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Research

Artificial Feeding and Laboratory Rearing of Endangered Saproxylic Beetles as a Tool for Insect Conservation

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Abstract

Conservation of threatened animals is frequently limited by lack of knowledge about their ecological preferences, and often artificial feeding is one of the few chances to save endangered species. We investigated the possibility to artificially feed two endangered flat bark beetles dependent on dead wood for their diet—namely, *Cucujus cinnaberinus* (Scopoli, 1763) and *Cucujus haematodes* Erichson, 1845—by examining their dietary preferences, life cycle duration, and survival in laboratory conditions. Individuals of the two species were caught in the wild and larvae and adults were fed in laboratory conditions by live or dead prey. Three species of saproxylic beetles: two cerambycids (*Acanthocinus griseus* Fabricius, 1793 and *Rhagium inquisitor* Linnaeus, 1758), one scolytid (*Ips sexdentatus* Börner, 1776) one tenebrionid (*Tenebrio molitor* (Linnaeus, 1758)) one dipteran (*Lucilia sericata* (Meigen, 1826)) and one ant (*Lasius* sp. Fabricius, 1804) were used as prey, with minced meat as a control. Our results indicated high survival and no difference in prey choice between the two flat beetle species. Larvae and adults preferred dead prey, but no significant preference was detected among dead prey taxa, supporting the hypothesis that the two species are opportunistic scavengers. Comparing data with previous results, both species and their developmental stages should be classified as obligate saproxylic organisms with preference to the dead and decaying organic material. Successful artificial feeding and rearing of these endangered species, followed by the release in the wild through rescue or reintroduction programs, therefore appear relevant for their protection and future conservation.

Graphical Abstract

- We observed high survival of two endangered saproxylic beetles *Cucujus cinnaberinus* and *C. haematodes* during artificial feeding.
- There was no difference in prey choice between these two flat beetle species.
- Larvae and adults preferred dead prey and no significant preference was detected among dead prey taxa.

Key words: diet preference, life cycle, *Cucujus cinnaberinus*, *Cucujus haematodes*, conservation

Many species in the world are presently facing decline and are therefore listed in the IUCN Red List of Endangered species (Cálix et al. 2018). Probably, the best way to prevent their loss and conserve their populations is the sustainable management of their habitats (Horák et al. 2012a). Nevertheless, in some cases, there is no possible way to protect the species in nature in a particular place (Conway 1995), but it is possible to preserve or repopulate the species in the wild or maintain them in zoological and botanical gardens (Kolter and Zimmermann 1988). When insect species are involved, it is also possible to raise them in laboratory conditions and improve their diet before returning them to natural habitats (Inoda et al. 2009). Among species facing the decline of their wild populations, are those strictly dependent on dead wood, such as saproxylic beetles (Grove 2002).

These species use decaying wood harboring other arthropods, plants, and fungi (Alexander 2008). Many beetles are generalists about their prey (Nieto and Alexander 2010), while others are specialized predators and scavengers. In this case, it is often difficult to establish whether the prey is consumed alive or dead. Within the large community of saproxylic insects, the diets of many taxa are still unknown, and few have been investigated for this biological aspect (Hilszczański et al. 2014, Yi et al. 2017). The genus *Cucujus* F. is composed of 12 species distributed throughout the Holarctic region and in Asia (Lee and Putz 2008, Horàk and Chobot 2009). Many endemic species have been recorded in China, Nepal, India, Taiwan, and Japan and, more recently from Asia, several new faunistic records have been reported (Hsiao 2020). In Europe, *Cucujus cinnaberinus* (Scopoli, 1763), *Cucujus haematodes* Erichson, 1845, *Cucujus clavipes* Fabricius, 1781 and *Cucujus tulliae* Bonacci, Mazzei, Horàk, and Brandmayr, 2012 have been described. To help in the identification of these rare and endangered species in literature, a set of characteristics for the adults and larvae were proposed (Palm 1941, Mamaev et al. 1977, Bonacci et al. 2012, Gutowski et al. 2014) that were observable in the field (Gutowski et al. 2014) and laboratory.

The genus *Cucujus* Fabricius, 1775 (Insecta: Coleoptera, Cucujidae) includes many threatened species throughout the world (Horák and Chobot 2009), mainly endangered because of restricted habitats. Moreover, some species of this genus are known as candidates for umbrella species recognition (Mazzei et al. 2011, 2018). Although the *Cucujus* species are generally considered predators (Essig 1926, Peterson 1951, Arnett 1968, Borror and White 1970, Swan and Papp 1972, Borror et al. 1976), up to now, no direct observations and reliable data have been reported on their foraging behavior.

In the mountains of the Region of Calabria (southern Italy), the *Cucujus* larvae complete their life cycle under the bark of deadwood, especially logs of *Pinus nigra* and *Pinus nigra laricio* (Poir.) Maire (Pinophyta: Pinaceae) (Fig. 1) and *Fagus sylvatica* L. 1753 (Magnoliopsida: Fagaceae) (Anderson and Nilssen 1978, Bonacci et al. 2012). Flat bodies of larvae and adults are well adapted for the narrow subcortical environment of dead trees (Bonacci et al. 2018). Larvae hide under the bark and the emerged adults spend most of their life on the trunks or logs and inside bark crevices where they feed and reproduce (Straka 2017).

Recent studies have been focused on organisms associated with the habitats of two flat bark species—*C. cinnaberinus* (Scopoli) and *C. haematodes* (Erichson) (Coleoptera: Cucujidae) (Horák et al. 2010, 2011)—to clarify the role of these beetles in the under bark environment. The *Cucujus* species have been considered scavengers that only occasionally prey on larvae and pupae of other beetles (Mamaev et al. 1977). Other studies showed facultative predatory behavior in adults and cannibalistic habits in larvae (Ślipiński 1982, Smith and Sears 1982). Based on the plant and fungal material found in their gut, *Cucujus* larvae have also been considered omnivorous (Lawrence 1991). Recently, adults of *C. cinnaberinus* have been reported as opportunistic omnivores (Přikryl et al. 2012). A more detailed knowledge based on experimental evidence about foraging and dietary preferences of these two endangered *Cucujus* species is, therefore, necessary for the correct management and conservation of their populations.

Aims

To increase knowledge about dietary preferences in two endangered species of the genus *Cucujus*, *C. cinnaberinus* and *C. haematodes,* experimental procedures were devised to test preference of adults and developmental stages for live or dead prey involving other insects. The following differences were examined:

1. Between species (interspecies and intraguild competition hypothesis);
2. Between larvae and adults (intraspecies competition hypothesis);
3. Preference of live or dead prey (functional trait hypothesis);
4. Preference for artificial or natural diet (prey selection hypothesis).

Materials and Methods

Sampling

All larvae were collected in forest sites of Natura 2000 Special Areas of Conservation of the Sila National Park (Calabria, Southern Italy) from May 2018 to May 2019. The under bark environment of suitable dead pine trees, either fallen or standing, was carefully inspected. In the field, larvae were collected of both species of different sizes and different ages. For this study, 120 selected larvae of both species at instars four and five (*C. cinnaberinus*, $n = 60$ and *C. haematodes*, $n = 60$) were manually chosen and placed in separate tubes and carried to the laboratory in a cooler at < 15°C. The average length of tested *C. cinnaberinus* larvae was 21.76 ± 1.15 mm; the average length of the tested *C. haematodes* larvae was 22.81 ± 0.32 mm. The adults used in the tests emerged in the laboratory from the collected larvae after pupation.

Laboratory Conditions

The larvae and adults of the studied species were brought to the entomological laboratory of Department of Biology, Ecology and Earth Sciences (University of Calabria, Arcavacata di Rende, Cosenza, Italy) individually placed in glass containers on a natural substrate (deadwood and bark) and stored in a thermostatic chamber at a constant temperature (15°C), 12:12 L: D photoperiod, under controlled humidity values (90–95%). Previous breeding investigations of the studied species in the laboratory suggest that the temperature of 15°C is optimal for the development and survival of immature individuals.

Before the experiments, the larvae were fed once a week exclusively with minced meat, considered suitable as food for laboratory

Fig. 1. High diameter logs of the Calabrian pine (*Pinus nigra*) are the most suitable habitat for two species of flat bark beetles (*C. cinnaberinus* and C. *haematodes*), where they find suitable prey (Sila National Park, Calabria, Italy).

rearing based on a previous study (Bonacci et al. 2018). A total of 120 larvae and a total of 48 emerged adults (*C. cinnaberinus* = 24 and *C. haematodes* = 24) were used for experiments on dietary preferences. Finally, all adults and larvae were released back in the sampled sites in the Sila National Park.

Feeding Experiments

To analyze the foraging behavior, food preference assays were performed on larvae and adults according to the protocol (Table 1). Three species of saproxylic beetles, one tenebrionid, one dipteran species at first instar (used in place of associated and unidentified soft-bodied larvae of Diptera at the collecting sites), and one ant genus were used as prey, with minced meat (mixed beef and pork) as the control. Namely, laboratory-reared larvae of common green bottle fly, *Lucilia sericata* (Meigen, 1826; Diptera: Calliphoridae) and larvae of mealworm beetle *Tenebrio molitor* (Linnaeus, 1758; Coleoptera: Tenebrionidae), and larvae and pupae of three saproxylic beetles species collected in the same microhabitat of the *Cucujus* larvae: longhorn beetle (*Acanthocinus griseus*, Fabricius, 1793; Coleoptera: Cerambycidae), bark beetle (*Ips sexdentatus*, Börner, 1776; Coleoptera: Scolytidae), and pupae of ribbed pine borer (*Rhagium inquisitor*, Linnaeus, 1758; Coleoptera: Cerambycidae) and pupae of an ant (*Lasius* sp., Fabricius, 1804; Hymenoptera: Formicidae) were used as potential prey.

All prey species were offered as live and dead (Table 1). The sets of living prey were indicated as 'lp' and those of dead prey as 'dp'. The live preys were kept in the laboratory under the same conditions as the studied species but in separated boxes.

The *L. sericata* larvae were reared on pork liver in the laboratory at 24°C (Humidity of 50–60%; 12:12 L: D photoperiod) and larvae of *T. molitor* were reared in a box with flour and pieces of bread inside a climate chamber at 22°C (Humidity 20–25%; 12:12/L:D photoperiod). The dead prey (dp) was killed by freezing at −20°C.

Laboratory Tests

For the experiment on prey preferences, larvae and adults of *C. cinnaberinus* or *C. haematodes* were tested individually and each individual was not fed for 3 d before testing. Feeding analysis of beetles was carried out by offering prey inside an experimental arena that consisted of a Petri dish 8 cm in diameter, suitably moisturized. Preys were put in the Petri dish according to the sets listed in Table 1. The recording of the behavior (foraging reaction on offered prey) started after placing the *Cucujus* individuals. The arrangement of the different prey in the arena was random. Each individual of *Cucujus* was tested only once.

Each test lasted 60 min and was recorded with a Sony compact digital camera DSC-HX60V (Sony, Minato, Tokyo, Japan) or by using a Canon digital camera EOS 450D (Ōta, Tokyo, Japan). The mean choice latency in adults and larvae and the mean duration of prey consummation were also recorded.

Based on the discovery of plant and fungal material (Lawrence 1991) in the gut of *Cucujus* larvae and to verify in laboratory conditions whether *Cucujus* spp are interested in feeding on the fungi discovered under pine bark, a preliminary analysis was carried out on larvae at instars four and five (n = 15 larvae of *C. haematodes* and n = 15 larvae of *C. cinnaberinus*) and adults (n = 7 adults of *C. haematodes* and n = 7 adults of *C. cinnaberinus*). For these trials, larvae and adults of *C. cinnaberinus* or *C. haematodes* were tested individually, and each individual was not fed for 3 d before testing. Inside the arena, only fungal material was supplied, and each individual of *Cucujus* was tested only once for 60 min. All observations were carried out in natural light conditions.

Table 1. Description of prey combination sets used to test feeding preferences of larvae and adults of the two flat bark beetles *C. cinnaberinus* and *C. haematodes*

Studied species	Developmental stage	Prey stage	Prey set	Prey combination
Cucujus cinnaberinus	Larva	Live	lp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			lp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
		Dead	dp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			dp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
	Adult	Live	lp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			lp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
		Dead	dp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			dp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
Cucujus haematodes	Larva	Live	lp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			lp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
		Dead	dp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			dp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
	Adult	Live	lp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			lp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat
		Dead	dp1	*A. griseus* + *I. sexdentatus* + *Lasius* + meat
			dp2	*L. sericata* + *T. molitor* + *R. inquisitor* + meat

The sets of living prey are indicated as 'lp' and dead prey as 'dp'.

Biology and Species Survival Analysis

The analysis of complete development from the eggs to the emergence of the adults of both species was recorded in laboratory conditions. The study was carried out on a different set of larvae ($n = 57$ larvae of *C. cinnaberinus* and $n = 48$ larvae of *C. haematodes*) collected in forest sites of the Sila National Park (Calabria, Southern Italy) from April 2016 to June 2019. We observed the mating behavior between a couple of *C. cinnaberinus* and *C. haematodes* in the laboratory and examined the duration of the complete life cycle from larvae at the first stage to adult emergence. The survival rate of the preimaginal stages was also examined. The experimental temperature was 15°C.

Statistical Analyses

All statistical analyses were performed using the SPSS (Statistics vers. 25 SPSS). The nonparametric Kruskal–Wallis test was employed to indicate differences in the prey choices by the adults and larvae in all feeding sets (larvae vs *lp1- lp2* and larvae vs *dp1- dp2*; adults vs *lp1- lp2* and adults vs *dp1- dp2*). The food preference was evaluated using the formulae for the preference index by Manly (1974).

$$\beta_{1(lp1-lp2)} = \text{Log}\,(e_1/A_1)\,/\text{Log}\;(e_1/A_1) + \text{Log}\,(e_2/A_2)$$

$$\beta_{2(dp1-dp2)} = \;\text{Log}\;(e_2/A_2)\,/\text{Log}\;(e_2/A_2)\, + \text{Log}\;(e_1/A_1)$$

where $\beta_{1(lp1\text{-}lp2)}$ is the preference (respectively for *Cucujus* larvae and adults) to prey set 1 (*lp1-lp2* live prey), and $\beta_{1(dp1\text{-}dp2)}$ is the preference (respectively for *Cucujus* larvae and adults) to prey set 2 (*dp1-dp2* dead prey), e_1 and e_2 are the number of prey set 1 and set 2 remaining after the experiment. A_1 and A_2 are the number or prey set 1 and 2 presented to the studied species (predators).

When the preference index $\beta_{1(lp1\text{-}lp2)}$ is close to 1, the studied species prefers prey set 1, and when it is close to 0, the prey set 2 is preferred. When the preference index $\beta_{2(dp1\text{-}dp2)}$ is close to 1, the studied species prefers prey set 2, and when it is close to 0, the prey set 1 is preferred. An index value close to 0.5 indicates no preferences (Cock 1978, Sherratt and Harvey 1993).

Results

Prey Preference

Grouping the recorded data, no significant difference was observed in prey choice between the studied species (*C. cinnaberinus* vs *C. haematodes*; $\chi^2 = 0.63$; gl = 1; $P = 0.427$) and between adults and larvae ($\chi^2 = 1.87$; gl = 1 $P = 0.171$).

Larvae (Fig. 2) significantly preferred prey set 2 (i.e., dead prey) than prey set 1 (live prey;$\chi^2 = 243.37$; df = 12; $P < 0.001$) and the preference indices for *Cucujus* larvae: $\beta_2 \pm SE = 0.83 \pm 0.04$ (*Cucujus* larvae with dead prey) compared to: $\beta_1 \pm SE = 0.37 \pm 0.04$ (*Cucujus* larvae with live prey) shows the preference of beetles for dead invertebrates.

Adults (Fig. 2) significantly preferred dead prey (dp1 and dp2) rather than live (lp1 and lp 2; $\chi^2 = 297.94$; df = 12; $P < 0.001$). The preference indices for *Cucujus* adults: $\beta_2 \pm SE = 1 \pm 0.02$ (*Cucujus* adults with dead prey) compared to $\beta_1 \pm SE = 0.28 \pm 0.02$ (*Cucujus* adults with live prey) shows also for the adults a clear preference for dead prey. The mean choice latencies in the *Cucujus* spp. larvae were 4 ± 2.5 min and 3 ± 5.3 min in the *Cucujus* spp. adults. Once chosen, the prey was consumed on average for 18 ± 5 min in the larvae and 16 ± 3 min in the adults. Our preliminary analysis focused on the feeding behavior of adults and larvae of studied species showed that in the laboratory the examined individuals refused fungal material as a food source.

Biology and Effects of Artificial Rearing on Species Survival

In the course of our observations focused on the study of the life cycle of *Cucujus* spp. in laboratory conditions, we evaluated the duration of complete development from eggs to adult emergence. The length of the complete life cycle for *C. cinnaberinus* was 218 ± 8.3 d. The length of complete development for *C. haematodes* was 222 ± 7.2 d.

The mean duration of the mating in *C. cinnaberinus* was 19.3 ± 2.4 min, and in *C. haematodes,* it was 18.7 ± 3.5 min. We found a high percentage of the success of the adult emergence of both species (85% on average). The highest mortality was observed in the first stages (I and II stages) for both studied species.

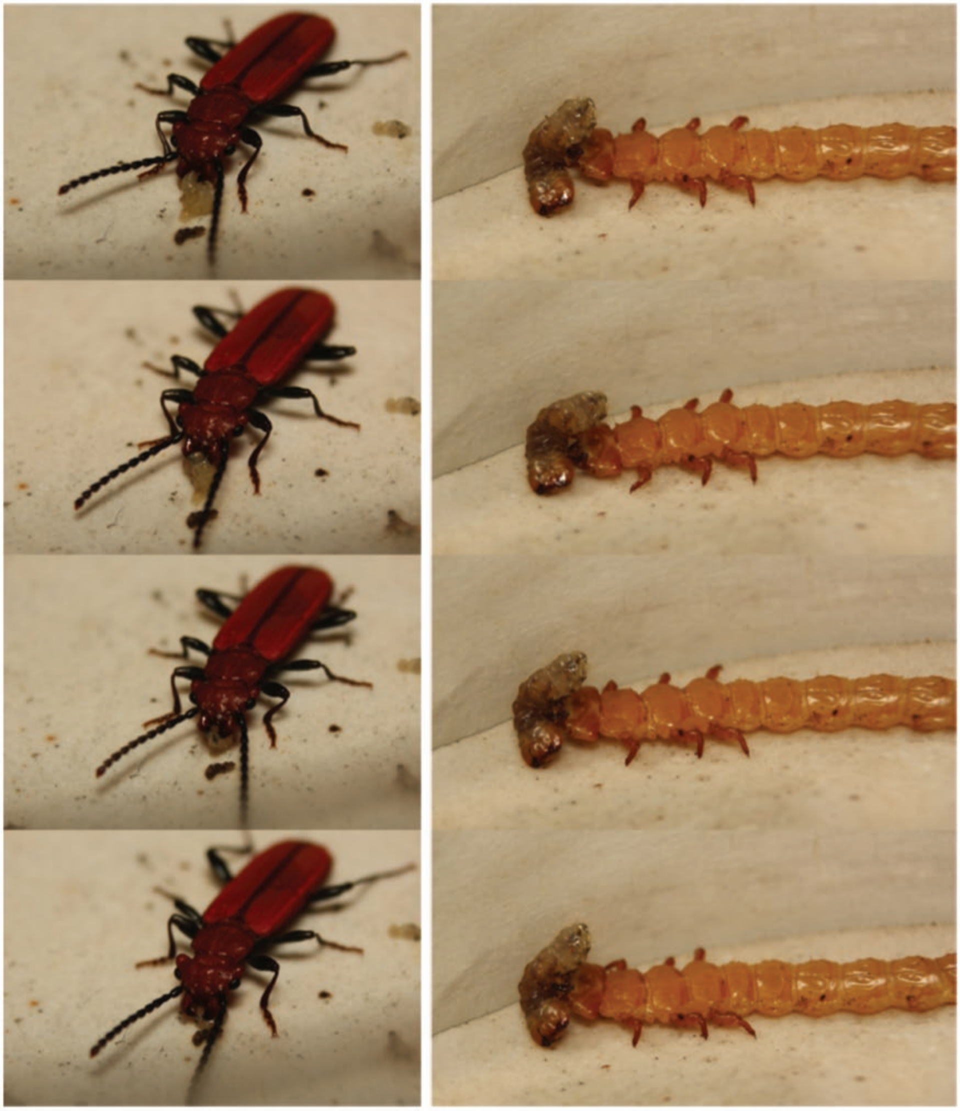

Fig. 2. Chronosequences of the foraging behavior of an adult *C. haematodes* (on the left) foraging on a dead larva of *L. sericata* reared in the laboratory. Larva of *C. cinnaberinus* (on the right) foraging on a dead larva of *R. inquisitor*.

Discussion

The results of the study indicated that the rearing of two IUCN red-listed (endangered) species of flat bark beetles (*C. cinnaberinus* and *C. haematodes*) from larvae to adults is possible in laboratory conditions and both species at the examined developmental stages clearly prefer dead prey. This information appears to highly relevant for management and the conservation of the species.

Both larvae and adults of *Cucujus* prey on dead individuals, refusing living ones. It is likely that the refuse to forage on live individuals due to the active movement of live prey. These findings partially support previous data considering the *Cucujus* species as scavengers only occasionally preying on live larvae and pupae of other beetles (Mamaev et al. 1977). The under bark environment is apparently populated by living individuals that could be potential prey, but in this subcortical environment, many trophic interactions may occur. Thus, for *Cucujus* species, it is possible to find eggs, dead larvae, pupae, and adults of other arthropod species.

We predicted that remnants of prey killed by other predator species and individuals killed pathogens might represent a suitable food for scavengers (e.g., Horák 2011). In some periods of the year (e.g., in winter), this food availability could be very important for the survival of *Cucujus* individuals, especially when low temperatures and snow limit the food search. *Cucujus* larvae are known to be highly freeze-tolerant (Sformo et al. 2010). Therefore, they can move all year long and search for prey killed by extreme abiotic conditions (e.g., low temperatures).

The investigated *Cucujus* species spend their immature life stages exclusively under bark, slowly moving inside this microhabitat until pupation and adult emergence. In addition, the adults spend most of their life inside the trunk crevices (Horák and Chobot 2011), where they lay the eggs after the adults mate (Bonacci, personal observations).

Since in nature the developmental time of *Cucujus* beetles is over a year long, the choice of dead prey (belonging to the scavenger functional group) can be more advantageous than predation. The choice of prey taxa in this study was based on the recent data indicating that the community associated with *C. cinnaberinus* and *C. haematodes* included species of the family Scolytidae, ants of the genus *Lasius* and dipteran larvae (Horák et al. 2012b, Horák 2015, Mazzei et al. 2018). A historical study also mentioned the presence of these arthropods in the environment of *C. cinnaberinus* (Palm 1941). The absence of significance in the choice of sets of dead prey indicated opportunistic feeding behavior on dead arthropods. Our investigations showed that larvae and adults of *Cucujus* spp. in the laboratory refused fungi as a food source.

These results agree with previous data indicating *Cucujus* preference of decaying bast (phloem; Přikryl et al. 2012) and may also explain why fungi are mentioned as a food in the study; as insect pathogens, fungi may directly cause death or develop on dead arthropods immediately after death. Beetles may therefore accidentally ingest the hyphae while feeding on dead individuals covered by moulds and other fungal species.

In conclusion, the data obtained in this study support the hypothesis that flat bark beetles may feed on dead insects (Horák 2011). The analysis of food preference in laboratory conditions showed a statistically significant preference for dead prey in comparison to living ones, supporting the scavenger feeding habits of adults and larvae of genus *Cucujus*. Therefore, the species is most probably saprophagous in the wild. However, many other aspects of the biology and behavior of these endangered beetles still require detailed investigation.

Species Conservation in the Wild

The two flat bark beetle species examined in this study live in highly similar microhabitat (Horák et al. 2011, 2012b; Mazzei et al. 2018, Jaworski et al. 2019). However, *C. cinnaberinus* probably has adapted to lands affected by anthropogenic uses (Jaworski et al. 2019), and it exhibits a higher dispersal ability (Della Rocca and Milanesi 2020). Therefore, *C. haematodes* appears to be affected by the conversion of traditionally managed forests into tree plantations (Jaworski et al. 2019), while *C. cinnaberinus* apparently manages to survive, with some limits, in less favorable conditions (Bełcik et al. 2019)

The main problem concerning the conservation of these beetle species is that potentially dangerous trees and their remnants (e.g., snags or high stumps) in the European landscape are frequently removed. These trees in open areas (such as riparian greenings or old avenues) or urban locations (e.g., parks) are under threat (Horák 2018). Such trees are sometimes inhabited by the two studied species (especially, *C. cinnaberinus*), and their larvae are often gregarious and abundant in one microhabitat (Mazzei et al. 2011). Therefore, the loss of these larval aggregations may be unfavorable for the local population of these beetles species, protected in pan-EU Natura 2000 network (https://www.eea.europa.eu/themes/biodiversity/natura-2000). To avoid loss of the population of these species when the inhabited tree is felled and has to be replaced or destroyed (e.g., due to public safety or other reason), the option for the larvae is their relocation in the laboratory and rearing by artificial feeding. This study shows for the first time that the rescue of the local populations of the two beetle species is possible through laboratory rearing and provides new data about diet preferences, life cycle duration, and mortality. Artificial feeding may be successfully applied for population increase or reintroductions. In conservation projects, the laboratory rearing of *Cucujus* individuals for future release could be very useful in rescue programs or reintroduction to habitats where the species have disappeared.

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