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# SIDE THREATS: FURTHER POSSIBLE EFFECTS OF WARMING ON THE HIGH ALPINE NARROW ENDEMIC CARABUS CYCHROIDES (COLEOPTERA: CARABIDAE)

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Carabus cychroides is an endemic ground beetle of a few high mountain slopes in the Cottian Alps. We have modelled the presence-absence data collected in a sample area, related to this species, the congener *C. depressus* with which it can hybridise and the small snail *Chilostoma glaciale*, specific prey of *Carabus cychroides*. The models were projected to three future periods according to four greenhouse gas emission scenarios. The results indicated the future increase in niche and spatial overlap of the two beetles, which could lead to more hybridisation and competition between them. If these threats occur over the entire geographic range of the already endangered *C. cychroides*, they could accelerate its predicted decline in the coming decades due to climate warming.

Key words: climate changes, Cottian Alps, endemism, hybrids, niche overlap

#### Introduction

The ground beetle Carabus cychroides Baudi di Selve, 1860 is a narrow endemic of a few high mountain slopes in the Cottian Alps (Northwestern Italy) (Casale et al., 1982; Cavazzuti & Ghiretti, 2020; Anselmo & Rizzioli, 2021), where most of its suitable area falls within the Cottian Alps Protected Areas (Anselmo & Rizzioli, 2021). Among the European species of the genus Carabus, this high alpine ground beetle is considered one of the two most localised species (Turin et al., 2003). Carabus cychroides is a cold-adapted insect, which mainly occupies the hollows of high altitude pastures and moraines (Casale et al., 1982) with prevalent northern exposure and where the snow remains longer in summer (Cavazzuti & Ghiretti, 2020), occupying an altitudinal range from 2162 m a.s.l. to 2776 m a.s.l. (Anselmo & Rizzioli, 2021). In these habitats, C. cychroides finds its specific prey Chilostoma glaciale Férussac, 1832, that it can effectively empty thanks to the thin and elongated anterior part of its body, a morphological adaptation present both in the adult and in the larvae (Sturani, 1962; Brandmayr et al., 2005). At the lower altitudes of its range, C. cychroides can be found in syntopy with Carabus depressus Bonelli, 1810 (Sturani, 1962), widely distributed in the Alps with various subspecies, below and above the treeline between 800 m a.s.l. and 2500 m a.s.l. (Casale et al., 1982). Carabus depressus feeds mainly on snails; however, its diet also includes

earthworms and small insects (Casale et al., 1982). These two Carabidae species can hybridise (Sturani, 1962; Casale et al., 1982, 1998; Deuve, 1994; Cavazzuti & Ghiretti, 2020), and this possibility demonstrates their recent separation, which probably ended in the late Tertiary (Casale et al., 1998). Carabus cychroides may have separated from *C. depressus* by isolation on the ice-free mountain summits (Sturani, 1962; Casale et al., 1982). Therefore, these species could have come into contact after the glaciations. The climate warming represents a serious threat for *C. cychroides*: according to the most negative greenhouse gas emissions scenario, it could face extinction at the end of the XXI century (Anselmo & Rizzioli, 2021).

Interspecific competition essentially leads to equilibrium or exclusion between species (Alley, 1982). In their optimal habitats, specialised species can displace generalist species (MacArthur & Levins, 1964; Wilson & Yoshimura, 1994). On the other hand, habitat loss and fragmentation can advantage generalist species (Jonsen & Fahrig, 1997; Warren et al., 2001; Nordén et al., 2013; Ramiadantsoa et al., 2018). The possibility of hybridisation between species can represent a serious threat, especially for the conservation of rare species (Rhymer & Simberloff, 1996; Allendorf et al., 2001; Todesco et al., 2016).

This study was aimed for deepening the knowledge on the ecology of *C. cychroides*, by compar-

ing its ecological niche and distribution to those of *C. depressus*. The relationship between these two species in the current period and in the nearby future under climate change was investigated to identify further threats to *Carabus cychroides*. Therefore, a loss of suitable areas of the specialist *C. cychroides* caused by rising temperatures (Anselmo & Rizzioli, 2021), and the possible effects of warming on the more generalist *C. depressus* were compared in order to estimate the niche and the spatial overlap between these two species, and the relative possibility of hybridisation by also assuming the existence of competition for the trophic source represented by *Chilostoma glaciale*.

# Material and Methods Presence-absence data

For the purposes of this study, an area of 0.7 km<sup>2</sup> was selected within the Cottian Alps Protected Areas, where both Carabus cychroides and C. depressus are present (Fig. 1). The study area was located between 2090 m a.s.l. and 2630 m a.s.l., being covered with pastures and meadows with a large percent of rocks and bushes. The area was divided into squared cells of 625 m<sup>2</sup> with QGIS (ver. 3.16.14), resulting in 1116 cells. Each cell was carefully inspected, looking for both Carabus species and the snail Chilostoma glaciale. Any accessible shelters of these three species, such as rocks and bush bases, were inspected from June to early August 2020, following the melting of the snow. We did not use pit-fall traps as they are poorly indicated for sampling low-mobility species living on small areas (Brandmayr et al., 2005) and to avoid reducing the already threatened population of C. cychroides (Anselmo & Rizzioli, 2021).

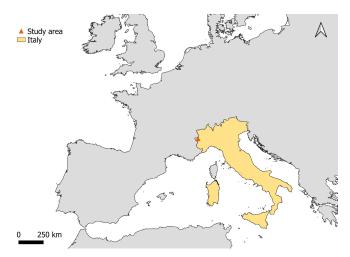


Fig. 1. Location of the study area in Europe.

#### Environmental variables

Some environmental variables (25-m resolution) were obtained for the study area, within the cells, where the three species were searched. These variables were: the Topographic Position Index (TPI), the Normalised Difference Vegetation Index (NDVI) and the maximum summer temperature at ground level (T<sub>max</sub>). The TPI was derived from a digital elevation model (DEM) with original resolution at 10 m (data were retrieved from https:// www.geoportale.piemonte.it). This index was considered important for the species because it allows us to distinguish the difference between ridges and valleys (Weiss, 2001), so their different habitats and different soil moisture. The NDVI was chosen among correlated vegetation indices provided by Copernicus Sentinel-2 mission (data retrieved by https://www.wekeo.eu/), at native resolution of 10 m, because it is linked to vegetation cover and widely used in ecological studies (Wegmann et al., 2016). Data deriving from the best image of completely cloud-free study area recorded in the summers between 2018 and 2020 were selected, corresponding to the captures made by Sentinel 2B on 03.08.2019. TPI and NDVI were brought to the final resolution by re-sampling them by aggregation. The variable  $T_{\text{max}}$  was derived by the interpolation of temperature measurements at ground level recorded in July 2020 (see Anselmo & Rizzioli (2021) for more details). This variable was found to be highly informative for predicting the distribution of C. cychroides. The management of the variables was carried out with QGIS (ver. 3.16.14).

#### Ecological niche modelling

The set of variables and the presence-absence data of Carablus cychroides, C. depressus, and Chilostoma glaciale were used to implement a series of ecological niche models (ENMs), which constitute common tools in ecology (Franklin, 2010; Peterson et al., 2011; Guisan et al., 2017). The absence of strong collinearity (< 0.7) between the explanatory variables was checked with the function «vifcor» of the «usdm» package (Naimi, 2017) in R ver. 4.1.2 (R Core Team, 2021). Four algorithms implemented with «biomod2» (Thuiller et al., 2021) in R (R Core Team, 2021) with default parameters, selected to model each of the three response variables represented by the presence-absence of the species: Generalised Linear Model (GLM), Generalised Additive Model (GAM), Generalised Boosted Model (GBM) and Random Forest (RF). These algorithms were calibrated with 70% of the data and tested with the remaining 30%. The entire procedure was replicated ten times and each model was evaluated by the True Skill Statistic (TSS). TSS ranges between -1 and 1; measures below 0.4 are considered poor, 0.4–0.8 useful, and more than 0.8 good-excellent (Allouche et al., 2006). These options led to 40 models being built (four algorithms × ten cross-validations) for each species. Thus the obtained models were filtered in order to produce ensemble models (Guisan et al., 2017) averaging only those useful with TSS higher than or equal to 0.6. Finally, each ensemble model was projected into current and future conditions, also building binary projections of presence-absence using the threshold that maximises TSS evaluation scores with «biomod2» (Thuiller et al., 2021) in R (R Core Team, 2021). The future conditions were estimated by increasing the current values of  $T_{\text{max}}$  with the difference between the near ground air temperatures of the future periods 2041–2060 (later named 2050), 2061-2080 (later named 2070) and 2081-2100 (later named 2090) and the values for the current period 2010–2018 (concurrent to the study), obtained from the pixel of 2.5 min of spatial resolution of the CNRM-CM6-1 climate model hanging on the study area and according to four greenhouse gas emission scenarios (RCPs): 2.6, 4.5, 7.0, and 8.5 (data from https://worldclim. org). This procedure made it possible to derive twelve future projections for each species, based on T<sub>max</sub> increases between 1.3°C and 7.7°C.

### Niche and spatial overlap

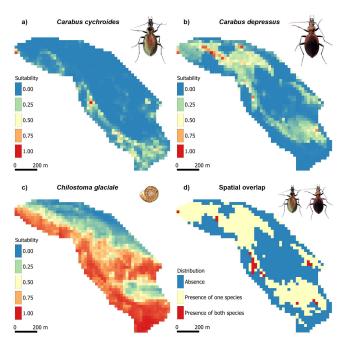
The current projections were employed to visualise the Predicted Niche Occupancy (PNO) profiles for each species related to  $T_{max}$ , following the procedure of Evans et al. (2009). The current and future projections of Carabus cychroides and C. depressus were used to estimate the niche overlap of each period and scenario, using the statistics Schoener's D and modified Hellinger's I (Warren et al., 2008). The values of these statistics range from 0 to 1, where 0 means no niche overlap and 1 means that niches are identical. These analyses were performed with the «phyloclim» package (Heibl & Calenge, 2018) in R (R Core Team, 2021). The spatial overlap was calculated for each period on the basis of binary maps, calculating the intersection area of presence of the two species ranges divided by the area of presence of C. cychroides. This metric ranges from 0 (no spatial overlap) to 1 (100% overlap).

#### Results

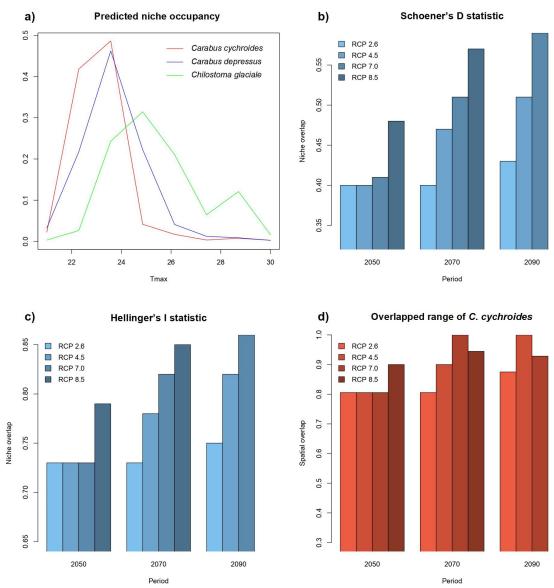
The presence of *Carabus cychroides* was found in 2.7% of the study area (30 cells), while in 3.4% for *C. depressus* (38 cells), and in 57.6% for *Chilostoma glaciale* (643 cells). The mean TSS of the ensemble ENMs was 0.96 for *C. cychroides* (36 models), 0.60 for *C. depressus* (six models), and 0.70 for *C. glaciale* (17 models). Therefore, the models were evaluated as useful.

Current projections confirmed the difference in distribution of the species emerged from the recorded data, showing a larger suitable surface for *C. glaciale* and smaller for *C. depressus* and *C. cychroides* (Fig. 2a,b,c). The spatial overlap of the binary ENMs of the *Carabus* species was found to be 27% (Fig. 2d). The niche overlap in the current period was 0.32 according to statistic D and 0.64 according to statistic I.

As regards the relationship between species distribution and T<sub>max</sub>, the PNOs showed a predilection for low temperatures for all the three species, more evident for carabid species than for the snail (Fig. 3a). Future projections showed a progressive increase in both niche overlap D and I statistics of the two beetles (Fig. 3b,c), and the spatial overlap progressively increases according to all scenarios (Fig. 3d).



**Fig. 2.** Ecological niche models projected in the current period for a) *Carabus cychroides*, b) *Carabus depressus*, c) *Chilostoma glaciale*, and d) spatial overlap between *C. cychroides* and *C. depressus* in the Cottian Alps Protected Areas.



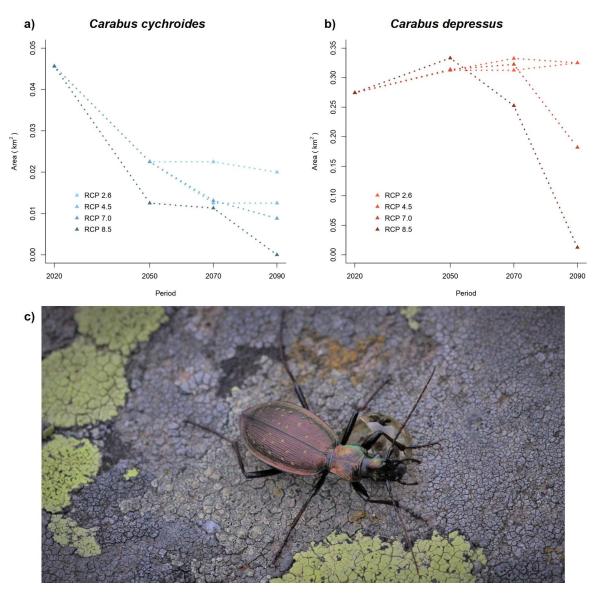
**Fig. 3.** Overlap between the two *Carabus* species. Designations: a) PNOs on  $T_{max}$  of the three species; b) niche overlap Schoener's D statistics of the two beetles; c) niche overlap Hellinger's I statistics of the two beetles; d) spatial overlap of *Carabus depressus* on *C. cychroides*.

Furthermore, the projections showed a rapid decline of *Carabus cychroides* toward its extinction in the study area in 2090 according to the more pessimistic greenhouse gas emissions scenario (Fig. 4a). On the other hand, the projection for *C. depressus* showed an increase in the occupied area in the first periods until its extinction in 2090 based on the more pessimistic scenario (Fig. 4b).

#### Discussion

The produced models underline the close link between low temperatures and the distribution of *Carabus cychroides*. Despite its prey show a wide distribution, this species could be able to exploit this food resource only where climatic conditions are sufficiently cold. *Carabus depressus* also appears to be related to colder areas, albeit to a lesser extent than *C. cychroides*.

The projections indicate a rather niche and spatial overlap between both Carabus species in the current period. However, as temperatures rise, the suitable area of Carabus depressus will increasingly overlap that of C. cychroides. According to the future projections, the two beetles will be more in contact in the study area, and therefore hybridisation between them could become more frequent. In addition, the expected emerging trends indicate a rapid decline of C. cychroides in line with the previsions on the entire geographical range (Anselmo & Rizzioli, 2021) and, at the same time, an increase (at least in the initial periods) of C. depressus. In this regard, hybridisation is considered a threat, especially for rare species that come into contact with other more abundant species (Rhymer & Simberloff, 1996; Allendorf et al., 2001).



**Fig. 4.** Predicted trends of the area occupied by a) *C. cychroides* and b) *C. depressus*; c) predation of *Chilostoma glaciale* by *Carabus depressus* observed in the study area (22.05.2016).

The increasing overlap could also concern the competition for *Chilostoma glaciale*, as *C. depressus* is able to feed on this snail species, even if it is not specialised like *C. cychroides* (Fig. 4c). The habitat loss and fragmentation can further advantage *C. depressus*, as it is a more generalist species (Jonsen & Fahrig, 1997; Warren et al., 2001; Nordén et al., 2013; Ramiadantsoa et al., 2018).

For future projections we assumed that the vegetation will remain constant over time. This is to be considered highly unlikely, as it is known that warming is affecting the distribution of plants in the Alps (Lenoir et al., 2008; Pauli et al., 2012; Steinbauer et al., 2018). The expected altitude rise of the treeline could further benefit *C. depressus*, as this species also inhabits forested environments (Casale et al., 1982).

### **Conclusions**

The upward shift is the consequence of warming for many mountain invertebrate species (Devictor et al., 2012; Platts et al., 2019; Termaat et al., 2019), causing the rarefaction and fragmentation of populations, including Carabus cychroides (Anselmo & Rizzioli, 2021). The obtained results indicate the presence of additional collateral threats related to the climate change on C. cychroides in the study area. If these threats occur over the entire geographical range of this species, they could accelerate its predicted decline in the coming decades caused by warming (Anselmo & Rizzioli 2021). Given the problems that arise from the conservation of this endangered species, we consider very important to limit further anthropogenic impacts on the microrefugia occupied and which will be occupied in the future by *C. cychroides*.

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# ПОБОЧНЫЕ УГРОЗЫ: ДАЛЬНЕЙШЕЕ ВОЗМОЖНОЕ ВЛИЯНИЕ ПОТЕПЛЕНИЯ НА УЗКОГО ЭНДЕМИКА АЛЬПИЙСКИХ ВЫСОКОГОРИЙ, *CARABUS CYCHROIDES* (COLEOPTERA: CARABIDAE)

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Carabus cychroides — эндемичный вид жужелиц, известный на нескольких высокогорных склонах Коттских Альп. Мы смоделировали данные о присутствии-отсутствии, собранные на территории исследования, относящиеся к этому виду, а также родственному ему виду Carabus depressus, с которым он может гибридизировать, и улитке Chilostoma glaciale, специфической добыче C. cychroides. Модели были спроецированы на три периода будущего в соответствии с четырьмя сценариями выбросов парниковых газов. Результаты показали увеличение в будущем перекрытия ниш и пространственного распределения обоих видов Carabus, что может привести к большей частоте гибридизации и конкуренции между ними. Если эти угрозы возникнут во всем географическом ареале C. cychroides, уже находящегося под угрозой исчезновения, они могут ускорить прогнозируемое сокращение численности популяции в ближайшие десятилетия из-за потепления климата.

Ключевые слова: гибриды, изменение климата, Коттские Альпы, перекрытие ниши, эндемизм