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Unexpected results obtained during the experimental crossing of *Carabus (Damaster) lafossei dabieshanus* Imura, 1996, with *Carabus (Damaster) fruhstorferi* Roeschke, 1900, a species endemic to the island of Tsushima (Coleoptera, Carabidae)

par

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Résumé

Le croisement de *Carabus (Damaster) lafossei dabieshanus* Imura, 1996, de Chine avec *Carabus (Damaster) fruhstorferi* Roeschke, 1900, endémique de l'île Tsushima de l'archipel Japonais produit des hybrides de première génération avec une bonne prolificité. En revanche, de façon surprenante, ces hybrides F1 se révèlent interfertiles, ce qui permet d'obtenir des hybrides de deuxième génération F2 également interfertiles conduisant à des hybrides de troisième génération F3 avec les mêmes taux de prolificité. Ces résultats sont à rapprocher de ceux obtenus lors du croisement de *C. (Damaster) lafossei dabieshanus* Imura, 1996, avec *C. (Damaster) nankotaizanus* Kanô, 1932, et posent la question du statut de *C. (D.) lafossei dabieshanus* au sein du sous-genre *Damaster*, ce dernier montrant de même une proximité génétique très inattendue avec l'espèce *C. (D.) frushtorferi*.

Abstract

Experimental crossing of *Carabus (Damaster) lafossei dabieshanus* Imura, 1996, from China with *Carabus (Damaster) fruhstorferi* Roeschke, 1900, from Japanese Tsushima Island allows to obtain hybrid individuals with a high success rate. On the other hand, surprisingly, these F1 hybrids are interfertile, which makes it possible to obtain second-generation F2 hybrids also interfertile leading to third-generation F3 hybrids with the same rates of prolificity. These results are reminiscent of those obtained when crossing *C. (Damaster) lafossei dabieshanus* Imura, 1996, with *C. (Damaster) nankotaizanus*

Kanô, 1932, and raise the issue of status of *C. (D.) lafossei dabieshanus* among the subgenus *Damaster*, the latter again showing a very unexpected genetic proximity with the species *C. (D.) frushtorferi*.

Mots-clés

Coleoptera, Carabidae, *Carabus*, *Damaster*, hybridization, China, Japan.

The taxonomy of the genus *Carabus* is in constant evolution as evidenced by the important review work carried out by T. Deuve (2021). It is now clear that the contributions of molecular biology studies both at the level of the nuclear genome (Sota *et al.*, 2004; Deuve, 2012; Deuve *et al.*, 2012) and the mitochondrial genome (Osawa *et al.*, 2004) allow a clarification of the relationships of the different subgenera and species between them, even if important gaps or difficulties of interpretation remain. Another way to better understand their relationships is to study the species *in vivo* in order to identify their phylogenetic proximity, thanks to the experimental hybridization results. This is the aim of many authors who continue to study the possibility of breeding and interspecific or even intersubgeneric experimental crossing experiments in order to better identify the genetic relationships of the species of the genus *Carabus* (Godeau *et al.*, 2007; Sapaly *et al.*, 2013).

We recently published our results on the study of experimental cross-breeding of species of the *Damaster* subgenus (Dubois, 2015). In particular, we observed that hybrids obtained by crossing *C. (Damaster) lafossei dabieshanus* Imura, 1996, with *C. (Damaster) nankotaizanus* Kanô, 1932, were interfertile over two generations (Dubois, 2013). This very unexpected result was interpreted as reflecting a high genetic proximity between these two species.

Here we present the results of another type of crossing involving again *C. (Damaster) lafossei dabieshanus* but with *C. (Damaster) fruhstorferi* Roeschke, 1900.

Material and methods

Breeding and hybridization experiments were conducted under the now well-established conditions (Malausa, 1977) which proved appropriate for the species *C. (D.) frushtorferi* which, to our knowledge, had never been bred in captivity. As with our previous study, we did not attempt to collect eggs to avoid any destruction. However, it is important to harvest and isolate the first stage (L1) larvae as soon as they appear in order to avoid any risk of cannibalism. The disadvantage of this method is that it does not allow to measure the fecundity of the females but only the prolificity which is expressed as a percentage of the imagos obtained compared to the L1 larvae harvested. It is also clear that the durations mentioned have no significant statistical value, the number of experiments carried out being too small.

Experimental results

For a good understanding of the text, we always mention the name of hybrid individuals starting with the name of the species of the female(s) used for the crossing followed by × and then the name of the species of the male(s) used. It is clear that the temperature at which the experiments were carried out affects the total duration of the development of individuals. On the other hand, the values indicated are the averages observed but the durations can range from single to triple.

1 – *C. (D.) fruhstorferi* strain (Fig. 1)

This type of pairing has been done several times to keep a strain of “pure” and virgin individuals. We did not observe any significant difference in the prolificity of the different generations despite the relative consanguinity of the individuals obtained. We describe an experiment performed in spring 2021 from a single female of *C. (D.) fruhstorferi*. One virgin female of *C. (D.) fruhstorferi* is put in the presence of two males of *C. (D.) fruhstorferi*. 56 L1 larvae and 42 imagos (21 females and 21 males) were obtained (75% prolificity). The average time from stage L1 to imago under the conditions used (temperature about 18°C) was 60 days.

2 – *C. (D.) lafossei dabieshanus* strain (Fig. 2)

Using similar conditions as for *C. (D.) fruhstorferi, C. (D.) lafossei dabieshanus* has been bred for many years to maintain a strain of pure and virgin individuals. The prolificity stays at a similar order of magnitude *c.a.* 80 %. We did not notice a variation of shape and coloration between the successive generations obtained in captivity.

3 – Crossing of *C. (D.) fruhstorferi* with *C. (D.) lafossei dabieshanus* (F1)

Two virgin females of *C. (D.) fruhstorferi* are placed in the presence of one male of *C. (D.) lafossei dabieshanus*. 72 L1 larvae and 66 imagos (30 females and 36 males) were obtained (91% prolificity). The average time from stage L1 to imago under the conditions used (temperature about 25°C) was about 40 days.

4 – Crossing of *C. (D.) fruhstorferi* with *C. (D.) lafossei dabieshanus* (F2)

Three virgin females F1 *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus* are placed in the presence of one male F1 *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus*. 27 L1 larvae and 21 F2 imagos (10 females and 11 males) were obtained (prolificity 77%). The average time from stage L1 to imago at 18-20°C was 58 days. The colors of the F2 imagos range from identical blue of *C. (D.) lafossei dabieshanus* to identical red of *C. (D.) fruhstorferi*, some individuals being green or of mixed colours. Note also that the shape of the pronotum, as

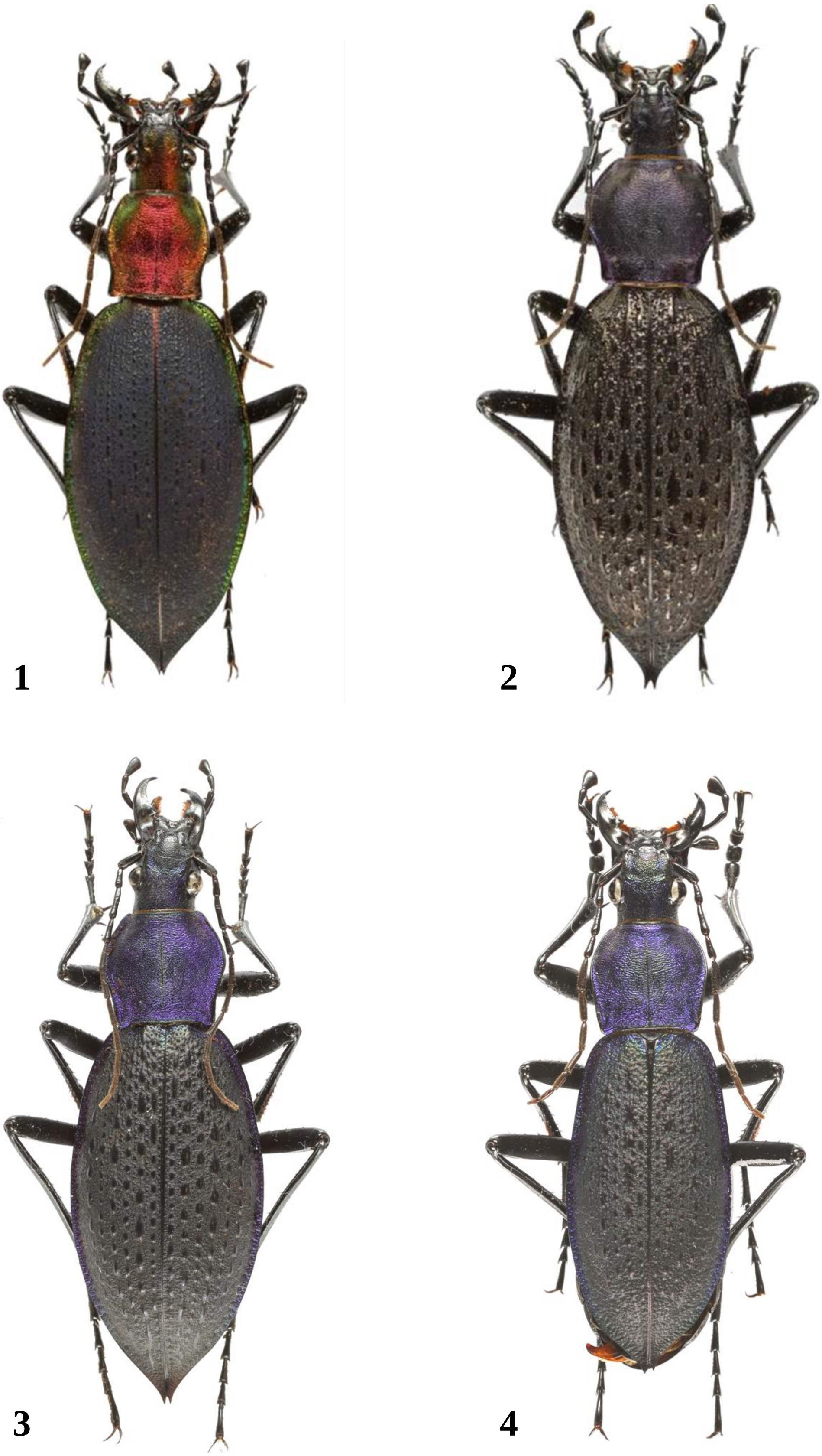

Fig. 1-4 : *Carabus* subgen. *Damaster* Kollar, 1836. – 1, *C.* (*D*). *fruhstorferi* Roeschke, 1900, ♀. – 2, *C.* (*D*). *lafossei dabieshanus* Imura, 1996, ♀. – 3-4, *C.* (*D*). *fruhstorferi* × *C.* (*D*). *lafossei dabieshanus*, F3 blue.

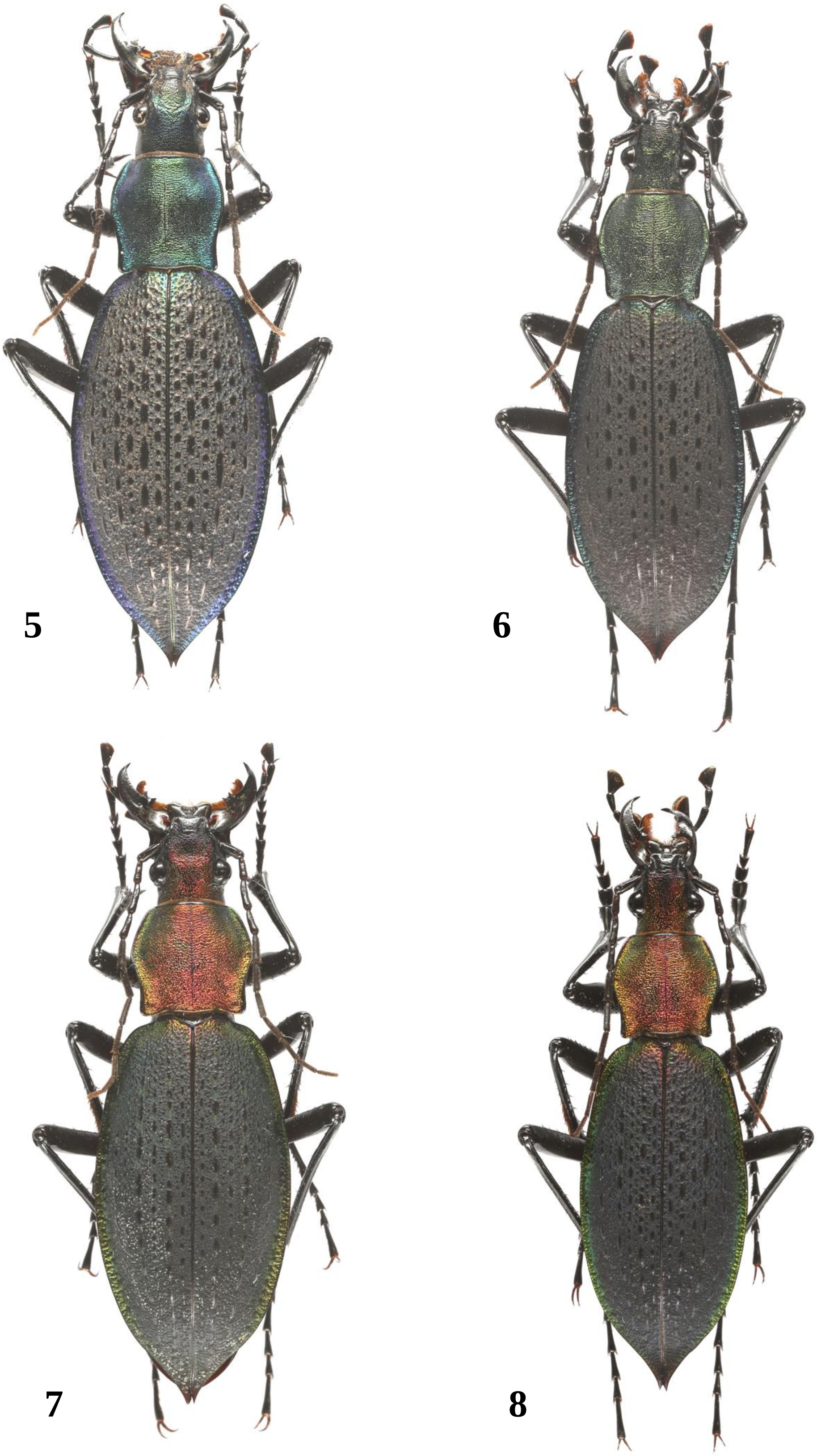

Fig. 5-8 : *Carabus* subgen. *Damaster* Kollar, 1836. – 5-6, *C.* (*D*). *fruhstorferi* × *C.* (*D*). *lafossei* dabieshanus, F3 green. – 7-8, *C.* (*D*). *fruhstorferi* × *C.* (*D*). *lafossei dabieshanus*, F3 red.

well as the costulation are quite variable: some individuals getting closer to the female parent, others to the male parent and the majority having intermediate characters.

5 – Crossing of *C. (D.) fruhstorferi* with *C. (D.) lafossei dabieshanus* (F3 blue, Fig. 3-4)

One virgin female F2 blue *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus* is presented with one male F2 blue *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus*. 25 L1 larvae and 15 F3 imagos (8 females and 7 males) were obtained (prolificity 60%). The average time from stage L1 to imago at 18-22°C was 51 days. The colour of the F3 imagos is identical to that of the spawners for 12 of the obtained imagos, the other 3 being mixed colors.

6 – Crossing of *C. (D.) fruhstorferi* with *C. (D.) lafossei dabieshanus* (F3 green, Fig. 5-6)

One virgin female F2 green *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus* is presented with one male F2 green *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus*. 8 L1 larvae and 3 F3 imagos (2 females and 1 male) were obtained (prolificity 37%). The average time from stage L1 to imago at 18-20°C was 52 days. The colour of females F3 imagos is green, the male is red.

7 – Crossing of *C. (D.) fruhstorferi* with *C. (D.) lafossei dabieshanus* (F3 red, Fig. 7-8)

One virgin female F2 red *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus* is presented with a male F2 red *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus*. 23 L1 larvae and 18 F3 imagos (6 females and 12 males) were obtained (prolificity 78%). The average time from stage L1 to imago at 18-20°C was 59 days. The colour of the F3 imagos is identical to that of the spawners for 17 of the 18 imagos obtained.

Discussion

The results of the breeding of *C. (D.) fruhstorferi* show that this species behaves like most species of the subgenus *Damaster*. In particular, the diet (mainly composed of *Thebas pisana* Müller, 1774), the number of larval stages (2) as well as the durations of the various pre-imaginal stages are quite comparable to those observed for the other *Damaster* species that we have bred. However, we can notice a particularly bellicose character of adults so it is important not to group in spaces too small at the risk of seeing individuals mutilate themselves. The prolificity remains correct despite the number of generations bred in captivity for four consecutive years. Finally, it is also observed that male and female individuals reach sexual maturity within a few weeks without having undergone a winter diapausis. This feature makes it easy to obtain two generations in the same year.

The *C. (D.) fruhstorferi* × *C. (D.) lafossei dabieshanus* (F1) hybrids obtained show an extensive colour palette and a variable elytra sculpture with relatively unmarked primary intervals for some individuals recalling their female parent *C. (D.) fruhstorferi* up to individuals having practically the characters of their male parent *C. (D.) lafossei dabieshanus*. It should also be noted that the shape of the pronotum is variable and presents either the characters of *C. (D.) fruhstorferi* (with a barely transverse pronotum when it is not even longer than broad), or those of *C. (D.) lafossei dabieshanus* (including a clearly transverse and cordiform pronotum). The majority of individuals with intermediate characters as expected for F1 hybrids.

Unexpectedly, the crossing of F1 hybrid females with F1 hybrid males led to the production of second-generation hybrids (F2) with a prolificity of the same order of magnitude as that of the parent individuals. This result is extremely surprising. Indeed, in general, hybrid females are almost always fertile, which has made it possible to obtain multispecies hybrids within the subgenus *Damaster*. Indeed, we were able to obtain heptaspecific hybrids (Dubois, 2015). This result already shows not only a good fertility of hybrid females but also clearly goes in the direction of an important genetic proximity for at least seven of the Chinese hybridized species crossed together. On the other hand hybrid males both in the subgenus *Damaster* and the subgenera *Chrysocarabus* or *Macrothorax*, which have been widely studied, are essentially sterile. These observations stem from the genetic barrier that maintains the status of the species.

The fecundity of F1 hybrid males has been verified in several experiments. The F2 imagos obtained are perfectly formed and show the variety of colors, elytral sculptures and shapes of the pronotum. We therefore sought to know if this characteristic was transmissible by crossing these F2 individuals. Having observed the wide colour palette of the F2, we took the opportunity to study how colours are transmitted by matching female F2 and male F2 of identical colours using a single individual of each sex. As indicated in the experimental part, the prolificity observed on two of the experiments conducted was of the same order of magnitude as for the previous crossing experiments. It is clear that this fertility character of the males (and females) of these hybrids is perfectly preserved. In addition, the transmission of colours is also very mostly univocal: the F2 individuals blue parents give a very large majority of blue F3, the same for the F2 individuals red which give almost exclusively red F3. These results seem to be in agreement with what has been observed in *C.* (*Chrysocarabus*) *auronitens* (Rasplus *et al.*, 2018), and which is explained by the mono-gene character determining the “metallic” coloration of the *Carabus*, resulting from a physical phenomenon and not from pigmentation of the teguments (see for instance, a study on a representative of *Cetoniidae*: McDonald *et al.*, 2017). It is more difficult to conclude for individuals of green "intermediate" colour, given the few individuals obtained but in this case also, the majority of individuals are green. Complementary experiments are underway to clarify this point because we have also found on some attempts to cross individuals of "mixed" colour a smaller number, or even absence of descendants.

The question then arises: how to explain our results ?

Recall that we have shown the fertility of hybrids F1 and F2 *C. (D.) lafossei dabieshanus* × *C. (D.) nankotaizanus* (Dubois, 2013). We find a similar situation with the crossing of *C. (D.) lafossei dabieshanus* with *C. (D.) fruhstorferi* for which the hybrids obtained also come from *C. (D.) lafossei dabieshanus* crossed with an endemic island species. In the previous case, the island of Taiwan, home to *C. (D.) nankotaizanus,* is located opposite continental China, to which it was attached about 5 million years ago (Ho, 1988). On the other hand, the island of Tsushima, home to *C. (D.) fruhstorferi,* is located further north and east of the Korean peninsula to which it was attached 15 million years ago (Tominaga *et al.*, 2000). Note that *C. (D.) lafossei* is currently unknown from this northern part of the Eurasian continent. The genetic proximity between these two species that seems to clearly show our results is therefore more difficult to explain although *C. (D.) lafossei* is found on the continent further south, compared to the latitude of the island of Tsushima.

The most plausible hypothesis that we can make in the current state of the hybridization data is that *C. (D.) lafossei*, *C. (D.) nankotaizanus* and *C. (D.) fruhstorferi* have a common ancestor. One of the likely speciation processes is the introgression of genes from another species through successive backcrosses such as those assumed in the formation of subspecies such as *C. (Chrysocarabus) lineatus* Dejean, 1826 or *C. (Chrysocarabus) punctatoauratus* Germar, 1824 (Rasplus 2001). This pattern of speciation leads to individuals genetically extremely close to the parent species. It would then be tempting to imagine that in the case of the two island species studied, the parent species is *C. (D.) lafossei* itself. Only the absence of *C. (D.) lafossei* in Korea does not militate in favour of this latter hypothesis but we do not know what it was at the time when the island of Tsushima was still attached to the Eurasian continent.

It is therefore important for us to better understand the place occupied by *C. (D.) lafossei* in the phylogeny of *Damaster*. To do this we propose to continue the studies of experimental crosses of this species with other representatives of the subgenus *Damaster* as well as, if possible, to refine the position of the latter in the phylogenetic trees resulting from the work on nuclear and mitochondrial DNA.

To conclude, we would like to send a call to the teams involved in the DNA analysis related to the study of the *Carabus* genus to examine the genomic characteristics of *C. (D.) lafossei dabieshanus* and its above mentioned hybrids (stabilized samples of them are available on request).

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