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First record of oviposition scars in two European *Platycerus* species: *P. caprea* (De Geer, 1774) and *P. caraboides* (Linnaeus, 1758) (Coleoptera: Lucanidae)

Davide SCACCINI

Via A. Moro 25, Zelo Buon Persico, Lodi, Italy (e-mail: davide.scaccini@alice.it)

Abstract

During sampling of *Platycerus* beetles in Northern Italy (2011-2015) and Belgium (April 2016), females of *P. caprea* and *P. caraboides* were observed to gnaw scars on the surface of deadwood during oviposition, similarly to East Asian species. This is the first record of such behavior for European *Platycerus*. More than 500 oviposition scars produced in both field and captivity conditions were examined and measured. The scars were never found in standing branches or trees, but only in fallen ones, and never onto the host's bark. Their major axis is perpendicular to the length of deadwood. Oviposition scars for both species can be roughly classified into three types: D-shaped, O-shaped, and droplet-shaped. In a "typical oviposition scar group" on the wood surface, three scars appear as follows: ᗡOD, one O-shaped scar with a D-shaped scar on either side of it, the left hand one is reversed. Droplet-shaped scars are often disposed in a random series and may enclose eggs as the previous ones. At the bottom of some D-shaped and droplet-shaped scars a jelly substance was found in one occasion.
In addition, any preference in diameter of the log as food source for larvae was investigated, showing differences between the two Lucanid species. Often, larvae of the two *Platycerus* species develop inside small logs, with 20 mm in diameter or less.
This study provides original information on the ecology and oviposition behavior for these two species, and underlines the possibility to establish an easy to use method for their monitoring.

Keywords: Deadwood, wood diameter, monitoring, behavior, stag beetles.

Introduction

Platycerus caprea (De Geer, 1774) and *P. caraboides* (Linnaeus, 1758) (Coleoptera: Lucanidae) are two widespread European saproxylic beetles (BALLERIO *et al.*, 2010; BARTOLOZZI *et al.*, 2016). They are red listed as Least Concern (LC) (NIETO & ALEXANDER, 2010) in Europe, as also reported in the Flanders Region (Endangered, THOMAES *et al.*, 2015) and in Italy (AUDISIO *et al.*, 2014). In some cases, in Italy these species are protected by special regional laws, such as the number 65/2000 for Tuscany (BARTOLOZZI & SFORZI, 2001).
Larvae of these two *Platycerus* species develop in deadwood of various tree species (*e.g.* KLAUSNITZER & KRELL, 1996; FRANCISCOLO, 1997). Females lay eggs during Spring/early Summer, and larvae develop through three larval instars up to late Summer, when they pupate and eclose; new adults overwinter in the pupal chamber to the subsequent Spring (*e.g.* MORETTO, 1984; FRANCISCOLO, 1997; HOLUŠA *et al.*, 2006).
Some aspects of the biology of these species are still poorly known, in particular concerning their oviposition behavior. Oviposition scars are known for different groups of beetles, such as Longhorn beetles, Cerambycidae (*e.g.* ANBUTSU & TOGASHI, 1996; 1997b; 2000; DODDS *et al.*, 2002; HAACK *et al.*, 2010; TURGEON *et al.*, 2010; HUSSAIN & BUHROO, 2012) and Lizard beetles, Erotylidae Languriinae (*e.g.* TOKI & TOGASHI, 2013; TOKI *et al.*, 2013; 2014).
In various species of stag beetles, Lucanidae, females have been reported to make oviposition scars (ARAYA, 1987; 1989; 1991). In particular, some Asian *Platycerus* species have been reported to gnaw

oviposition scars on deadwood (*e.g.* ADACHI, 1987; ARAYA, 1989; HUANG & CHEN, 2009; IMURA, 2010), and here this behavior is reported for the first time for the European species *P. caprea* and *P. caraboides*.

In Asian *Platycerus* species there seem to be also a preference of oviposition sites related to the diameter of branches/trunks where oviposition occurs. ARAYA (1993) reported differences in diameter of the food source for two Japanese *Platycerus* species: *P. acuticollis* Kurosawa, 1969 and *P. delicatulus* Lewis, 1883. Also IKEDA (1987) wrote about diameter and other differences in decayed wood preferences for the previous two Asian species and for *P. kawadai* Fujita & Ichikawa, 1982.

Monitoring of stag beetles is important because the presence of several species is considered as an ecological indicator (LACHAT *et al.*, 2012). *P. caraboides* occurrence does not depend on the amount of available deadwood, and its presence is an indicator for sites with higher temperatures than *P. caprea* (LACHAT *et al.*, 2012). In Italy, *P. caraboides* is often observed at lower altitude than *P. caprea* (*e.g.* FRANCISCOLO, 1997; MELLONI & LANDI, 1997). *P. caprea* is reported for cooler sites, and with the presence of higher amount of deadwood (LACHAT *et al.*, 2012), preferring in general higher altitudes (KLAUSNITZER, 1995; FRANCISCOLO, 1997).

The aim of this study is to report the presence of oviposition scars of *P. caprea* and *P. caraboides* and, at the same time, to provide data about their shape, size and other features. The function of oviposition scars for these species is also briefly discussed. Furthermore, any preference related to the oviposition log was investigated, showing differences in deadwood diameter between the species.

Material and methods

From 2011 to 2015 various rotten branches and trunks were collected in several places in Northern Italy. Some branches with oviposition scars were also collected in the Sonian forest, Flanders, Belgium, during the 9th Symposium on the Conservation of Saproxylic Beetles (Genk, 21st-24th April 2016), on 21st April 2016 (data not included in statistical analysis of the present study) (Fig. 1). Oviposition scars are also recently observed in the Meerdaal forest (near Leuven) by A. Thomaes (pers. comm.).

About 150 larvae (Figs 6 and 9), pupae, and adults (Fig. 1) (but no eggs) of the two Italian *Platycerus* species found inside the decaying wood were identified by using available keys. For larvae, species identification was made following special diagnostic characters reported by HŮRKA (1975), FRANCISCOLO (1997) and SCACCINI (2015b), not including their head capsule width, which can lead to misclassification (SCACCINI, 2015a). Pupae were reared to obtain adults to check the identification.

More than 500 oviposition scars produced by the two species in both field and captivity conditions were examined. The maximum height, width and depth of each scar was measured with a caliper (Fig. 2). The distances between scars were also measured.

In addition, the diameter of the food source (represented by decaying wood showing the presence of individuals) was also measured. Data were analyzed using SPSS (Statistical Package for Social Science).

Results

Females of *P. caprea* and *P. caraboides* have been observed to gnaw oviposition scars (Fig. 3) mainly in white-rot wood with an adequate moisture content and in advanced stage of decay. Gnawing onto the host's bark has never been observed, scars always occurred on wood parts free from the bark cover.

Scars for both species can be roughly classified into three types: D-shaped, O-shaped, and droplet-shaped (Figs 1-2 and 4-14 respectively). On the surface of the wood, the main axis of the scar is always perpendicular to the wood length.

In a "typical oviposition scars group" on the wood surface, three scars appear as follow: ᗡOD, one D-shaped scar at either side of an O-shaped one, the left hand D-shaped scar is reversed (Figs 1- 2, 4, 7-8).

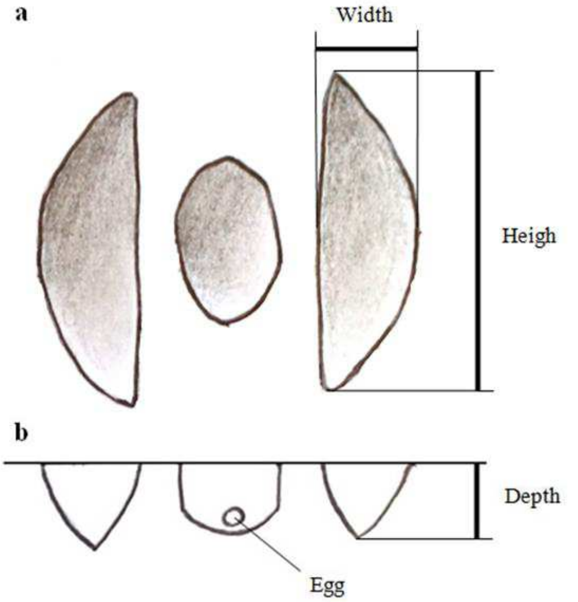

Fig. 1. A male of *P. caraboides* on the deadwood in which it was found, in the Sonian forest, Flanders, Belgium (April 2016). There are visible oviposition scars. Scale bar: 5 mm. Photographed by M. Fremlin.

Fig. 2. Diagram of a "typical oviposition scars group" of *Platycerus* spp. Top view, indicating the maximum width and height (a). Vertical section through the center indicating the depth; the O-shaped scar contains an egg (b).

Fig. 3. A female of *Platycerus caprea* gnawing a scar on moist deadwood, in April. Piacenza province (Northern Italy). Scale bar: 5 mm.

Fig. 4. Oviposition scars of *P. caprea* with some "typical oviposition scars groups". Piacenza province (Northern Italy), abt. 800 m a.s.l., April 2015. Scale bar: 5 mm.

Fig. 5. Big oviposition scars of *P. caprea*. Bergamo province (Northern Italy), abt. 1000 m a.s.l., December 2015. Scale bar: 5 mm.

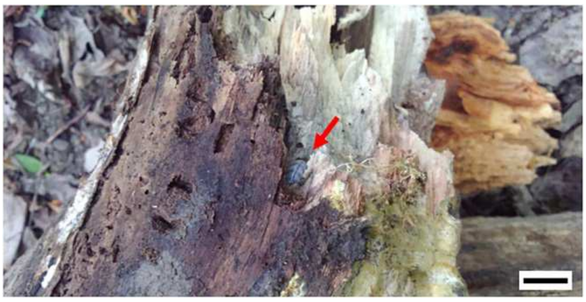

Fig. 6. Oviposition scars on a big piece of deadwood. The arrow indicates a larva of *P. caprea*. Pavia province (Northern Italy), abt. 1000 m a.s.l., July 2015. Scale bar: 5 mm.

Fig. 7. Oviposition scars of *P. caraboides* also with a "typical oviposition group", on the right. Piazzolo, Bergamo (Northern Italy), abt. 750 m a.s.l., November 2014. Scale bar: 5 mm.

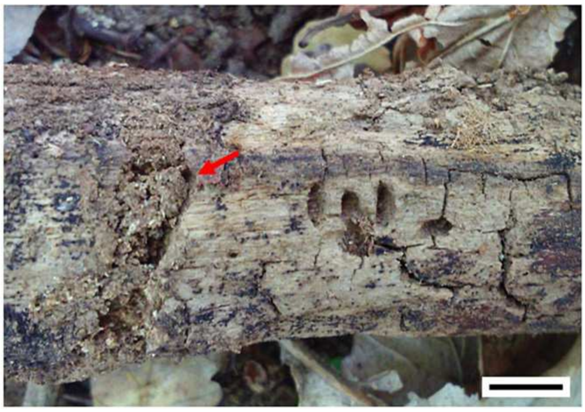

Fig. 8. Oviposition scars of *P. caraboides* in a "typical oviposition group". Tunnels of a mature larva are visible (arrow). Piacenza province (Northern Italy), abt. 850 m a.s.l., April 2015. Scale bar: 5 mm.

Fig. 9. Oviposition scars of *P. caraboides*, with a third instar larva found inside the log. Piacenza province (Northern Italy), abt. 700 m a.s.l., April 2015. Scale bar: 5 mm.

Fig. 10. Very old oviposition scars of *P. caraboides*. Piazzolo, Bergamo (Northern Italy), abt. 750 m a.s.l., November 2014. Scale bar: 5 mm.

Fig. 11. Various droplet-shaped oviposition scars of *P. caprea* on a big branch of *Fagus sylvatica*, in captivity. Scale bar: 5 mm.

Fig. 12. Various droplet-shaped oviposition scars of *P. caraboides* on a small log. Bergamo province (Northern Italy), abt. 750 m a.s.l., November 2014. Scale bar: 5 mm.

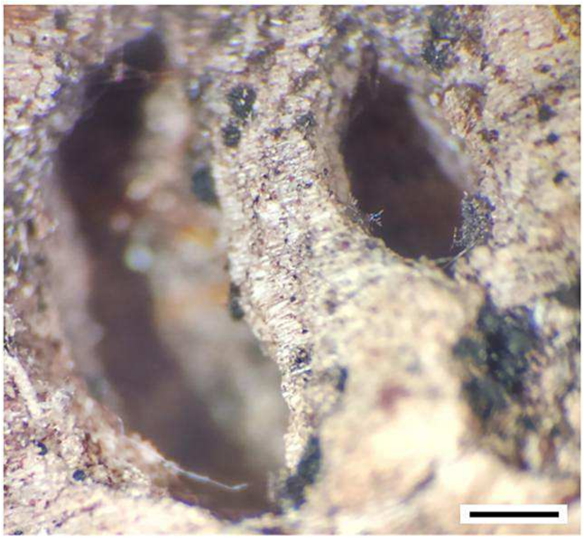

Fig. 13. Oviposition scars of *P. caprea* photographed on a stereomicroscope; D- and O-shaped ones. Scale bar: 1 mm.

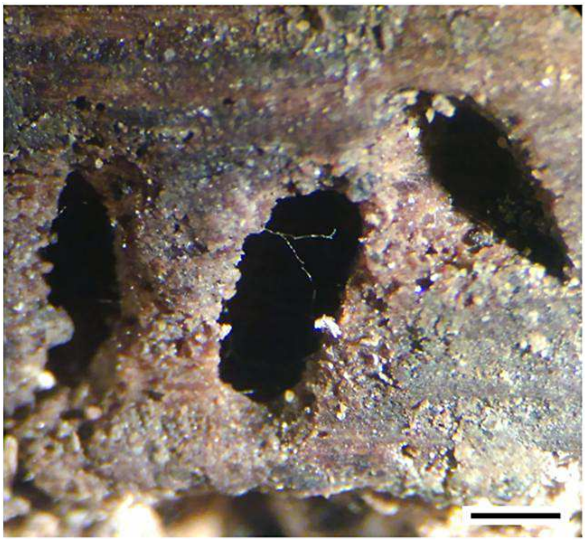

Fig. 14. Oviposition scars of *P. caraboides* photographed on a stereomicroscope. Scale bar: 1 mm.

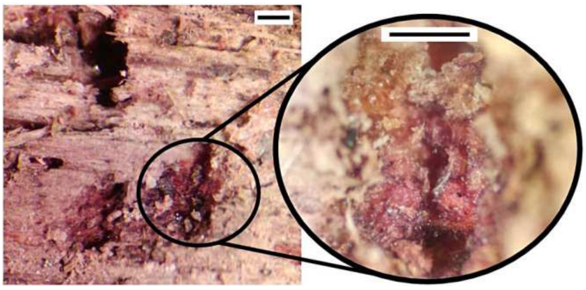

Fig. 15. Two opened oviposition scars of *P. caraboides*, showing a jelly substance at their bottom. Deadwood collected in Piacenza province (Northern Italy), November 2015. Scale bar: 1 mm.

Fig. 16. Attempted oviposition scars on a hard deadwood, probably by *P. caprea*. Bergamo province (Northern Italy), abt. 900 m a.s.l., December 2015. Scale bar: 5 mm.

No eggs were found in this survey, but young larvae were collected in the wood near these scars during the oviposition periods of the two Italian *Platycerus*. Droplet-shaped scars are often disposed in random series (Figs 11-12) and could enclose eggs as the O-shaped ones, one for each scar (*e.g.* ARAYA, 1989; IMURA, 2010). These scars could represent just "incomplete oviposition scars groups".

Measurements of height, width and depth of scars (Fig. 2) are reported for *P. caprea* and *P. caraboides* in Table 1 and 2. Both Kolmogorov-Smirnov's and Shapiro-Wilk's tests show that data are not normally distributed, for each species regarding the scar type and the wood diameter. From the Mann-Whitney's *U* test, no differences between the two species are reported for each type of scar, for height, width or depth (Table 3).

Data regarding the diameter of the branches and trunks with oviposition scars varied a great deal, from 16 to 130 mm (Table 4). *P. caprea* has a significant preference for thicker branches (mean 51.70 ± 29.40 mm), compared to *P. caraboides* (mean 34.30 ± 16.90 mm) ($U = 561.5$, $p < 0.001$, $n = 95$) (Table 4).

Both species were found in deadwood of deciduous trees, for *P. caprea* mainly in beech (*Fagus sylvatica*) and other trees, and for *P. caraboides* in chestnut (*Castanea sativa*) and lime (*Tilia* sp.).

Finally, in one case a jelly substance was found at the bottom of D- and droplet-shaped scars (Fig. 15).

Table 1. Dimensions of *P. caprea* oviposition scars.

P. caprea	**Measures (mm)**											
Scar shape	**Height**				**Width**				**Depth**			
	Mean (± st. dev.)	**Min**	**Max**	***n***	**Mean (± st. dev.)**	**Min**	**Max**	***n***	**Mean (± st. dev.)**	**Min**	**Max**	***n***
D	3.92 (± 1.17)	1.40	7.40	126	1.88 (± 0.44)	1.00	3.60	126	1.99 (± 0.99)	0.40	5.50	107
O	2.60 (± 0.70)	1.60	4.80	39	1.76 (± 0.33)	1.00	2.50	40	1.89 (± 0.75)	0.50	3.90	30
Droplet	3.25 (± 0.79)	1.80	6.10	96	1.77 (± 0.35)	0.80	2.90	101	1.89 (± 0.77)	0.40	4.50	69

Table 2. Dimensions of *P. caraboides* oviposition scars.

P. caraboides	**Measures (mm)**											
Scar shape	**Height**				**Width**				**Depth**			
	Mean (± st. dev.)	**Min**	**Max**	***n***	**Mean (± st. dev.)**	**Min**	**Max**	***n***	**Mean (± st. dev.)**	**Min**	**Max**	***n***
D	3.87 (± 0.74)	1.80	5.70	154	1.86 (± 0.31)	1.00	2.70	155	1.77 (± 0.72)	0.30	4.20	123
O	2.74 (± 0.52)	1.70	4.20	52	1.85 (± 0.36)	0.80	2.60	52	1.81 (± 0.85)	0.20	4.50	37
Droplet	3.38 (± 0.74)	2.00	5.50	112	1.81 (± 0.32)	1.00	3.05	114	1.67 (± 0.67)	0.60	3.60	84

Table 3. Output of Mann-Whitney's *U* test on oviposition scars of the two *Platycerus* species. * is for $p < 0.05$.

	Scar shape								
	D			**O**			**Droplet**		
	***U* value**	***p* value**	***n***	***U* value**	***p* value**	***n***	***U* value**	***p* value**	***n***
Height	3715.5	0.768	280	833.5	0.241	91	4656.5	0.144	208
Width	5444.0	0.904	281	798.5	0.099	92	5177.0	0.391	215
Depth	3579.0	0.412	230	450.0	0.422	67	2183.5	0.048*	153

Table 4. Wood diameter for *P. caprea* and *P. caraboides* logs collected in field; *n*: the number of logs. [a] and [b] show the difference in wood diameter for the two species (Mann-Whitney's *U* test, $U = 561.5$, $p < 0.001$, $n = 95$).

Species	**Wood diameter (mm)**			***n***
	Mean (± st. dev.)	**Min**	**Max**	
P. caprea	51.70 (± 29.40)[a]	22.50	130.00	40
P. caraboides	34.30 (± 16.90)[b]	16.00	105.00	55

Discussion

This study provides the first data on oviposition behavior of *P. caprea* and *P. caraboides*, of which oviposition scars are reported for the first time. Despite the "primitive position" of the genus *Platycerus* in the phylogeny of the stag beetles (KIM & FARRELL, 2015), a typical oviposition behavior on the surface of their hosts occurs.

Probably due to the size similarity of adult females of the two species (*e.g.* FRANCISCOLO, 1997), no differences in size of scar between the species are reported.

Normally, larvae of European *Platycerus* species develop inside very small logs, with diameter less than 20 or 30 mm. However, they can live also in deadwood with 100 mm in diameter or more (Table 4). In general the female of *P. caraboides* leaves its eggs in smaller wood than the *P. caprea* female (Table 4); differences in wood diameter are reported in studies on Japanese *P. acuticollis* and *P. delicatulus* (ARAYA, 1993), and *P. kawadai* (IKEDA, 1987). Only in few cases, the two Italian species have been found inside lying dead tree trunks, while they are usually found in dead branches on the soil surface.

Probably, at the bottom of the O-shaped *Platycerus* scar (the middle one) an egg is dropped, as reported for the Lucanid beetle *Prosopocoilus occipitalis* Hope & Westwood, 1845 (ARAYA, 1991) or shown for Asian *Platycerus* species (IMURA, 2010). In general, eggs laid into a cavity in the wood are less exposed to severe weather conditions and to predators.
Quite similarly to *Platycerus* species, various Cerambycidae are reported to excavate wounds before the oviposition with their mandibles, but on the bark surface (see for example ANBUTSU & TOGASHI, 1996). Both scars and oviposition behavior are well studied in some taxa, *e.g. Monochamus saltuarius* Gebler, 1830 by ANBUTSU & TOGASHI (1997b). For *Monochamus alternatus* Hope, 1843, oviposition scars are distributed uniformly on the bark surface and enhance survival of young larvae (ANBUTSU & TOGASHI, 1997a). In *M. saltuarius* and *M. alternatus* a jelly substance is found at the bottom of the scars, which is deposited after the oviposition (ANBUTSU & TOGASHI, 2000). It seems that the female can discriminate scars (with or without eggs) gnawed by other females by chemical cues (ANBUTSU & TOGASHI, 2000). Also the female of Lizard beetles makes oviposition scars on bamboo internodes, and they provide vital symbiotic yeasts to their larvae (TOKI *et al.*, 2012; 2013; TOKI & TOGASHI, 2013). Equally, it could be hypothesized that the jelly substance found in only few *Platycerus* scars could come from the female. In this case, it could be a rich-in-symbionts substance useful for larval growth. In general, microorganisms are important for larvae of stag beetles feeding on decaying wood (TANAHASHI *et al.*, 2009). It is known that the females provide microorganisms to the larvae via its microbe-storage organ (mycangium) when ovipositing. This is proven for Lizard beetle (TOKI *et al.*, 2012) and Lucanids (TANAHASHI *et al.*, 2010; TANAHASHI & FREMLIN, 2013; FREMLIN & TANAHASHI, 2015; TANAHASHI & HAWES, 2016). The presence of the mycangium was first reported in nine genera of the subfamily Lucaninae, *Platycerus* included (TANAHASHI *et al.*, 2010). More recently, it has also been studied in *P. caprea* and *P. caraboides* (M. Tanahashi, pers. comm.) as well as other European stag beetles: *Dorcus parallelipipedus* (Linnaeus, 1758), *Lucanus cervus* (Linnaeus, 1758) and *Sinodendron cylindricum* (Linnaeus, 1758) (TANAHASHI & FREMLIN, 2013; FREMLIN & TANAHASHI, 2015; TANAHASHI & HAWES, 2016).
Moreover, scars can also maintain moisture for the egg, as reported for some Lucanid beetles (ARAYA, 1991), and also in the Japanese *Dorcus rectus* (Motschulsky, 1857) and in other species of genus *Platycerus* (ADACHI, 1987). Furthermore, ADACHI (1987) speculated that oviposition marks of *Platycerus* actually accelerate moisture absorption, produce humidity retention effect, preventing drying, and accelerate the decay of the deadwood. In various cases, I have found oviposition scars near the soil surface, in parts of the deadwood with higher moisture.
Other hypothesis about scars' utility could be related to their physical presence. Scars around O-shaped ones could be useful to "calibrate" the construction of the central "egg scars" (the O-shaped), or maybe to distance them from other inhabited scars, gnawed by other females. ARAYA (1989) suggested that oviposition scars on wood can control the density of the eggs.
In some cases, I have found some "attempted scars" (Fig. 16), without individuals of *Platycerus* inside the deadwood, in wood that is too hard or without a good moisture content, showing that females occasionally abort oviposition sites.

Conclusion

Oviposition scars of *Platycerus* can be a useful indicator to wood colonized by these species.
Platycerus scars are typical in shape (D-, O- or droplet-shaped), and they are often present in large numbers. Also their location is typical: they are always found in bark-stripped branches and lie along the length of them. Differently from East Asian species, they are found only on lying branches or dead trees, not in standing ones. More often scars are found on dead branches than on trees, and in moist parts of the wood.
The detection of oviposition scars can make monitoring of these species easier. *Platycerus* scars should therefore be considered as important and easy-to-use tool in deadwood ecology studies; for example calculating density, richness and diversity of species as by using the Saproxylic Activity Index (BURNS *et al.*, 2014). In addition, it is possible to use these scars to know more on the biology of the species, as reported for the Longhorn beetle *Rosalia alpina* based on their exit holes (*e.g.* CASTRO *et al.*, 2012; CIACH & MICHALCEWICZ, 2013; CASTRO & FERNÁNDEZ, 2016). Further studies

should be conducted to find scars' utility, also related to the energy cost of such behavior and to the fitness of the species.

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