the Romanian studied provenances (Bucegi, Hășmașul Mare) and the provenances of the Alps Queyras (Abries, La Monta 1800 m.), which have the highest percentage of dissimilarity.

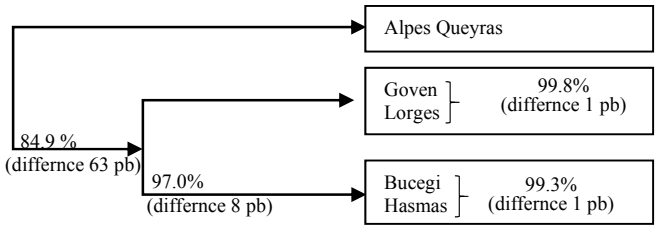

Figure 5. Concatenation COI I + cyt b

C.2. Concatenation cyt b+ND4+ND5 (1871 pb)

The figure 6 indicates the percentage of dissimilarity and the in base pairs differences.

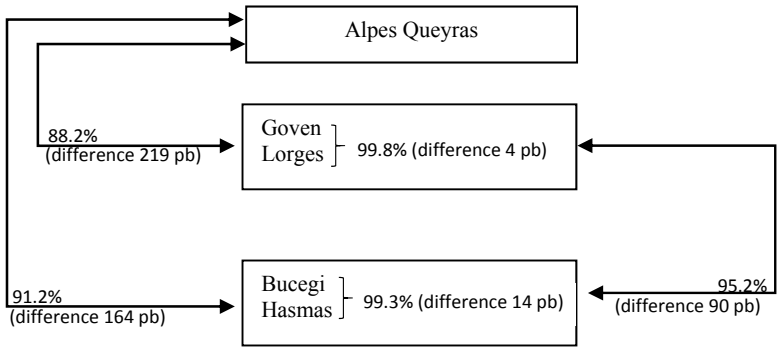

Figure 6 Concatenation cyt b+ND_4+ND_5

The populations of the Breton forests (natio *solutus* Oberthür, 1884), taken as reference type *problematicus* are homogeneous (4 base pair difference). Less different between them the Romanian provenances (14 base pair) are clearly distinguished from the French reference group (4.8% dissimilarity or 90 base pair). This suggests a more distant maternal origin.

Curiously and in spite of high rates of dissimilarity, the provenances of the Alps of Queyras are closer to the Romanian origins than to those from the Breton forests.

D. Discussions and perspectives

Insofar as the sample of studied populations represent the diversity of *C. (Mesocarabus) problematicus* Herbst, only 13% thereof (analysis cyt b) have a infraspecific variability (dissimilarity rate) greater than 4% with high values (5 to 16% cyt b; 12% in concatenation).

A as high intraspecific variability do not seem to have been never reported, but must be noted that this species occupy a vast geographical area, her origin being ancient, the fossil being known of Galicia (late glacial, WALKER *et al*. 2001), of England and Belgium (interglacial), Denmark (postglacial).

It was verified that the observed mutations do not form pseudogenes but correspond to substitutions of nucleotide not changing the corresponding acide amine (translation by using the invertebrate mitochondrial genetic code trans l. Table 5).

It was besides verified that the populations at the strong rate of dissimilarity do not belong to another species (recourse to the nuclear marker 28RNA).

Despite some difference between them, the both Romanian populations distinguish themselves little from the most represented type (category 0 for cytochrome b, dissimilarity 3%, dissimilarity 4.8% in concatenation). These two provenances, most probably have the same genetic origin, nearby of type.

These preliminary results incite to intensify the prospection in Romania, to verify these initial data and increase the provenances from the neighbouring countries. (Czech and Slovak Republic, Hungary) in order to possibly situate the origins Romanian.

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Annex 2

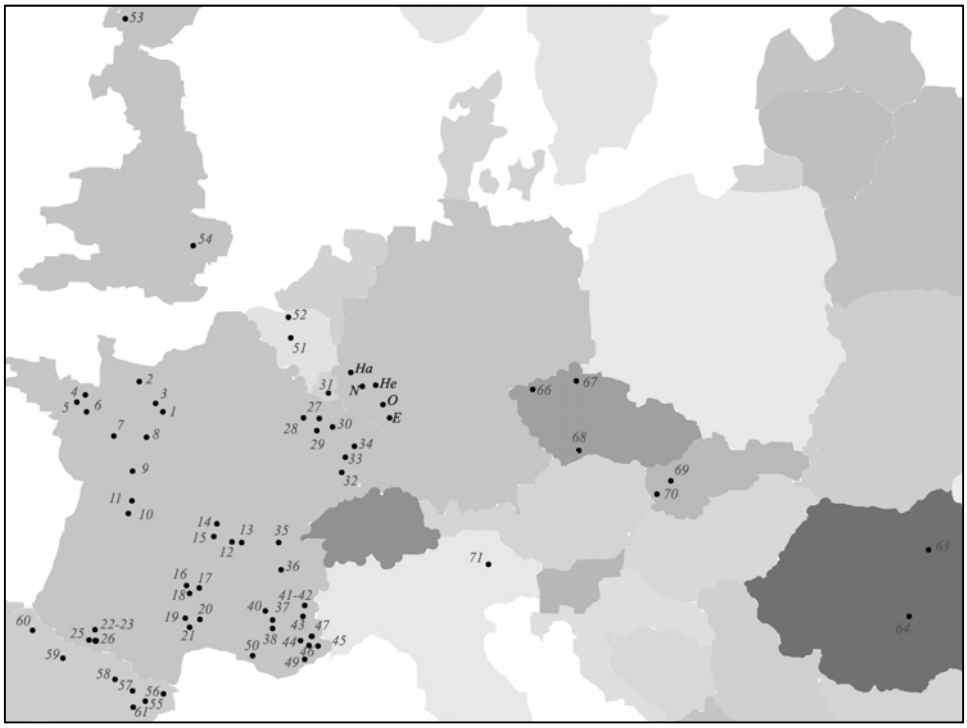
Provenances of studied C. *(Mesocarabus) problematicus* Herbst.

	Provenance	Geographical coordinates	Altitude	ssp or natio
	FRANCE			
	● Forests of West			
	•Normandia			
1.	Bellême Forest (Andujar *et al*.)	48 22 N 00 33 E	205 m	n. *problematicus* Herbst, 1786
2.	Cerisy Forest	49 11 N 00 53 E	83 m	
3.	The Ecouves forest	48 32 N 03 53 E	310 m	
	•Bretagne			
4.	Lorge Forest	48 33 N 02 49 E	260 m	
5.	Paimpont Forest	48 01 N 02 10 E	255 m	
6.	Goven Forest	48 00 N 01 50 E	104 m	
	•Anjou			n. *solutus* Oberthür, 1884
7.	Forest of Ancenis	47 33 N 01 19 E	80 m	
8.	Forest of Monnoye	47 28 N 00 08 E	70 m	
	•Vendée			
9.	The Mervent-Vouvant Forest	46 31 N 45 19 E	65 m	
	•Centre West			
10.	Forest of Braconne	45 43 N 00 19 E	120 m	n. *alluaudi* Colas, 1936
11.	Bois-Blanc Forest, La Bouffarde	45 39 N 00 17 E	117 m	
	● Massif Central			
	•Monts Forez			
12.	Col des Supèyres	45 59 N 03 81 E	1365 m	
13.	Jasserie du Coq Noir	45 58 N 03 86 E	1253	n. *arvernus* Lapouge, 1902
	•Chaine des Puys			
14.	RN Vallée De Chandefour	45 31 N 02 50 E	1137	
15.	Lac Pavin Forest (Besse)	45 29 N 02 53 E	1197 m	
	•Segala			
16.	Grésigne	44 01 N 01 45 E	500 m	
17.	Massif forestier Montech	43 57 N 01 16 E	133 m	
18.	Forest of Sivens	43 55 N 01 46 E	240 m	
19.	Montagne Noire Forest	43 33 N 02 14 E	950 m	
20.	Soulanes de Nore	43 24 N 02 29 E	825 m	
21.	La Loubatière	43 25 N 02 16 E	764 m	n. *occitanus* Lapouge, 1910
	●Pyrenees			
22.	Col du Tourmalet, La Mongie	42 54 N 08 42 E	2300 m	
23.	Lac d'Oncet	42 55 N 08 06 E	2250 m	
24.	Lac des Espécières	42 42 N 03 39 E	2195	
25.	Port de Boucharo	42 42 N 00 03 E	2270	
26.	Cirque de Troumousse	42 43 N 00 06 E	2103 m	
	●Lorraine			
27.	Jonville-en-Woevre Forest	49 04 N 0547 E	231 m	n. *gallicus* Géhin, 1885
28.	Beaulieu Argonne Forest	49 01 N 0504 E	223 m	
29.	Bois de la Montagne	49 00 N 0540 E	453 m	
30.	Forest of Saint-Hubert	49 13 N 06 20 E	220 m	n. *belgicus* Lapouge, 1913
	LUXEMBOURG			
31.	Remich	49 33 N 06 22 E	196 m	
	FRANCE			
	●Vosges			
32.	Carspach (Andujar)			n. *gallicus* Géhin, 1885
33.	Hautes Vosges (Kastelberg)	47 N 55 07 01 E	1880 m	
34.	Bois Birkenwald Dabo	48 39 N 07 14 E	345 m	
	●Jura Vercors Diois	46 16 N 06 00 E	1557 m	n. *clairvillei*, Lapouge 1916
35.	Crozet Forest			
36.	Col de la Chau	44 54 N 05 21 E	1337 m	
37.	Col de l'Allimas	44 52 N 05 24 E	1352 m	n. *iserensis* Breuning, 1933
38.	Font d'Urle	44 53 N 05 19 E	1600 m	
39.	*iserensis* (Deuve *et al*.)			
40.	Forest of Saou (Horta, Pas de Lauzun)	44 39 N 05 07 E	435 m	
	●Queyras			
41.	La Monta - Abriès	44 76 N 06 98 E	1607 m	n. *mayeti* Géhin, 1885
42.	Abriès, La Roche Écroulée	44 73 N 06 99 E	1700 m	

	Provenance	Geographical coordinates	Altitude	ssp or natio
	●The French Prealps			n. *clairvillei*, Lapouge 1916
43.	Signal de Lure- Jabron Forest	44 07 N 05 48 E	1828 m	
	●Mercantour			n. *moderatus*, Raynaud, 1971
44.	Annot Forest	43 57 N 06 40 E	820 m	
45.	Authion massif (Turini)	43 58 N 07 23 E	1580 m	
46.	Gordolasque Valley (Belvédère)	44 07 N 07 25 E	1500 m	
47.	Breil-sur-Roya Forest	43 56 N 07 30 E	1600 m	
	●Var			n. *inflatus* Kraatz, 1878
48.	Var (Andujar *et al*.)			
49.	Le Plan-de-la-Tour (Henidrich *et al*.)			
	●Divers			
50.	Montferrier-sur-Lez (Deuve *et al*.)			
	BELGIUM (Heindrich *et al*.)			n. *belgicus* Lapouge, 1913
51.	Vlaams- Flemish Brabant			
52.	Anvers			
	SCOTLAN			
53.	Auchincruive			
	ENGLAND (Andujar *et al*.)			n. *procedens* Csiki, 1927
54.	Essex-Saffron Walden			
	GERMANY (Heindrich *et al*.)			ssp. *harcyniae* Sturm, 1815
55.	Oberheimbach			
56.	Edenkoben			
57.	Hamminkeln			
58.	Heimbach Blens			
59.	Rhineland			
60.	Niederrhein (region)			
61.	Palatinat (Mayence — Bingen)			
62.	Palatinat (Südliche Weinstraße)			
63.	Westfalia (Wesel)			
64.	Westfalia (Düren)			
65.	North Rhine-Westphalia			
	SPAIN (Andujar *et al*.)			ssp. *planiusculus* Géhin, 1885
66.	Turó de l'Home			
67.	Ull de Ter			
68.	Sierra del Serral			
69.	Boscue de Gaujac			
70.	Puerto de Sahún			
71.	Ochagavía - Aduana			
72.	Sant Hilari Sacalm	41 52 N 02 30 E	820 m	
73.	Montseny (Girona)			n. *xaxarsi* Born, 1917
74.	Vallter (Girona)			
75.	Vielha (Lleida)			n/ *andorranus* Lapouge, 1902
76.	Huesca (Aragon)			
77.	Navarre			n. *navarrensis* Breuning, 1933
	NORWAY			ssp. *wockei* Born 1898
78.	Finse	60 36 N 07 30 E	1250 m	
	ROMANIA			n. *holdhausi* Born, 1911
79.	Hasmasu Mare	46 40 N 25 49 E	1700 m	
80.	Bucegi Mountains	45 35 N 25 50 E	1800 m	
	CZECH Republic			n. *diluvialis* Blumenthal & Nussler, 1967
81.	Hora Svatého Šebestiána (Ergebirge Au/CZ)	50 30 N 13 15 E	1230 m	
82.	Svor u Nového Boru (Lusatian Mountains, cz: Lužické hory) N Bohemia	50 47 N 14 35 E	793 m	
83.	Častrov (Vysočina Region)	49 18 N 15 10 E	604 m	n. *austriacu*s Sturm, 1815
	SLOVAK Republic			n. *cyanescens* Sturm, 1815
84.	Trstin (*Malé Karpaty)*	48 31 N 17 27 E	236 m	
85.	Trenčín (NW SK near CZ)	48 53 N 18 02 E	217 m	
	ITALY			n. *inflatus* Kraatz, 1878
86.	Passo di Pennes (Dolomites)	46 49 N 11 25 E	2211 m	

Annex 3

Geographical distribution of studied provenances



Annex 4

Single-nucleotide polymorphism cyt b (464 pb)

	2	17	48	53	57	59	62	65	66	71	73	74	77	78	92	113	116
Forêt Goven (FR)	C	T	C	C	G	T	T	T	T	A	C	A	A	T	G	C	T
Forêt Lorges (FR)	A	T	C	C	G	T	T	T	T	A	C	A	A	T	G	C	T
Bucegi	A	T	C	A	G	T	T	T	T	A	T	A	G	T	G	C	T
Hasmas	A	T	C	A	G	T	T	T	T	A	T	A	G	T	G	C	T
Alpes Gueyras (FR)	A	A	T	A	A	C	C	C	C	G	C	T	A	A	A	T	C

	119	125	134	140	154	158	161	167	168	169	174	175	179	188	192	196	197
Forêt Goven (FR)	A	A	T	T	C	T	C	T	G	T	G	C	A	C	T	C	C
Forêt Lorges (FR)	A	A	T	T	C	T	C	T	G	T	G	C	A	C	T	C	C
Bucegi	A	G	T	T	C	T	C	T	G	T	G	C	G	C	T	C	C
Hasmas	A	A	T	T	C	T	C	T	G	T	G	C	G	C	T	C	C
Alpes Gueyras (FR)	C	A	C	A	T	C	T	A	A	C	A	T	A	T	C	T	T

	200	209	212	215	244	254	263	269	272	275	281	285	303	306	313	315	322
Forêt Goven (FR)	C	A	A	T	T	T	C	T	T	A	A	A	C	T	C	C	C
Forêt Lorges (FR)	C	A	A	T	T	T	C	T	T	A	A	A	C	T	C	C	C
Bucegi	T	A	A	T	T	T	C	T	T	A	A	A	C	T	C	T	C
Hasmas	T	A	A	T	T	T	C	T	T	A	A	A	C	T	C	T	C
Alpes Gueyras (FR)	A	T	G	A	C	C	T	C	C	C	G	T	T	A	T	C	T

	329	330	332	333	338	341	344	350	359	365	368	370	374	377	380	383	389
Forêt Goven (FR)	C	T	A	T	C	C	T	A	T	C	C	C	T	G	C	C	A
Forêt Lorges (FR)	C	T	A	T	C	C	T	A	T	C	C	C	T	G	C	C	A
Bucegi	C	T	A	T	C	C	T	A	C	C	C	C	T	A	C	C	A
Hasmas	C	T	A	T	T	C	T	A	C	C	C	C	T	A	C	C	A
Alpes Gueyras (FR)	G	C	T	C	T	A	C	C	C	T	T	T	A	A	T	T	T

	392	395	396	410	413	444	446
Forêt Goven (FR)	T	T	A	A	G	A	G
Forêt Lorges (FR)	T	T	A	A	G	A	G
Bucegi	C	C	A	A	G	G	A
Hasmas	C	T	A	A	G	G	A
Alpes Gueyras (FR)	A	A	G	T	A	A	A

Annex 5

Single-nucleotide polymorphism in concatenation cytb+ND_4+ND_5

	5	9	10	18	19	38	50	51	53	62	74	86	89	95	98	110	125
Forêt Goven (FR)	G	T	A	T	C	A	G	C	T	T	A	A	A	G	A	C	A
Forêt Lorges (FR)	G	T	T	T	C	A	G	C	T	T	A	A	A	G	A	C	A
Bucegi	A	G	T	A	G	G	A	T	A	A	T	T	T	A	G	G	T
Hasmas	A	G	T	A	G	G	A	T	A	A	T	T	T	A	G	G	T
Alpes Gueyras (FR)	A	G	T	A	G	G	A	T	A	A	T	T	T	A	A	G	T

	161	170	173	176	179	191	206	209	224	242	243	257	260	263	275	278	282
Forêt Goven (FR)	G	A	C	A	A	T	A	T	T	A	T	G	T	A	G	G	G
Forêt Lorges (FR)	G	A	C	A	A	T	A	T	T	A	T	G	T	A	G	G	G
Bucegi	A	T	A	G	G	T	G	G	A	T	C	A	A	T	A	A	A
Hasmas	A	T	A	G	G	T	G	G	A	T	C	A	A	T	G	A	A
Alpes Gueyras (FR)	A	T	A	G	G	A	G	G	A	T	C	A	A	T	A	A	A

	284	285	288	290	302	308	332	362	366	371	383	384	395	422	437	443	446
Forêt Goven (FR)	A	C	T	T	A	T	G	A	A	G	G	T	G	T	T	T	G
Forêt Lorges (FR)	A	C	T	T	A	T	G	A	A	G	G	T	G	T	T	T	G
Bucegi	T	T	G	T	A	C	A	G	T	A	A	A	A	A	G	C	A
Hasmas	T	T	G	A	G	C	A	G	T	A	A	A	A	A	G	C	A
Alpes Gueyras (FR)	T	T	G	T	A	C	A	G	T	A	A	A	A	A	G	C	A

	464	467	470	492	494	497	542	548	551	552	553	555	591	609	612	613	627
Forêt Goven (FR)	T	A	A	A	T	A	G	T	A	A	A	A	C	G	A	A	T
Forêt Lorges (FR)	T	A	A	A	T	A	G	T	A	A	A	A	C	G	A	A	T
Bucegi	A	G	C	G	A	G	A	A	G	G	G	C	C	G	A	G	T
Hasmas	A	G	C	G	A	G	A	A	G	G	G	C	C	G	A	G	T
Alpes Gueyras (FR)	A	G	C	G	A	G	A	A	G	G	G	C	T	A	G	A	G

	639	643	651	660	670	693	705	708	709	717	724	726	730	744	750	768	783
Forêt Goven (FR)	C	G	A	G	A	T	A	G	T	G	T	A	A	G	C	T	A
Forêt Lorges (FR)	C	G	A	G	A	T	A	G	T	G	T	A	A	G	C	T	A
Bucegi	C	G	A	G	A	T	A	G	T	G	T	A	A	G	T	T	A
Hasmas	C	G	A	G	A	T	A	G	C	G	T	A	A	G	C	T	A
Alpes Gueyras (FR)	T	A	T	A	G	A	G	A	T	A	C	T	T	A	C	A	G

	804	828	831	834	837	846	855	861	888	892	900	903	912	921	933	936	963
Forêt Goven (FR)	A	A	T	C	A	A	A	A	A	T	T	A	T	C	G	A	A
Forêt Lorges (FR)	A	A	T	C	A	A	A	A	A	T	C	A	T	C	G	A	A
Bucegi	G	A	C	T	A	A	A	A	A	T	T	A	T	C	G	A	A
Hasmas	G	A	T	T	A	A	A	A	A	T	T	A	T	C	G	A	A
Alpes Gueyras (FR)	A	G	T	A	G	T	T	G	T	A	T	T	A	T	A	C	G

	975	985	987	994	1006	1008	1017	1020	1023	1029	1047	1050	1074	1092	1119	1141	1143
Forêt Goven (FR)	A	A	T	A	C	G	A	A	A	G	T	T	T	G	A	G	G
Forêt Lorges (FR)	A	A	T	A	C	G	A	A	A	G	T	T	T	G	A	G	G
Bucegi	A	A	T	A	C	A	A	A	A	G	T	T	T	A	A	G	G
Hasmas	A	A	T	A	C	A	A	A	A	G	T	T	T	A	A	G	G
Alpes Gueyras (FR)	T	T	A	T	T	A	T	T	T	A	A	G	G	T	G	T	T

	1152	1158	1159	1167	1173	1176	1185	1200	1210	1221	1222	1239	1242	1255	1257	1263	1266
Forêt Goven (FR)	A	T	A	A	T	T	G	C	A	T	A	G	A	G	T	G	T
Forêt Lorges (FR)	A	T	A	A	T	T	G	C	A	T	A	G	A	G	G	G	T
Bucegi	A	T	A	C	T	T	G	C	G	T	A	G	A	G	G	G	T
Hasmas	A	T	A	C	T	T	G	C	G	T	A	G	A	G	G	G	T
Alpes Gueyras (FR)	T	A	G	A	A	A	A	G	A	A	T	A	T	T	T	A	A

	1270	1272	1282	1287	1290	1299	1313	1314	1347	1350	1356	1369	1380	1386	1346	1398	1404
Forêt Goven (FR)	T	T	T	A	A	A	G	T	C	G	G	A	T	T	G	T	A
Forêt Lorges (FR)	T	T	T	A	A	A	G	T	C	G	G	A	T	T	G	T	A
Bucegi	T	T	T	A	A	A	G	T	C	G	G	A	T	T	G	C	T
Hasmas	T	T	T	A	A	A	G	T	C	G	G	A	T	T	G	T	A
Alpes Gueyras (FR)	A	A	A	G	G	G	A	C	A	A	A	T	G	A	A	T	G

	1407	1420	1453	1458	1462	1464	1467	1470	1471	1476	1478	1479	1482	1483	1498	1518	1521
Forêt Goven (FR)	T	T	C	C	G	T	T	T	T	A	C	A	A	T	G	C	T
Forêt Lorges (FR)	T	T	C	C	G	T	T	T	T	A	C	A	A	T	G	C	T
Bucegi	T	T	C	A	G	T	T	C	T	A	T	A	G	T	G	C	T
Hasmas	T	T	C	A	G	T	T	C	T	A	T	A	G	T	G	C	T
Alpes Gueyras (FR)	~~A~~	~~A~~	~~T~~	A	A	C	C	C	C	G	C	T	A	A	A	T	C

	1524	1530	1539	1545	1558	1563	1566	1572	1573	1574	1579	1580	1584	1593	1597	1602	1603
Forêt Goven (FR)	A	A	T	T	C	T	C	T	G	T	G	C	A	C	T	C	C
Forêt Lorges (FR)	A	A	T	T	C	T	C	T	G	T	G	C	A	C	T	C	C
Bucegi	A	G	T	T	C	T	C	T	G	T	G	C	G	C	T	C	C
Hasmas	A	A	T	T	C	T	C	T	G	T	G	C	G	C	T	C	C
Alpes Gueyras (FR)	C	A	C	A	T	C	T	A	A	C	A	T	A	T	C	T	T

	1605	1614	1617	1620	1649	1659	1668	1674	1677	1680	1686	1690	1708	1718	1720	1727	1734
Forêt Goven (FR)	C	A	A	T	C	T	C	T	T	A	A	A	C	T	C	C	C
Forêt Lorges (FR)	C	A	A	T	C	T	C	T	T	A	A	A	C	T	C	C	C
Bucegi	T	A	A	T	C	T	C	T	T	A	A	A	C	T	T	C	C
Hasmas	T	A	A	T	C	T	C	T	T	A	A	A	C	T	T	C	C
Alpes Gueyras (FR)	A	T	G	A	T	C	T	C	C	C	G	T	T	C	C	T	G

	1735	1737	1738	1740	1743	1746	1749	1753	1755	1764	1770	1773	1775	1779	1782	1785	1788
Forêt Goven (FR)	T	A	T	A	C	C	C	T	A	T	C	C	C	T	G	C	C
Forêt Lorges (FR)	T	A	T	A	C	C	C	T	A	T	C	C	C	T	G	C	C
Bucegi	T	A	T	A	C	C	C	T	A	C	C	C	C	T	A	C	C
Hasmas	T	A	T	A	T	C	C	T	A	C	C	C	C	T	A	C	C
Alpes Gueyras (FR)	C	T	C	T	T	A	T	C	C	C	T	T	T	A	A	T	T

	1795	1797	1800	1815	1818	1848	1851	1871
Forêt Goven (FR)	A	T	T	A	T	A	G	G
Forêt Lorges (FR)	A	T	T	A	T	A	G	G
Bucegi	A	C	C	A	T	G	A	A
Hasmas	A	C	T	A	T	G	A	A
Alpes Gueyras (FR)	T	A	C	T	A	A	A	G