Coordinated actions for the conservation of *Osmoderma eremita* (Scopoli, 1763), *Rosalia alpina* (Linnaeus, 1758), *Coenagrium mercuriale castellanii* (Roberts, 1948), *Graphoderus bilineatus* (De Geer, 1774) in Emilia-Romagna

Con il contributo dello strumento finanziario LIFE dell'Unione Europea

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Prefaces

Stopping the loss of biodiversity and the deterioration of natural environments is among the great challenges of the planet. This one of the most serious threats to the environment in which we live, together with the great theme of combating the effects of climate change. These objectives feature prominently in the 2030 Agenda for Sustainable Development (UN 2015), which aims to outline a possible future for the planet Earth and its inhabitants, combining economic development, environmental protection, and respect for human rights.

The collective awareness on these issues is growing. As we are publishing this volume, the 14th meeting of the **Convention on Biological Diversity**, to which Italy adheres along with 195 other countries, is taking place in Egypt. This assembly works to put the theme of biodiversity at the centre of every sectoral policy at the various institutional levels.

The **Emilia-Romagna Region** has acted in time and made challenging choices in this direction.

The system of protected areas and sites of the Natura 2000 network, the issuing of specific laws for the protection of flora and fauna: these are the main tools of our region to contribute to achieving the goal of halting the loss of biodiversity and ensuring the maintenance of ecosystem services connected to it.

Recently, within this framework, the Emilia-Romagna Region has co-funded, together with the European Community and the beneficiary managing bodies, the project *Life Eremita 2016/2021*, a concrete program of actions aimed at saving some endangered species, which are essential for the reproduction of complex and functional ecosystems, from extinction.

These are, more specifically, four species of insects that have seen a progressive reduction of their life habitats and are at risk of disappearing forever also from our territory and from the European one. Two of them depend on the sustainable management of our forests, the other two on the sustainable management of aquatic environments.

Promoting the *Life Eremita* project, the Emilia-Romagna Region reaffirmed the value and relevance of the Regional Law 15/2006 "Provisions for the protection of minor fauna", which has long protected all species of amphibians, reptiles, chiropterans and even small mammals that are unfortunately often given little consideration, yet indispensable for the functioning of natural systems.

This volume is born in this context of positive actions. It has a main purpose: to offer everyone an instrument of knowledge about the conservation issues of these species, as well as illustrating the concrete actions of the Emilia-Romagna Region for the protection of biodiversity. The hope is that it may stimulate each of us to learn more about our environment so that we may protect it in the best way possible.

Paola Gazzolo
Councillor for the Defence of the soil and the coast, civil protection
and environmental and mountain policies of the Emilia-Romagna Region

Millions of species live on our planet. Of these over 1.5 million have been identified and described. 50% are insects. Every year new species are catalogued, though many will never be because they disappear without us realising it.

This project wanted to do something to save at least some of these animals, nearly invisible and often forgotten, or even worse, considered useless.

The Hermit beetle, the Rosalia longicorn, the Southern damselfly and the Water beetle are splendid examples of this unknown biodiversity.

Their names alone already intrigue us. The Hermit who lives for years as a larva in the trunk of old trees, the Rosalia with its coloured livery, or the Water beetle, an insect diver.

Why should we protect them, you might wonder. Why spend public money on a long-horned insect or a blue dragonfly?

Because they are beautiful, and they are rare.

Because they are useful by carrying out a specific task in the great game of nature.

Because they have the right to live on this planet as much as we do and finally, because we Homo sapiens are depriving them of this right by stealing and destroying their habitat.

Yet they do not ask for much: an old tree or a pond.

This project demonstrates in practice that cohabitation is possible.

Thank you to the team of this beautiful LIFE-Eremita for giving these fellow travellers a chance.

I hope that many others will follow this example; the LIFE programme will be ready to support them.

Angelo Salsi
Head of LIFE and CIP Eco-Innovation Unit
Executive Agency for Small and Medium-sized Enterprises (EASME)
European Commission

The LIFE14 project NAT/IT/000209 EREMITA "Coordinated actions to preserve residual and isolated populations of forest and freshwater insects in Emilia-Romagna"

Monica Palazzini Cerquetella

The Emilia-Romagna Region is the beneficiary responsible for the coordination of the European project LIFE14 / NAT / IT / 000209 EREMITA "Coordinated actions to preserve the residual and isolated populations of forest and freshwater insects in Emilia-Romagna", launched on January 1st 2016, and funded by the European Commission Directorate General for Environment (DG ENV).

The project involves four managing bodies of the regional protected areas and two National Parks, as associated beneficiaries. The area included by the activities involves of more than 70 sites of the Natura 2000 Network, managed by different beneficiaries.

The project's goal aims to ensure, in the medium and long term, the best conditions for the conservation of the residual populations in Emilia-Romagna of two saproxylic insects of primary conservation interest (*Osmoderma eremita* and *Rosalia alpina*) and of two insects of European Community interest (*Graphoderus bilineatus* and *Coenagrion mercuriale castellanii*), acting on the threat factors of anthropic origin.

Concrete conservation actions are being implemented in an integrated and coordinated way among all the partners to pursue specific objectives that contribute to the improvement of the conservation status of these insect species and their habitats. At the same time, this will allow the expansion of their distribution area in Emilia – Romagna, and thus ensure their survival over time.

The specific objectives, are:

- Increase the knowledge related to the presence/absence and distribution of sub-populations in Emilia-Romagna;
- Increase the availability of habitats for residual populations and improve their connectivity
- Develop a long-term management strategy, through the development and implementation of management plans and specific conservation measures;
- Promote correct behaviour among interest groups that is compatible with the needs of habitat and species protection;
- Involve the citizens and diverse stakeholders in order to disseminate a wider view of naturalistic culture, to raise awareness and respect for ecosystem balances and to overcome stereotyped attitudes and paradigms on insects, with an increased awareness of the importance of their role for man, in the ecosystem.

The concrete conservation actions, partly already implemented, concern: habitat creation and restoration interventions to guarantee adequate availability of reproductive sites; *ex situ* reproduction (captive breeding), in particular for *O. eremita*, to provide specimens for restocking and reintroduction to reinforce natural populations; installation of nest boxes -w*ood mould boxes (WMB)* - for the *in situ* reproduction of *O. eremita*, thus favouring the natural dispersion of the species; drafting of multi-year operational programs for the management / conservation of the species; carrying out information and awareness campaigns aimed at public opinion and stakeholders.

The wide geographic scale, to which the project actions refer, undoubtedly constitutes a unique experience also for the European context. The joint work between various parties involved (Management Bodies for Parks and Biodiversity, National Parks and the Emilia-Romagna Region) and the various stakeholders, is a practice that will remain stable over the medium and long term to allow for a synergic and systemic effort in the conservation of the four target species, also in favour of other insect species that need to be protected, linked to the forest and aquatic environment.

Aspects of biology, conservation status and ecology of the target species of the LIFE Eremita project: *Osmoderma eremita* (Scopoli, 1763) and *Rosalia alpina* (Linnaeus, 1758)

Roberto Fabbri, Margherita Norbiato

Osmoderma eremita (Scopoli, 1763)

The Hermit beetle (*Osmoderma eremita*) is an obligate saproxylic Cetoniinae Beetle, saprophytic from larva, lymphophagous and frugivorous as an adult (Dubois, 2009, Smolis & Kadej, 2017, Micó, 2018). As the term of the genus indicates (*Osmoderma* is composed of *osmos* = odor and *derma* = skin), the species owes the name to the characteristic odour emitted by the males to attract the females, which can be perceived several meters away (Micó, 2018). In some countries it is identified as the smell of ripe plums, or apricots, or even of Russian leather. It has a squat body and a length between 2.4 and 3.7 cm, its colour is black-bronze with metallic reflections. It is considered one of the most charismatic species that inhabit tree cavities and in recent decades has become a flag species for conservation in Europe (Audisio *et al*., 2007; Bezborodov, 2015). *O. eremita* has a high value as an indicator of the richness of saproxylic beetles in the arboreal cavities and can be assumed as an "umbrella species" because the measures taken for its conservation favour many other species settled in cavities (Ranius, 2002).

It is widespread in north-western Europe up to Germany, Slovenia and southern Sweden. In Italy it is distributed throughout the peninsula, with two different species in central and southern Italy (Audisio *et al*., 2009). It is present from the sea level up to the mountain plain, generally from the plain to the high hill in the northern Italian regions, while in southern Italy and in Sicily the species has been found up to over 1500 m a.s.l.

Until a recent past *O. eremita* was considered as a single species, but following recent morphological and genetic studies it has been attributed to two main taxa groups (Audisio *et al*., 2007; Audisio *et al*., 2009; Landvik *et al*., 2013):

- The *O. eremita* (*sensu lato*) group, widespread in the countries of Western Europe, which includes three taxa: *O. eremita* (*sensu stricto*), *O. cristinae* and *O. italicum*
- The *O. barnabita* (*sensu lato*) group, widespread in Eastern Europe, which includes two taxa: *O. barnabita* (*sensu stricto*) and *O. lassallei*.

Figure 1. Distribution of the *Osmoderma* genus in Europe (from Audisio *et al*., 2009).

In Italy only the first group is present:

- *Osmoderma eremita* (s.s.), widespread in different regions of central and northern Italy (including Emilia-Romagna), from the plain to about 1500 m a.s.l. in the Apennines
- *Osmoderma italicum*, present with populations scattered in southern Italy, from Campania to Calabria
- *Osmoderma cristinae*, which is endemic to northern Sicily.

Although the taxonomic status of the last two taxa is still debated, recent studies conducted on both molecular and morphological basis (Audisio *et al*., 2009) support the species rank for *O. cristinae* and that of subspecies for *O. italicum*, which should therefore be called *O. eremita italicum*.

The three taxa are listed in Appendix II of the Bern Convention and in Annexes II and IV of the Habitats Directive (92/43 / EEC), as a priority species, under the common name of *O. eremita* (Bologna *et al*., 2016).

O.eremita (*sensu stricto*), the only Osmoderma present in Emilia-Romagna, is included in the European Red List of saproxylic Coleopterans as a Near Threatened species (NT) (Nieto *et al*., 2010). In Italy it is included in the Italian Red List of saproxylic Coleopterans as a vulnerable species (Audisio *et al*., 2014) and is included in the lists of protected species of various Regions. At the regional level it has been attributed the EN category (Agnelli *et al*., 2010). Moreover, in Emilia-Romagna *O. eremita* is an expressly protected species according to Regional Law 15/2006, which contains provisions for the protection of minor fauna.

The adult is visible between the end of May and September depending on the altitude (Campanaro *et al*., 2011). In Emilia-Romagna from late May to mid-August; in breeding facilities they also survive through October. In autumn, adults generally die but there are exceptions (Ranius *et al*., 2005). However, the longevity is around 3 months in captivity (6 months at most, Dubois, 2009), one month at most in the wild (Tauzin, 1994, Ranius, 2001). The species is active both during the day and in the crepuscular and even nocturnal hours. After mating, each female lays 20-80 eggs in the depths of tree cavities, and these, just before hatching, have a diameter of about 5 mm (Luce, 1996). The incubation lasts 2-3 weeks and the larva feeds on the rotting wood, the humus and the leaves that accumulate in the cavity. The larva may measure up to 75 mm and can reach a weight of 12 g (Ranius *et al*., 2005, Life Eremita data). Beginning in October, the larva builds an oval cocoon, using debris, fragments of rotting wood and excrement, and it remains there until the following spring, first in the pre-pupa stage and later in the pupa stage.

Its development takes 2-4 years to complete the cycle (Ranius *et al*., 2005) and up to 6 years (Luce, 1995), depending on the conditions in the cavity, mainly in relation to the hygrometric conditions, the quality of nourishment (Tauzin 2005) and the presence of microbial activity (Landvik *et al*., 2016a).

In the Emilia-Romagna plains, as well as in lab experiments with *O. cristinae* (Dubois, 2009), it has transpired that several larvae develop into adults over a year (Life Eremita data).

Figure 2. Male of *O. eremita* at the entrance of a beech tree cavity, while emitting the pheromone to attract the female.

The species lives within the cavities of large trees broad-leaved trees that are still alive, rich in boring dust, which constitute the optimal habitat, even if there are finds in dead trees, standing or fallen. It is usually found in mature and large trees (Ranius *et al*., 2005; Dubois, 2009), but trees do not necessarily have to be large (even only 20 cm in diameter). The appropriate cavities can be located both in the lower part of the trunks, where there must be good solar irradiation, and at considerable heights (up to 25 m, Ranius *et al*., 2005) as it frequently happens in the beech-woods of the Emilia-Romagna Apennines, where the cavities are also found even over 15 m. The amount of substrate inside the cavities suitable for hosting the species is generally very high but a minimum of 4 litres may suffice (Chiari *et al*., 2012). The debris that fills the cavities constitutes a protective substrate as it fixes atmospheric humidity and mitigates the temperature variation, in association with good insulating action of the tree trunk (Dubois, 2009). The substrate is generally barely wet and contains boring dust and fragments of wood, and anything that can enter from the outside as leaves, organic remains of insects and other animals (birds, bats, rodents, etc.) (Landvik *et al*., 2016). *O. eremita* prefers cavities exposed to the sun, necessary to maintain a suitable microclimate within the colonized cavity (Chiari *et al*., 2012), but this depends on the latitude because in the southern part of its distribution area the cavities that are very exposed are generally too dry (Dubois, 2009). The host trees are of various species of broad-leaved trees. They are, in order of importance: *Quercus* spp. (*Q. robur, Q. ilex, Q. petraea, Q. pubescens*), *Castanea sativa, Salix* spp., *Fagus sylvatica, Tilia* spp., *Morus* spp., *Acer* spp., *Ulmus* spp., *Platanus* spp., *Populus tremula , Populus nigra, Juglans regia*, various fruit trees and many others. The preference for one species or another varies geographically (Dubois, 2009); in rare cases, there were also findings on conifers (Tauzin, 1994; Ranius *et al*., 2005; Dubois, 2009).

In the Po Valley, as well as in other European areas, ~~it~~ the species is also commonly found in cultivated willows (e.g. *Salix viminalis*, *S. triandra, S. alba*) and mulberries (*Morus* spp.), as well as in urban areas in the avenues and gardens with old trees (Ranius *et al*., 2005; Sebek *et al*., 2012).

In Emilia-Romagna, in the monitoring area of the Life Eremita project, the species was found everywhere in the chestnut groves and well established only in the beech woods of the upper Romagna Apennines; secondarily it was also found in tree-lined avenues and parks in urban areas and in rows of willow and mulberry trees in rural areas.

The species was originally settled in the veteran hollow trees of woods and forests; later, the action of man has considerably reduced the primary habitat, creating however new types of wooded environments and areas occupied by isolated and scattered trees or arranged in rows. Although it is known that *O. eremita* uses hollow trees even in highly anthropic areas, the probability that the species is present in such areas decreases with increasing distance from the forest habitats (Kadej *et al*., 2016).

The species is a useful indicator both of the presence of trees with natural cavities, a threatened and relatively rare element, and also for a very wide range of wildlife species (Ranius *et al*., 2005).

Cavities in live trees are considered an extremely stable habitat for decades and are therefore a suitable habitat to host *O. eremita* and other saproxylics for many generations (Ranius & Hedin, 2001; Feldhaar & Schauer, 2018). These species generally have a limited capacity and propensity to scatter compared to the species that inhabit the dead wood in general. Using radio telemetry, it was in fact found that *O. eremita* has a maximum dispersion comprised between 190 m and 1500 m. Physically, however, the species is able to fly at greater distances, up to 2300 m, as demonstrated experimentally (Ranius & Hedin 2001; Hedin & Ranius, 2002; Dubois & Vignon, 2008; Dubois *et al*., 2010; Svensson *et al*., 2011; Chiari *et al*., 2013). At any rate, females show greater flight capacity (Dubois *et al*., 2010). Although *O. eremita* is a good flyer, studies on its dispersion have anyway shown that 85% of adults remain in their original trees (Ranius & Hedin, 2001; Ranius, 2007).

The larvae have the ability to fix nitrogen through fixative bacteria and to digest polysaccharides and lignin. In this way they produce excrement that enriches the internal substrate of the cavities with nutrients, thus facilitating the establishment of other saproxylic organisms that exploit this microhabitat (Micó *et al*., 2011; Birkemoe *et al*., 2018; Brin & Bouget, 2018). Moreover, by feeding on the dead wood, they enlarge the dimensions of the cavities themselves (Luce, 1995; Dubois, 2009; Brin & Bouget, 2018). There does not appear to be interspecific competition between the larvae of *O. eremita* and other *Cetoniinae* species inhabiting the tree cavities, although both taxa appear to have a similar diet (Hilszczański *et al*., 2014; Chiari *et al*., 2014).

Figure 3. Female of *O. eremita* in beech-wood.

Figure 4. Pair of adults of *O. eremita* within black poplar cavities.

The main predator of the larvae is the Rusty Click Beetle, (*Elater ferrugineus*) and there have been observed predations by European rollers and other corvids. Several mites, nematodes and fungi can parasitize larvae and adults, killing them (Ranius *et al*., 2005).

Adults can feed on fruit, lymph and flowers, but such observations in nature are not common (Dubois & Vignon, 2008; Dubois, 2009; Smolis & Kadej, 2017). The presence of both wild and cultivated fruit trees (cherry, apricot, plum, mulberry, etc.) a short distance from hollow trees is a positive factor for their greater longevity.

Although northern Italy shows one of the highest densities (Ranius *et al*., 2005), in many regions of North-western Europe the species is extinct or there are surviving relict groups. In Europe there is a marked decline in the populations of the *Osmoderma* species throughout their distribution area, due to habitat loss and intensive management of woods and forests (Audisio *et al*., 2009). The main threat factor for the species is the extreme fragmentation and isolation of populations, often corresponding to a strong localisation of suitable habitats. The causes are to be found in the ways forest management is carried out, exclusively or mainly for productive purposes and the lack of large decayed and dying trees - even isolated or in groups - in pastoral-forestry contexts, in agro-ecosystems and in other anthropized environments, from the plains to the mountains. Due to this threat, Individual populations often face frequent local extinctions (Audisio *et al*., 2014). In fact, the conversion of areas with the presence of mature woods or old trees in plots of intensive cultivation abates vital populations and at the same time increases the isolation of the remaining populations. Similarly, when the species is present in urban areas, the demolition of old trees or old gardens to make way for new buildings and parking areas, entails further local extinctions (Carpaneto *et al*., 2010). Fires can completely destroy entire local populations. The widespread use of pesticides and insecticides is dangerous especially for the populations living in agricultural areas and in suitable habitats on their margins.

Figure 5. Old beech with cavity hosting the Hermit beetle in the Casentinesi Forests National Park.

Figure 6. Row of mulberry trees, pollarded for silkworm breeding, with *O. eremita* in the Bolognese plain.

Rosalia alpina (Linnaeus, 1758)

The Beech cerambycidae (*Rosalia alpina*). It is a long-horned coleopteran (= longicorn), obligate saproxylic, 1.5 to 3.8 cm long and velvety grey-blue or light blue with black spots on the prothorax and on the elytra.

Rosalia alpina, the only European representative of the genus *Rosalia*, is one of the rarest species in Europe, highly vulnerable due to the smallness of its mostly localised populations, and to the continuous reduction and destruction of the particular habitats in which it lives (Duelli & Wermelinger, 2005). The distribution area of *Rosalia alpina* is of the Euro-Iranian-Anatolian type (Sama, 1988). This cerambycidae is widespread from southern Scandinavia, through central and South-eastern Europe, to the south, up to Corsica, Sicily, Greece, and Turkey and to some isolated areas in Anatolia. It is absent in Great Britain and the Netherlands (Sforzi & Bartolozzi, 2001; Audisio & Sama, 2004).

In Italy the species has a wide distribution area in the Alps and the Apennines, along the entire peninsula, and in Sicily, but with localized populations (Ruffo & Stoch in CkMap, 2005); it is absent only in Valle d'Aosta and in Sardinia (LIFE MIPP, Zapponi *et al*., 2017).

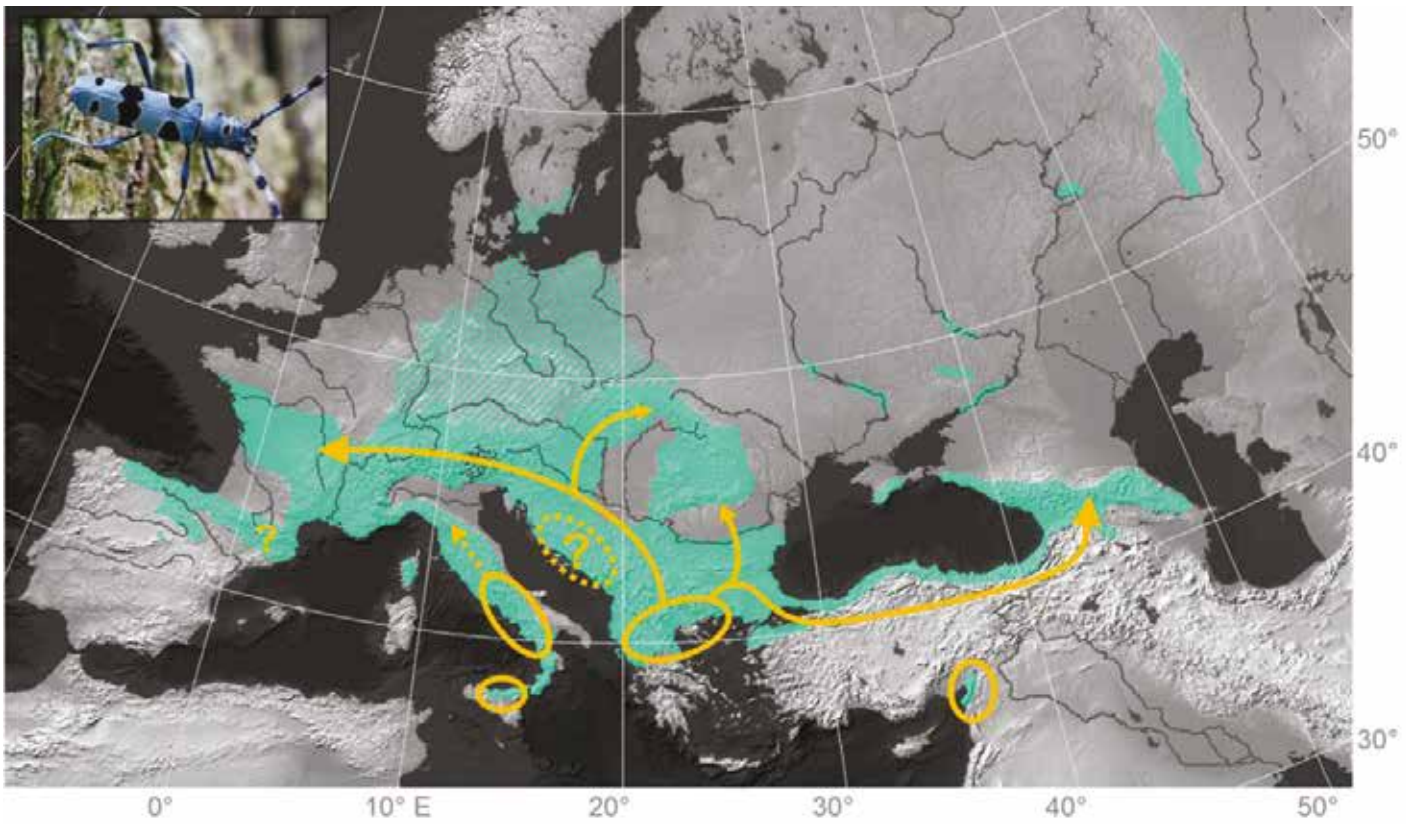

Figure 7. Distribution range of *Rosalia alpina* and its tentative location of refugia and the postglacial recolonization routes (Drag *et al*., 2018).

The species is included in the IUCN Red List of species threatened with extinction, as vulnerable (LC) version 3.1 (Horák J. *et al*., 2009) and is included in the Italian Red List of saproxylic Beetles as a Near Threatened species (NT) (Audisio *et al*., 2014); at the regional level it has been given the category VU (Agnelli *et al*., 2010). *R. alpina* is a species of European Community interest, listed in Annex II of the Habitats Directive (Directive 92/43 / EEC) as a "priority" species and in Annex IV as a species whose protection requires strict protection (Council of the European Communities 1992); it is also included in Annex II of the Berne Convention, among the protected species throughout Europe. In Emilia-Romagna the Beech cerambycidae is a particularly protected species under Regional Law 15/2006, which contains provisions for the protection of minor fauna.

The adult has a variable phenology based on latitude, altitude and climatic conditions. Although the eclosion may occur in May, the period of greatest activity is between July and early August (Duelli & Wermelinger, 2005; Drag *et al*., 2011). This has been confirmed by the monitoring carried out by LIFE Eremita in beech-woods of the Casentinesi Forests National Park, and by the LIFE MIPP in the same Park and in the National Park of Abruzzo, Lazio and Molise (Rossi de Gasperis *et al*., 2017). The males generally eclose one week before the females and defend their territory from the other males (Duelli & Wermelinger, 2005). After copulation, females lay their eggs in cracks in the wood of old trees, partially alive or dead, generally exposed to the sun. The females seem to prefer wood without bark for oviposition (Campanaro *et al*., 2017) and it has been noted that they prefer trunks or branches at least 20 cm thick (Castro *et al*., 2012) with dry or decomposing wood (Bense, 1995). The females prefer trunks rather than branches (Castro *et al*., 2012) but occasionally lay their eggs on stumps or large branches fallen to the ground (Duelli & Wermelinger, 2005; Campanaro *et al*., 2011; Castro *et al*., 2012). This preference for large-diameter standing trunks can be explained by the greater availability of *pabulum*, greater isolation from wood decay conditions, typical of dead or fallen wood, and the fact that larger trunks represent a more durable habitat, both in terms of nourishment and humidity conditions (Castro *et al*., 2012).

Larval development takes place in 2-3 years depending on the climatic conditions and the quality of the wood (Sama, 1988; Sama, 2002). In Europe, larvae have been observed in most cases on beech (*Fagus* spp.) but in some cases they grow in the wood of other broad-leaved trees such as maple, apple, elm, ash, hazel, chestnut, poplar, alder, linden and hornbeam (Sama, 2002; Duelli & Wermelinger, 2005; Ciach *et al*., 2007; Čížek *et al*., 2009; Horák *et al*., 2009; Michalcewicz *et al*., 2013;

Trizzino *et al*., 2013).

Before the last winter season, the larvae move towards the bark and the nymphosis takes place in a pupation cell built between the end of spring and the beginning of summer. The eclosion occurs through characteristic elliptical exit holes, of 4.9-12 mm in length by 3-8 mm in width, with the major axis generally oriented according to the direction of the wood fibres (Campanaro *et al*., 2017). The width and length of the exit holes are favorably correlated with the adults' size, in particular with the pronotum width (Ciach & Michalcewicz, 2013).

The selection of the preferential habitat and its host plants are well known in Europe (Sama, 2002; Duelli & Wermelinger, 2005; Ciach *et al*., 2007; Horák *et al*., 2009; Čížek *et al*., 2009; al., 2011; Trizzino *et al*., 2013; Michalcewicz *et al*., 2013; Di Santo & Biscaccianti, 2014; Castro & Fernandez, 2016). In contrast, only a few studies have been published on larval and adult biology and their behavior (Drag *et al*., 2011; Russo *et al*., 2011) and this is why there are still several gaps in this regard. *Rosalia alpina* is considered a mountain species, associated with mature beech forests, but is able to colonize a wide variety of deciduous tree species (such as *Aceraceae, Betulaceae, Fagaceae, Oleaceae, Tiliaceae, Ulmaceae*). It is also considered an excellent biological indicator of mature broad-leaved forests and therefore in good ecological condition (Pignataro & Vicidomini, 2007). It can be found from the mountain to the subalpine, between 500 and 2000 m a.s.l., even if there exist in Italy populations of frigophile relictual character at lower altitudes, down to sea level (Policoro, Basilicata). From the point of view of the landscape, *Rosalia alpina* prefers open or semi-open areas rather than forests with too dense tree cover (Russo *et al*., 2011). On a smaller scale, the species can reproduce on a certain variety of trees, but shows a preference for mature trees, dead or dying and exposed to the sun, in open areas and/or in sites with a low percentage of tree cover. Moreover, the species prefers trees that are not surrounded by excessive undergrowth, which could therefore prevent them from flying. Finally, the trees occupied by *R. alpina* have, on average, a thick bark compared to unoccupied trees (Russo *et al*., 2011). These specific ecological needs mean that forest management practices are important to the population trend of this species and therefore also sometimes responsible for local extinctions. In general, the limited dispersion capacity of adults strongly exposes this species to the risk imposed by the fragmentation of its habitat (Drag *et al*., 2011; Russo *et al*., 2011; Bosso *et al*., 2013).

In Emilia-Romagna the species is present with a sizable population, located in the south-east area of the Apennine belt (in the Casentinesi Forests National Park), both in pure and mixed beech forests, the latter mixed with white fir and yew. The beech woods are mostly high trees forests resulting from the conversion of old coppices. The species is also present in the westernmost Apennines, in the Tuscan - Emilian Apennine National Park, where some observations have been reported. The species is found in particular in the most ancient beech woods, with ample availability of mature and senescent trees and with a lot of dead wood on the ground and standing, in the most open areas exposed to the sun as in the top of ridges, at heights between 750 and 1200 m.

During the sunny and warm days the adults of *R. alpina* can be active from 10-11 am, with peaks around noon and 2 pm (Drag *et al*., 2011).

Although adults normally move within a certain variety of habitats, they are able to fly long distances. Marking-recapture studies have shown that movements within a given habitat are quite common, ranging from tens to hundreds of meters, with no observed difference in mobility between the sexes (Drag *et al*., 2011). The longest recorded dispersal distance is 1.5 km (Drag *et al*., 2011; Rossi De Gasperis, 2016). In any case, it should be borne in mind that marking-recapturing techniques can significantly underestimate the dispersion distances. The maximum duration of life recorded in the wild, estimated during a marking-recapture study carried out in the Czech Republic, was 24 days for males and 15 for females (Drag *et al*., 2011). The adults do not seem to depend on the nectar of the flowers (Lachat *et al*., 2013) and may not eat at all, as also observed in other species of different subfamilies of Cerambycidae.

With regard to conservation, in Europe this species is undergoing a fragmentation of its habitat, which has led to the existence of isolated populations, which may pose a threat to this species, as it is a saproxylic coleopteran characterized by a low dispersion capacity (Drag *et al*., 2011; Bosso *et al*., 2013). The key factors in the state of the population of *R. alpina*, including local extinctions, are: the abandonment of traditional forest management practices (such as the management of pastures) and/or their conversion into high trees forests, which reduce the availability of trees exposed to the sun (Drag *et al*., 2011; Lachat *et al*., 2013); the removal of dead wood or veteran trees, which causes a marked decrease in the availability of dead wood, and negatively affects the survival of *R. alpina* (Duelli & Wermelinger, 2005; Čížek *et al*., 2009; Russo *et al*., 2010); the senescence of dead standing trees can in fact host a large quantity of larvae and adults, therefore they are "key trees" for a population or a community of saproxylics (Audisio *et al*., 2014); the practice of stacking the fallen trees, which attract females ready to lay the eggs and which represent ecological traps, if this wood is then removed and used by humans before the adult beetles leave it (Duelli & Wermelinger, 2005; Adamski *et al*., 2016)); forest fires (Duelli & Wermelinger, 2005; Trizzino *et al*., 2013).

Figure 8. Male of *Rosalia alpina* on the dead grounded beech trunk.

Figure 9. Eclosion hole(s) of *Rosalia alpina* on a beech tree trunk.

Figure 10. Old beech broken grounded, populated by *Rosalia alpina*.

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Update of the distribution of *Osmoderma eremita* (Scopoli, 1763) and *Rosalia alpina* (Linnaeus, 1758) in Emilia-Romagna

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Introduction

As part of the Life Eremita project monitoring was carried out on *Osmoderma eremita* and *Rosalia alpina*. The *ex ante* monitoring was aimed at defining the presence and distribution of the two species in the project area, in order to plan the interventions for the improvement of their conservation status. In particular, the project includes interventions in habitats as well as restocking and reintroduction, with the ultimate goal of strengthening existing populations and expanding the distribution area of the target species in the regional territory. This resulted in a set of data that was analysed together with the previous data already available in the regional databases, thus updating the knowledge on the two species in the intervention area of the Life Eremita project. Starting from 2010, prompted by the Habitats Directive 92/43/EEC, the Emilia-Romagna Region had considerably expanded its knowledge on regional biodiversity, commissioning studies and research on its natural heritage, through the funding of Measure 323 of the 2007-2013 PSR (*Rural Development Plan*). In this way, knowledge was accrued as to the actual presence of animal and plant species of conservation interest within the sites of the regional Natura 2000 network. Nevertheless, for many species, in particular for many invertebrates, the information available was still rather incomplete and fragmentary. This was mainly due to the lack of studies conducted on this group of animal species: *O. eremita* was reported in Emilia-Romagna in all the provinces, except that of Rimini, especially in the plains and the foothills, up to 1000 m, but there were no data regarding several regional areas. *R. alpina* was known only in the provinces of Modena and Forlì-Cesena, and for the latter recent data was available only within the Casentinesi Forests National Park (Agnelli *et al*., 2010). The monitoring carried out with the project at least partially filled the knowledge gaps for the two species, among the most relevant of the regional and national natural heritage. The knowledge framework will be further enriched after the conservation measures envisaged by the European project have been completed, when a further monitoring campaign will be carried out *ex post* with the aim of verifying the effectiveness of those measures and the trend over time of the populations of the two target species.

Figure 1. Survey area of *Osmoderma eremita* monitoring within the *ex ante* actions of the Life Eremita project (2016-2017). Monitoring has been developed on 32 Natura 2000 sites in Emilia-Romagna.

Figure 2. Survey area of the new monitoring campaign of *Rosalia alpina* as part of the *ex ante* actions of the Life Eremita project (2016-2017). Monitoring has been developed on 13 Natura 2000 sites, one of which falls in Tuscany.

Data source

The previous data have been extracted from the database of the species reports, available at the Servizio aree protette, foreste e sviluppo della montagna (*Protected Areas, Forests and Mountain Development Service of the Emilia-Romagna Region*). The database, updated in 2014, is the result of a substantial work started in 2010 with funds from 2007-2013 PSR (*Rural Development Plan*). This work systematised all the reports of species of conservation interest in the region through an in-depth analysis of the existing bibliography, contacts with the managing bodies of Natura 2000 sites, research institutions, naturalistic groups, specialist scholars, and the study of findings kept in museums and other public institutions or in private collections. After extracting the data, as part of the Life project all the reports locations have been geo-referenced, making it possible to create a graphic representation on the geographical map of the Emilia-Romagna Region. In addition, the reports from the monitoring carried out within the LIFE MIPP project (2012-2017) were used. Overall, the previous data on the presence of the two species cover a period of more than a century, from 1891 to 2015. To these were added new data from the surveys of the campaign conducted within the LIFE Eremita project during the monitoring *ex ante*, in the two-year period 2016 and 2017.

Study area

The *ex-ante* monitoring area for the two saproxylic species covers a total area of 54,812 ha and includes 39 Natura 2000 sites (one of which in Tuscany), of the 158 present in Emilia-Romagna, in addition to some external areas near the sites themselves. The selection of the Natura 2000 sites to be investigated was carried out using three criteria: the first, analysing the official forms and examining previous data on the presence of the species, then taking field trips to verify preliminarily, based on the vegetation maps and the presence of suitable habitats. The other two criteria were based on aptness, excluding from the monitoring activities those sites where it would not have been possible to carry out the conservation measures envisaged by the Life project. Additionally, those where the monitoring program of the Life MIPP project was in progress were excluded, so as to optimise resources and to concentrate the field effort in the areas less investigated to date. The territories of the two national parks that fall between Emilia-Romagna and Tuscany are also within the project area. For this reason, the project area also included, for the sole *Rosalia alpina* species, a limited area of the Tuscan side.

The monitoring areas of the Natura 2000 sites are characterized in general by the presence of 13 types of forest habitats of Annex I of the Habitats Directive, for a total extension of 28,701.29 ha (Table 1).

Natura 2000 Habitat	Area (ha)
9110 - *Luzulo-Fagetum* beech forests	4.972
9130 - *Asperulo-Fagetum* beech forests	9.896
9180 - *Tilio-Acerion* forests of slopes, screes and ravines	1.354
91AA - Eastern white oak woods	678
91E0 - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior (Alno-Padion, Alnion incanae, Salicion albae)*	370
91F0 - Riparian mixed forests with *Quercus robur, Ulmus laevis* and *Ulmus minor, Fraxinus excelsior* or *Fraxinus angustifolia (Ulmenion minoris),* along the large rivers	8
91L0 - Illyrian oak-hornbeam forests *(Erythronio-Carpinion)*	483
9210 - Apennine beech forests with *Taxus* and *Ilex*	1.636
9220 - Apennine beech forests with *Abies alba* and beech forests with *Abies nebrodensis*	3.686
9260 - *Castanea sativa* woods	4.163
92A0 - *Salix alba* and *Populus alba* galleries	1.305
9340 - *Quercus ilex* and *Quercus rotundifolia*	103
9430 - Subalpine and montane *Pinus uncinata* forests	45
TOT	28.701

Table 1. Nature 2000 forest habitats and their extension in the study area (n. 38 Nature 2000 sites in Emilia-Romagna).

Monitoring methods

The field monitoring was carried out according to protocols (Fabbri R., 2017a, Fabbri R., 2017b) developed for each of the target species by the technical coordinator of the Life Eremita project, and reviewed by a scientific supervisor, both appointed by the beneficiary responsible for the project. A staff of 8 entomologists appointed by the project beneficiary partners took the field trips. Groups of specially trained volunteers joined them.

The monitoring of *Osmoderma eremita* took place in 2016 and 2017 between June and August. Within each Natura 2000 site a number of transects have been identified and traced, and accordingly identified with an identification code (ID). The code is composed of the site code of the Natura 2000 site, the acronym of the Park or Macro-area, the transect code composed of the identification code of the species (Os = Osmoderma eremita) and the letter L (linear) and a progressive number (e.g. IT4080003_PNFC_Os_L2). The choice of transects was made following the path that connects likely hollowed trees, located in rows, tree-lined avenues, parks, tree-lined hedges, woods, identified as "habitat trees". Each is identified with the same method of coding, except for the last abbreviation, which identifies the tree with a P followed by the progressive number. The area of the transects has been identified taking into account the distances from the sites of confirmed presence of the species (previous data already available), i.e. distance no greater than 2.5 km, in relation to the dispersal capacity of the species. The choice priority was given to the areas of public ownership, in order to ensure greater ease and speed of operation and a future sustainability of the restoration interventions of the habitats envisaged in the Life Eremita project, and the presence of forest roads that would allow easier access to the site. As a general criterion, the monitoring transects have been traced with a length of at least 1 km. Overall, for the monitoring of *Osmoderma eremita*, 80 transects and 424 habitat trees were monitored at 32 sites of the Natura 2000 network, including some locations outside the sites falling in protected areas or outside them (Table 2).

Natura 2000 sites	N° of transects monitored	N° of habitats trees monitored
IT4080003	7	44
IT4080002	1	9
Total PNFC	**8**	**53**
IT4030002	4	31
IT4030003	2	8
IT4030005	4	18
IT4030009	4	9
Outside SCI	3	8
Total PNATE	**17**	**74**
IT4050004	2	15
IT4070011	5	30
IT4070016	2	9
IT4070024	1	5
IT4080004	1	2
IT4080008	1	1
IT4090001	1	5
IT4090003	2	5
Outside SCI	3	6
Total MAR	**18**	**78**
IT4050001	2	9
IT4050002	3	19
IT4050003	2	6
IT4050013	1	2
IT4050020	3	25
Outside SCI	1	2
Total MEOR	**12**	**63**
IT4030007	1	2
IT4030011	1	1
IT4040001	2	36
IT4040002	1	24
IT4040003	5	20
IT4040004	2	15
Parco del Frignano	1	2
Outside SCI	3	15
Total MEC	**16**	**115**
IT4010008	1	2
IT4010017	1	2
IT4020001	1	6
IT4020003	1	6
IT4020009	1	8
IT4020021	1	3
IT4020026	1	6
Parco Reg. Cento Laghi	2	8
Total MEOC	**9**	**41**
Total	**80**	**424**

Table 2. Number of transects and habitat trees monitored in the survey area for *O.eremita* (PNFC = National Park of the Casentinesi Forests; PNATE = National Park of the Tuscan-Emilian Apennines; MAR = macro-area Romagna; MEOR = macro-area Eastern Emilia; MEC = macro-area Central Emilia, MEOC = macro-area Western Emilia).

The verification of the presence of *Osmoderma eremita*, and of other species of conservation interest, took place using four methods:

- Visual encounter survey (VES);
- Wood mould sampling (WMS);
- Black cross window traps (BCWT);
- Pitfall trap (PT)

VES, that is the direct visual search of adults, was carried out on the trunks or within the cavities without going deep into the boring dust. This method was used to verify if the monitoring transects were suitable for the application of the subsequent sampling methods.

WMS, or the search for remains, larvae, pupal cocoons and pellets in the boring dust contained in the cavities, was carried out also using tools such as bowls and scoops to extract the boring dust and analyse it.

BCWT (Ranius & Jansson, 2002; Larsson & Svensson, 2009; Chiari *et al*., 2013; Trizzino *et al*., 2013) are intercepting attractive traps. The trap, carrying an open *Eppendorf* tube containing the attractive pheromone, is hung from the branches of the trees using a hook. After installation the BCWTs are checked and/or withdrawn after 1 or 3 days.

Pitfall traps are fall traps consisting of plastic cans of 500 cc volume, not primed with attractive substances, buried in the boring dust of the cavities up to the upper edge, and containing a saturated solution of sodium chloride and water. The traps were renewed every 3-7 days and kept active for at least 30 days. They have been placed mainly within chestnuts cavities (they have large cavities, generally easy to access).

Specimens captured during monitoring were determined, in many cases marked, and all of them released on neighbouring trees (CMR capture-marking-recapture method). Based on the ratio between recaptured marked individuals and captured unmarked individuals, it is possible to quantify the population size (Campanaro *et al*., 2011). The marked specimens were photographed, measured and catalogued. The marking was made using coloured and numbered labels (queen marking stickers) placed on the right elytron and glued with a small drop of Attack Supergel.

All the data collected were gathered on special field cards, which were subsequently uploaded on Excel tables and entered in the Database.

The monitoring of *R. alpina* took place in the high-mountain areas, in 2016 and 2017. Also for this species transects have been identified on the basis of previous reports of presence, the identification of beech woods and, during preliminary field trips, on the presence, in open and sunny areas, of veteran trees with clear signs of deterioration (fungal fructifications, parts of dead wood both in the trunk and in the foliage, woodpecker holes), stumps of standing dead trees (volis) or uprooted trees (chablis), stumps with buried roots, large branches broken on the ground (with a diameter of more than 20-25 cm), piles of wood.

In particular, the area of the transects has been identified considering a distance of no more than 3.0 km from the sites where the species presence was certain or had been reported, in stations which are primarily located in the territory of the Natura 2000 sites pertaining the project. Also for *R. alpina* priority has been given to the areas of public ownership, and the presence of forest paths and carriageways that allowed easier access to the site has been considered positive. Preliminary field trips were taken before the start of the monitoring, between April and early June, in a variable number according to the number of stations to be checked. They were expeditious field trips, to check the environmental suitability, track with the GPS hypothetical transects, and identify a suitable number of habitat trees (considered as monitoring stations) that were marked with appropriate metal plates.

Rosalia alpina has been monitored in 13 Natura 2000 sites, as well as some locations in protected areas outside Natura 2000 sites. In total, 38 transects including 540 habitat trees were monitored. (Table 3).

Natura 2000 sites	N° of transects monitored	N° of habitat trees monitored
IT4080002	1	3
IT4080003	5	110
IT5140005	1	2
Outside SCI	1	1
TotalePNFC	**8**	**116**
IT4030004	1	2
Outside SCI	1	1
Total PNATE	**2**	**3**
IT4080008	4	4
Totale MAR	**4**	**4**
IT4050002	6	19
Total MEOR	**6**	**19**
IT4040001	5	193
IT4040002	2	74
IT4040005	1	1
Parco del Frignano	2	12
Total MEC	**10**	**280**
IT4010003	1	21
IT4020007	3	51
IT4020008	1	7
IT4020020	2	19
Parco Reg. Cento Laghi	1	20
Total MEOC	**9**	**118**
Total	**38**	**540**

Table 3. Number of transects and habitat trees monitored in the survey area for *R.alpina* (PNFC = National Park of the Casentinesi Forests; PNATE = National Park of the Tuscan-Emilian Apennines; MAR = macro-area Romagna; MEOR = macro-area Eastern Emilia; MEC = macro-area Central Emilia, MEOC = macro-area Western Emilia).

The assessment of the presence of *Rosalia alpina*, and of others of conservation interest, took place using the following two methods:

- Direct search (visual encounter survey, VES);
- Capture-marking-recapture (CMR).

Initially, direct research (VES) of adults was carried out on the trunks, branches and on the ground, or of remains, larvae, tunnels and pupation cells under the bark and of eclosion holes in the trunks. In the following monitoring phase, the capture-mark-recapture (CMR) method was used, in which the specimen recognition was based on the shape of the black spots on the elytra, documented through digital photography (photographic markings) (Duelli & Wermelinger, 2005). This easy to perform and non-invasive method, is repeatable over time and also practical for personnel with minimal technical preparation. The identification of *R. alpina* is very simple thanks to the colouring of the elytra of this coleopteran, which allows unique identification of the specimens. Applying the CMR method, based on the ratio between recaptured individuals and not captured before individuals, it is possible to directly obtain an

estimate of the absolute population size, an estimate of the sex ratio and an adult survival parameter. The data collected in the field were collected on a special data sheet, and then transferred to Excel tables and databases.

Results

The following tables show nominally and in chronological order the locations where the presence of *O. eremita* in Emilia-Romagna was reported overall, from 1891 to 2012. The first table refers to the data extracted from the database of species reports of the Emilia-Romagna Region, the second to data from the monitoring carried out within the Life MIPP project. It should be noted that the localities are numerically smaller than the presence reports in the various archives, as some data refer to the same survey location.

Report date	Location or Natura 2000 site	Municipality and Province
1891	Calestano	Calestano (PR)
1897	Cotignola	Cotignola (RA)
1934	Bologna	Bologna (BO)
1956	Granarolo Faentino	Granarolo Faentino (RA)
1963	Le Mose	Piacenza (PC)
1968	Fusignano	Fusignano (RA)
1970	Piacenza	Piacenza (PC)
1970	Castel San Giovanni	Castel San Giovanni (PC)
1973	Russi	Russi (RA)
1974	Massa Lombarda	Massa Lombarda (RA)
1976	Balze di Verghereto	Verghereto (FC)
1977	Lugo	Lugo (RA)
1977	Sant'Agata sul Santerno	Sant'Agata sul Santerno (RA)
1978	Sala Bolognese	Sala Bolognese (BO)
1980	Collecchio	Collecchio (PR)
1981	Tossignano	Tossignano (BO)
1981	Fontanellato	Fontanellato (PR)
1982	Passo dei Mandrioli, Bagno di Romagna	Passo dei Mandrioli, Bagno di Romagna (FC)
1982	Pieve Modolena, Reggio nell'Emilia	Pieve Modolena, Reggio nell'Emilia (RE)
1984	Podere Pantaleone	Bagnacavallo (RA)
1984	Faenza	Faenza (RA)
1984	Sant'Agata sul Santerno	Sant'Agata sul Santerno (RA)
1985	Collagna	Collagna (RE)
1985	Campotto	Argenta (FE)
1986	Calerno,	Sant'Ilario d'Enza (RE)
1986	Castelnuovo,	Borgonovo Val Tidone (PC)
1989	Olmo,	Bettola (PC)
1989	Cadelbosco di Sotto	Cadelbosco di Sotto (RE)
1990	Balze di Verghereto	Verghereto (FC)
1990	Campotto	Argenta (FE)
1990	San Benedetto in Alpe	San Benedetto in Alpe (FC)
1990	Massenzatico	Reggio Emilia (RE)
1990	Podere Pantaleone	Bagnacavallo (RA)
1992	Barbiano,	Cotignola (RA)
1993	Balze di Verghereto	Verghereto (FC)
1993	Brisighella	Brisighella (RA)
1995	Campotto	Argenta (FE)
1995	Fontanili di Corte Valle Re	Fontanili di Corte Valle Re (RE)
1995	Gessi Triassici	Gessi Triassici (RE)
1997	Noceto	Noceto (PR)
1997	Sestola	Sestola (MO)
2000	Sestola	Sestola (MO)
2002	Podere Pantaleone,	Bagnacavallo (RA)
2002	Ponte Taro,	Fontevivo (PR)
2008	Riserva Naturale di Sasso Fratino	Bagno di Romagna (FC)
2012	Bosco del Crociale,	Crevalcore (BO)
2012	San Martino dei Manzoli	Minerbio (BO)
2012	Mezzolara	Budrio (BO)
2012	Campotto, Valle Santa	Argenta (FE)

Table 4. Occurence of *O. eremita* in Emilia-Romagna from 1891 to 2012 - Data extracted from the database of species reporting (2014) of the Servizio Aree protette, Foreste e Sviluppo (*Protected Areas, Forests and Mountain Development Service)* of the Emilia-Romagna Region.

Report date	Location or Natura 2000 site	Municipality and Province
2010	Foresta della Lama,	Badia Prataglia (AR)
2010	Riserva Integrale Sasso Fratino,	Bagno di Romagna (FC)

Table 5. Reports from the Life MIPP data.

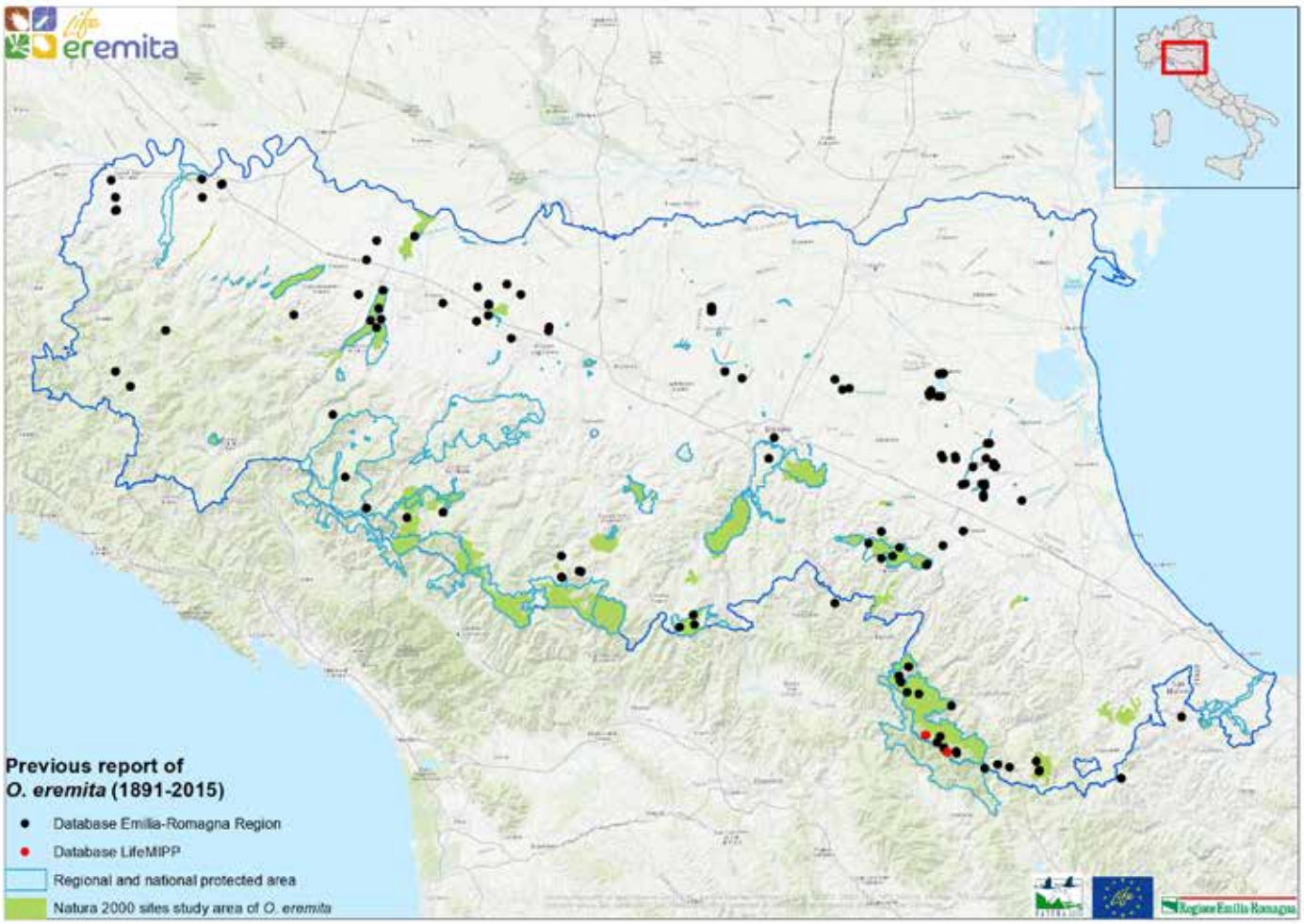

Figure 3. Distribution of previous reports (1891-2015) of O. Eremita presence in Emilia-Romagna. Data extracted from the database of species reports of the Servizio Aree protette, foreste e sviluppo della montagna (*Protected Areas, Forests and Mountain Development Service*) of the Emilia-Romagna Region (2014 update).

In the *ex ante* monitoring campaigns of the LIFE Eremita project, carried out in 2016 and 2017, the species was overall found with 85 individuals, of which 20 in 2016 and 65 in 2017. Most reports (No. 77) fall within the Natura 2000 Network, 8 specimens have been reported in non-SCI areas but in any case close to the Natura 2000 network (Table 6).

Natura 2000 site	Transect ID	N° of reported specimens Year 2016	N° of reported specimens Year 2017	N° of reported specimens Total	Monitoring method
IT4080002 Acquacheta	IT4080002_PNFC_Os_L5	0	1	1	BCWT
IT4080003 Monte Gemelli	IT4080003_PNFC_Os_L2	3	11	14	BCWT, VES
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Os_L3	1	0	1	BCWT
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Os_L5	0	2	2	WMS
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Os_L7	0	6	6	BCWT, PT, WMS
IT4030002 Ventasso	IT4030002_PNATE_Os_L5	0	2	2	BCWT
IT4030003 Monte la Nuda Cima Belfiore Passo Cerreto	IT4030003_PNATE_Os_L10	0	5	5	BCWT
IT4030005 Abetina Reale Alta Val Dolo	IT4030005_PNATE_Os_L7	0	7	7	BCWT, PT
IT4030005 Abetina Reale Alta Val Dolo	IT4030005_PNATE_Os_L8	0	3	3	BCWT
IT4030005 Abetina Reale Alta Val Dolo	IT4030005_PNATE_Os_L9	0	2	2	BCWT
Outside SCI	FuoriSIC_PNATE_Os_L13	0	1	1	BCWT
IT4050004 Bosco della Frattona	IT4050004_MAR_Os_L12	0	2	2	WMS
IT4070011 Vena del Gesso Romagnola	IT4070011_MAR_Os_L4	2	0	2	WMS
IT4070011 Vena del Gesso Romagnola	IT4070011_MAR_Os_L7	1	3	4	WMS
IT4070011 Vena del Gesso Romagnola	IT4070011_MAR_Os_L22	0	3	3	PT, WMS
IT4070016 Alta Valle del Torrente Sintria	IT4070016_MAR_Os_L16	0	2	2	WMS
IT4070016 Alta Valle del Torrente Sintria	IT4070016_MAR_Os_L25	0	2	2	WMS
IT4070024 Podere Pantaleone	IT4070024_MAR_Os_L13	0	5	5	BCWT
IT4090003 Rupi e Gessi Valmarecchia	IT4090003_MAR_Os_L20	0	3	3	BCWT
Outside SCI	FuoriSIC_MAR_Os_L13	0	5	5	BCWT

Outside SCI	FuoriSIC_MAR_Os_L14	0	1	1	WMS
IT4050020 Parco dei laghi Suviana e Brasimone	IT4050020_MEOR_Os_L2	0	3	3	BCWT, VES
IT4050020 Parco dei laghi Suviana e Brasimone	IT4050020_MEOR_Os_L3	0	1	1	BCWT, VES
IT4040002 Monte Rondinaio, Monte Giovo	IT4040002_MEC_Os_L010	0	1	1	BCWT
IT4040002 Monte Rondinaio, Monte Giovo	IT4040002_MEC_Os_L002	0	1	1	BCWT
IT4020021 Medio Taro	IT4020021_MEOC_Os_L02	3	2	5	BCWT, VES
Outside SCI	Fuori SIC_MEOC_L09	0	1	1	BCWT

Table 6. Sites where the presence of *Osmoderma Eremita* was detected from 2016 to 2017 within the ex-ante monitoring of the LIFE Eremita project; transects per site; number of individuals either captured or spotted along the transects in the survey area. The name of the transect "outside SCI" refers to areas outside the Natura 2000 site, even though close to them.

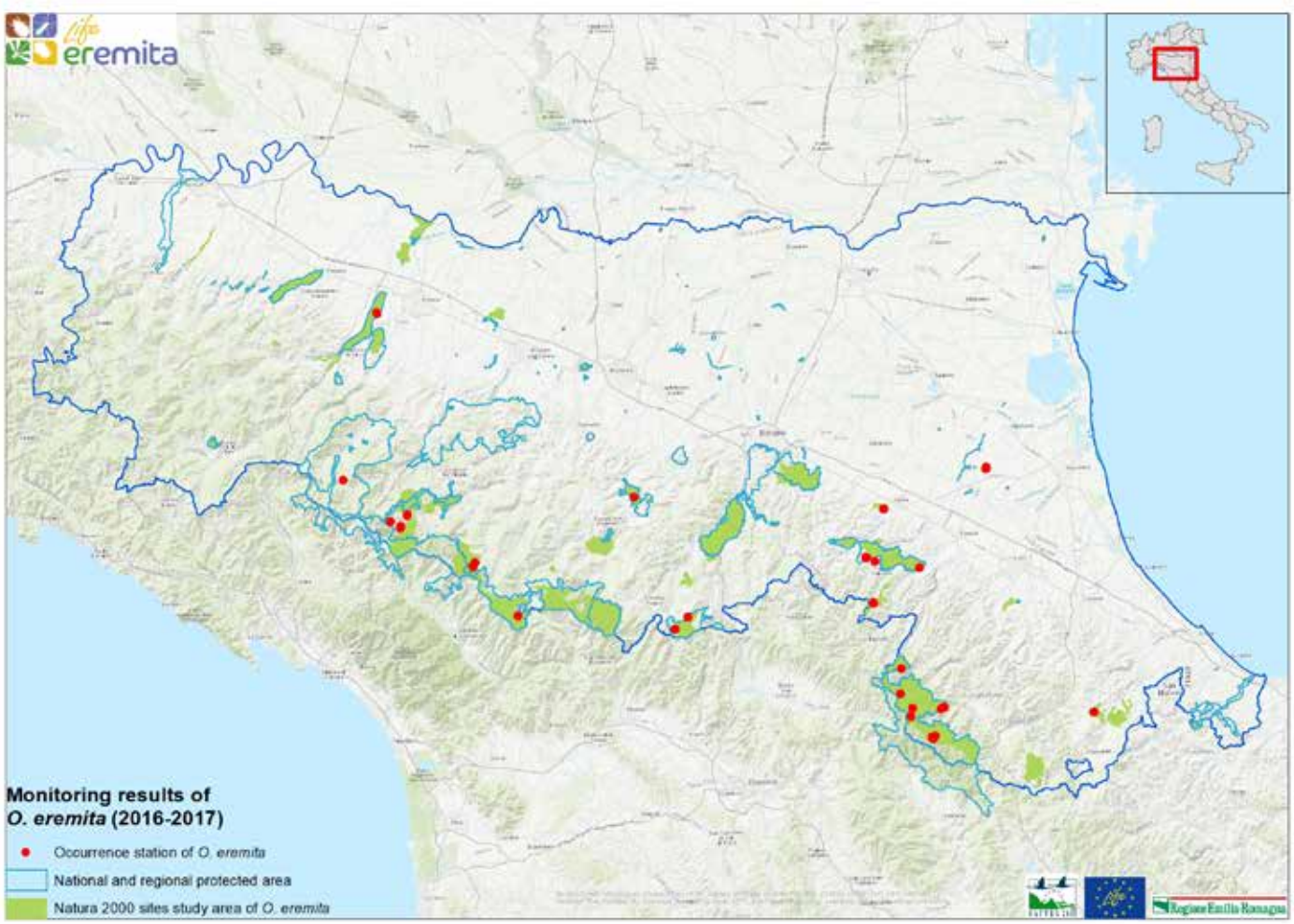

Figure 4. Distribution of the new records of *Osmoderma Eremita* presence in Emilia-Romagna (data LIFE Eremita project 2016-2017).

As for the sampling methodology, 47 specimens (45 F; 2 M) were captured through the use of BCWTs and 1 specimen (F) using PT traps; 27 reports (1 M, 1 F, 29 remains) were detected with the WMS method and 6 reports (2 M and 4 F) through VES. There were no recaptures of the marked specimens (Table 6).

Of the 77 specimens captured and/or spotted in Natura 2000 sites, 39 fall into 3 Natura 2000 habitats (9130 - *Asperulo-Fagetum* beech forests; 9220 - Apennine beech forests with *Abies alba* and beech forests with *Abies nebrodensis* and 9260 - *Castanea sativa* woods), among these, 31 specimens were found in habitat 9260 - *Castanea sativa* woods (Table 7).

SCI	Habitat code	N° of specimen reported
IT4030005	9260	12
IT4030002	9260	2
IT4030003	9260	5
IT4040002	9130	1
IT4050002	9260	1
IT4050020	9260	3
IT4070011	9260	4
IT4080002	9130	1
IT4080003	9260	1
IT4080003	9130	1
IT4080003	9220	5
IT4090003	9260	3

Table 7. Number of specimens captured or spotted within Natura 2000 habitats (Annex I).

Taking a more detailed look at each habitat tree, it is noted that the species has been found especially on chestnut trees (*Castanea sativa*), present both within Natura 2000 habitats (9260) and in cultivated chestnut groves, but also on Beeches (*Fagus sylvatica*), oaks (*Quercus robur*), willows (*Salix sp.*), poplars (*Populus nigra*) and in the plains also on alien species such as Japanese Sophora (*Sophora japonica*) (Table 89).

Transect ID	Habitat Tree ID	Environment	Tree species	Tree age	Girth (cm)
IT4080002_PNFC_0s_L5	IT4080002_PNFC_0s_P221	Beech wood	*Fagus sylvatica*	50-80	165-265
IT4080003_PNFC_0s_L2	IT4080003_PNFC_0s_T10				
	IT4080003_PNFC_0s_T12				
	IT4080003_PNFC_0s_T6	Beech wood	*Fagus sylvatica*	100	340
	IT4080003_PNFC_0s_T13	Beech wood	*Fagus sylvatica*	90-100	320
	IT4080003_PNFC_0s_P159				
	IT4080003_PNFC_0s_T14				
	IT4080003_PNFC_0s_P83	Beech wood	*Fagus sylvatica*	50	225
	IT4080003_PNFC_0s_P138	Beech wood	*Fagus sylvatica*	50-60	280
IT4080003_PNFC_0s_L3	IT4080003_PNFC_0s_P156	Beech wood	*Fagus sylvatica*	>100	260
IT4080003_PNFC_0s_L7	IT4080003_PNFC_0s_P131				
IT4030002_PNATE_0s_L5	IT4030002_PNATE_0s_T32				
	IT4030002_PNATE_0s_T33	Mesophilic broadleaf forest	*Castanea sativa*	619	387
IT4030003_PNATE_0s_L10	IT4030003_PNATE_0s_T52				
	IT4030003_PNATE_0s_T71				
IT4030005_PNATE_0s_L7	IT4030005_PNATE_0s_T44				
	IT4030005_PNATE_0s_T46	Mesophilic broadleaf forest	*Castanea sativa*	800	680
	IT4030005_PNATE_0s_T47				
IT4030005_PNATE_0s_L8	IT4030005_PNATE_0s_T49				
IT4030005_PNATE_0s_L9	IT4030005_PNATE_0s_T50				
FuoriSIC_PNATE_0s_L13	FuoriSIC_PNATE_0s_T66				
IT4050004_MAR_0s_L12	IT4050004_MAR_0s_P93	Public Park	*Quercus robur*	200	515
	IT4050004_MAR_0s_P94	Public Park	*Sophora japonica*	40-50	145
IT4070011_MAR_0s_L4	IT4070011_MAR_0s_P34	Rows of pollarded trees	*Salix sp.*	30	100
IT4070011_MAR_0s_L7	IT4070011_MAR_0s_P61	Avenue with pollarded lindens	*Tilia sp.*	30-100	100-285
	IT4070011_MAR_0s_P62	Avenue with pollarded lindens	*Tilia sp.*	30-100	100-285
	IT4070011_MAR_0s_P63	Avenue with pollarded lindens	*Tilia sp.*	30-100	100-285
IT4070011_MAR_0s_L22	IT4070011_MAR_0s_PT2				
	IT4070011_MAR_0s_P127	Cultivated chestnut grove	*Castanea sativa*	60	210
	IT4070011_MAR_0s_P131	Cultivated chestnut grove	*Castanea sativa*	140	390
IT4070016_MAR_0s_L16	IT4070016_MAR_0s_P112	Cultivated chestnut grove	*Castanea sativa*	100	300
	IT4070016_MAR_0s_P113	Cultivated chestnut grove	*Castanea sativa*	100	300
IT4070016_MAR_0s_L25	IT4070016_MAR_0s_P122	Cultivated chestnut grove	*Castanea sativa*	100	300
	IT4070016_MAR_0s_P123	Cultivated chestnut grove	*Castanea sativa*	100	300
IT4070024_MAR_0s_L13	IT4070024_MAR_0s_T20	Tree-lined avenue	*Sophora japonica*	40-100	180-235
	IT4070024_MAR_0s_T22	Woods	*Populus nigra*	70-90	350-440
	IT4070024_MAR_0s_T23	Woods	*Populus nigra*	70-90	350-625
IT4090003_MAR_0s_L20	IT4090003_MAR_0s_T24				
	IT4090003_MAR_0s_T26				
FuoriSIC_MAR_0s_L13	FuoriSIC_MAR_0s_T19	Tree-lined avenue with 4 parallel rows	*Sophora japonica*	40-100	180-235
	FuoriSIC_MAR_0s_T21	Tree-lined avenue with 4 parallel rows	*Sophora japonica*	40-100	180-235
FuoriSIC_MAR_0s_L14	FuoriSIC_MAR_0s_T102				
IT4050020_MEOR_0s_L2	IT4050020_MEOR_0s_P1	Broadleaf forest, cultivated chestnut	*Castanea sativa*	secolare	715
	IT4050020_MEOR_0s_P3	Broadleaf forest, cultivated chestnut	*Castanea sativa*	secolare	600
	IT4050020_MEOR_0s_P4	Broadleaf forest, cultivated chestnut	*Castanea sativa*	secolare	420
IT4050020_MEOR_0s_L3	IT4050020_MEOR_0s_P6	Broadleaf forest, cultivated chestnut	*Castanea sativa*	Secolare	310
IT4040002_MEC_0s_L010	IT4040002_MEOR_0s_T047				
IT4040003_MEC_0s_L002	IT4040003_MEOR_0s_P008	Natural environment with vegetation: chestnut grove	*Castanea sativa*	40	110
IT4020021_MEOC_0s_L02	IT4020021_MEOC_0s_T004				
ParcoCentoLaghi_MEOC_L09	ParcoCentoLaghi_MEOC_T038	Chestnut grove	*Castanea sativa*	50	167

Table 8. Description of the environment and of the habitat tree where specimens of *O. Eremita* have been captured or spotted within Natura 2000 sites.

The following tables show nominally and in chronological order the locations where the presence of *R. alpina* has been comprehensively reported in Emilia-Romagna from 1900 to 2015.

The first table refers to data extracted from the Emilia-Romagna Region database; the second table refers to data from the monitoring carried out within the Life MIPP project.

Report date	Location or Natura 2000 site	Municipality and Province
1900	Monte Modino	Frassinoro (MO)
1955	Alfero, loc. Alpe della Moia	Verghereto (FC)
1955	Campigna, Foresta di Campigna	Santa Sofia (FC)
1959	Campigna, Foresta di Campigna	Santa Sofia (FC)
1959	Le Balze	Verghereto (FC)
1960	Castel dell'Alpe	Castel dell'Alpe (FC)
1960	Monte Fumaiolo	Verghereto (FC)
1964	La Lama, Foresta della Lama	Bagno di Romagna (FC)
1969	Campigna, Foresta di Campigna	Santa Sofia (FC)
1984	Passo della Calla	Passo della Calla (FC)
1989	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
1990	SIC IT4080005 Monte Zuccherodante (FC)	Bagno di Romagna (FC)
1990	Le Grigiole	Bagno di Romagna (FC)
1991	La Lama, Foresta della Lama	Bagno di Romagna (FC)
1991	Foresta della Lama, Vetreria	Bagno di Romagna (FC)
1994	Campigna, Foresta di Campigna	Santa Sofia (FC)
1994	Passo dei Mandrioli	Passo dei Mandrioli (FC)
1995	Campigna, Foresta di Campigna	Santa Sofia (FC)
1995	Lago di Pratignano dintorni, SIC-ZPS Monte Cimone, Libro Aperto, Lago di Pratignano	Fanano (MO)
1995	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
1995	SIC IT4010012 Val Boreca, Monte Lesima	(PR)
1995	SIC IT4020010 Monte Gottero	Albareto (PR)
1995	SIC IT4010003 Monte Nero, Monte Maggiorasca, La Ciapa Liscia	Ferriere (PC)
1995	SIC IT4020008 Monte Ragola, Lago Moo', Lago Bino	(PR)
1995	SIC-ZPS IT4030004 Val D'Ozola, Monte Cusna	(RE)
1999	La Lama, Foresta della Lama	Bagno di Romagna (FC)
1999	Monte Penna	Bagno di Romagna (FC)
1995	SIC IT4020007 Monte Penna, Monte Trevine, Groppo, Groppetto	(PR)
1999	Poggio La Guardia	Bagno di Romagna (FC)
1999	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
2000	Campigna, Foresta di Campigna	Santa Sofia (FC)
2000	Alfero, loc. Alpe della Moia	Verghereto (FC)
2000	Foresta della Lama, Vetreria	Bagno di Romagna (FC)
2000	Le Balze	Verghereto (FC)
2000	Monte Falco	Monte Falco (FC)
2000	Monte Fumaiolo	Verghereto (FC)
2000	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
2003	Lago di Pratignano dintorni, SIC-ZPS Monte Cimone, Libro Aperto, Lago di Pratignano	Fanano (MO)
2004	Campigna, Foresta di Campigna	Santa Sofia (FC)
2006	Corniolo, Lago di Corniolo	Santa Sofia (FC)
2006	La Lama, Foresta della Lama	Bagno di Romagna (FC)
2006	San Benedetto al Bucine	Premilcuore (FC)
2007	tra Libro Aperto e Monte Cimone	Fanano (MO)
2008	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
2009	Le Grigiole	Bagno di Romagna (FC)
2009	Riserva Integrale di Sasso Fratino	Bagno di Romagna (FC)
2012	Strada per le Tagliole, SIC IT4040002 Monte Rondinaio, Monte Giovo	(MO)
2013	San Paolo in Alpe, SIC/ZPS IT4080003 Monte Gemelli, Monte Guffone	Santa Sofia (FC)
2013	San Paolo in Alpe verso Poggio della Serra, SIC/ZPS IT4080003 Monte Gemelli, Monte Guffone	Santa Sofia (FC)

Table 9. Occurrence of *R. alpina* in Emilia-Romagna from 1891 to 2013 – Data extracted from the database of species reporting (2014 update) of the Servizio Aree protette, Foreste e Sviluppo della Montagna (*Protected Areas, Forests and Mountain Development Service*) of the Emilia-Romagna Region.

Report date	Location or Natura 2000 site	Municipality and Province
2010	Casa forestale della Lama e Foresta La Lama	(FC)
2010	Camaldoli	Camaldoli (AR)
2012	Metaleto di Camaldoli	Metaleto di Camaldoli (AR)
2014	Metaleto di Camaldoli	Metaleto di Camaldoli (AR)
2014	Poggio Scali	Poggio Scali (AR)
2015	Monte Penna	Monte Penna (FC)
2015	Casa forestale della Lama e Foresta La Lama	(FC)
2015	Faltroncella	Faltroncella (FI)
2015	Bivacco Citerna	Bivacco Citerna (FI)

Table 10. Reporting from Life MIPP data.

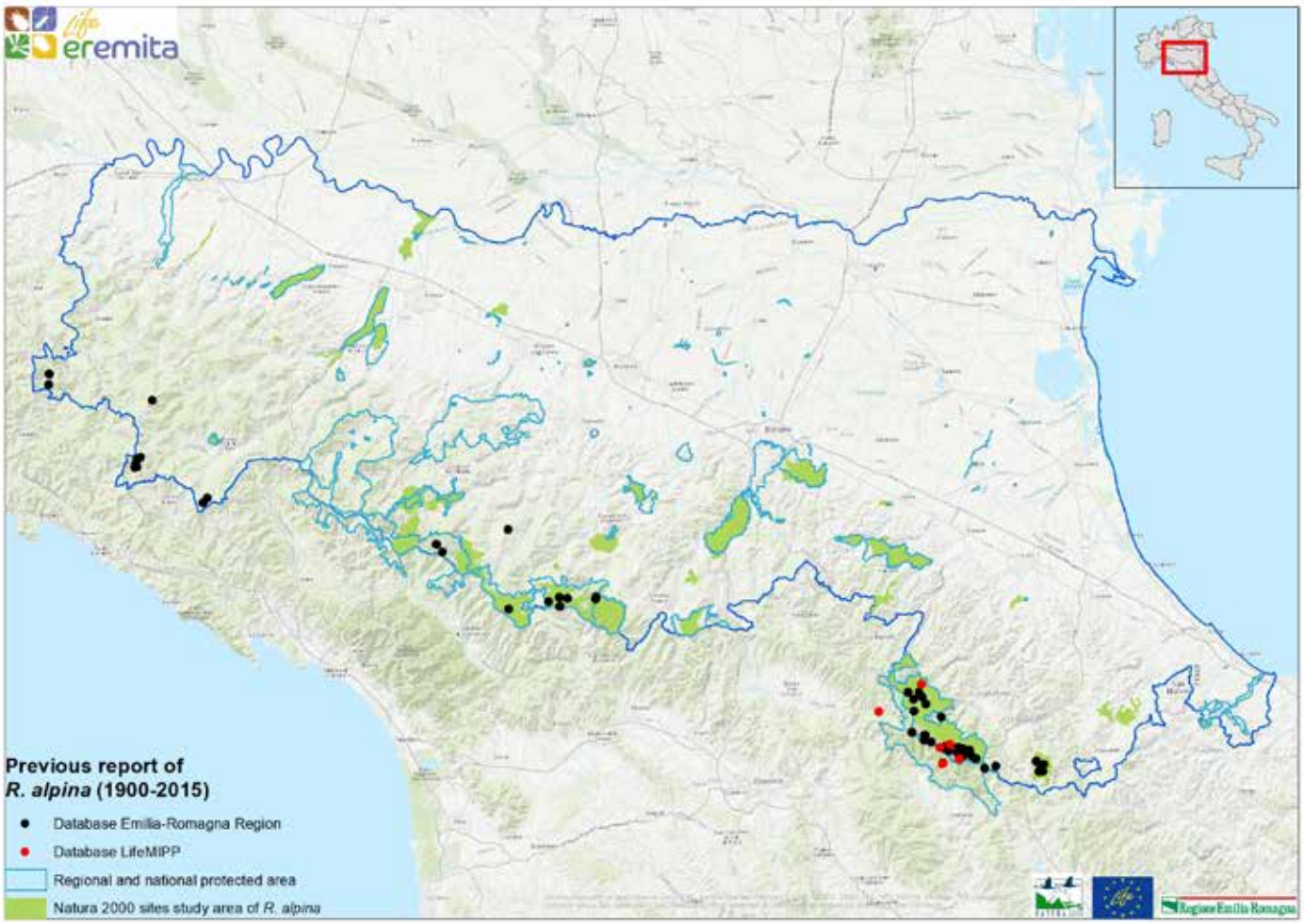

Figure 5. Distribution of previous reports of *R. alpina* presence in The Emilia-Romagna Region (1900-2015 data).

Within the monitoring of the LIFE Eremita project (2016-2017 biennium), 110 specimens were contacted inside four Natura 2000 sites (IT408003, IT408002, IT514005, SIC IT4030004) and in two stations outside SCI. Furthermore, three habitat trees have been identified with the presence of holes ascribable to the species, two in the IT408003 site and the other one in the IT405002 site. Also for *R. alpina*, most of the detections (no. 90) fall within the Natura 2000 network; 20 specimens have been reported in non-SCI areas but in any case adjacent to the Network. There were no re-captures of the marked specimens (Table 11).

Natura 2000 site	Transect ID	N° of reported specimens 2016	N° of reported specimens 2017	N° of reported specimens total	Monitoring method
Outside SCI	Fuori SIC_PNFC_Ros_L009	12	0	12	CMR
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Ros_L001	0	5	5	CMR
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Ros_L002	25	24	49	CMR
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Ros_L003	6	10	16	CMR
Fuori SIC	FuoriSIC_PNFC_Ros_L005	0	2	Holes (2)	VES
IT4080003 Monte Gemelli, Monte Guffone	IT4080003_PNFC_Ros_L006	0	16	16	CMR
IT5140005 Muraglione Acquacheta	IT5140005_PNFC_Ros_L008	2	0	2	CMR
Outside SCI	FuoriSIC_PNATE_Ros_L014	0	8	8	VES and CMR
IT4030004 Val d'Ozola, Monte Cusna	IT4030004_PNATE_Ros_L010	2	0	2	VES
IT4050002 Corno alle Scale	IT405002_MEOR_Ros_L007	0	1	Hole (1)	VES

Table 11. Sites of presence of *Rosalia alpina* detected from 2016 to 2017 as part of the *ex-ante* monitoring of the LIFE Eremita project; transects per site; number of individuals captured or sighted along the transects in the survey area. The "outside SCI" transect name refers to areas outside the Natura 2000 network, albeit bordering it.

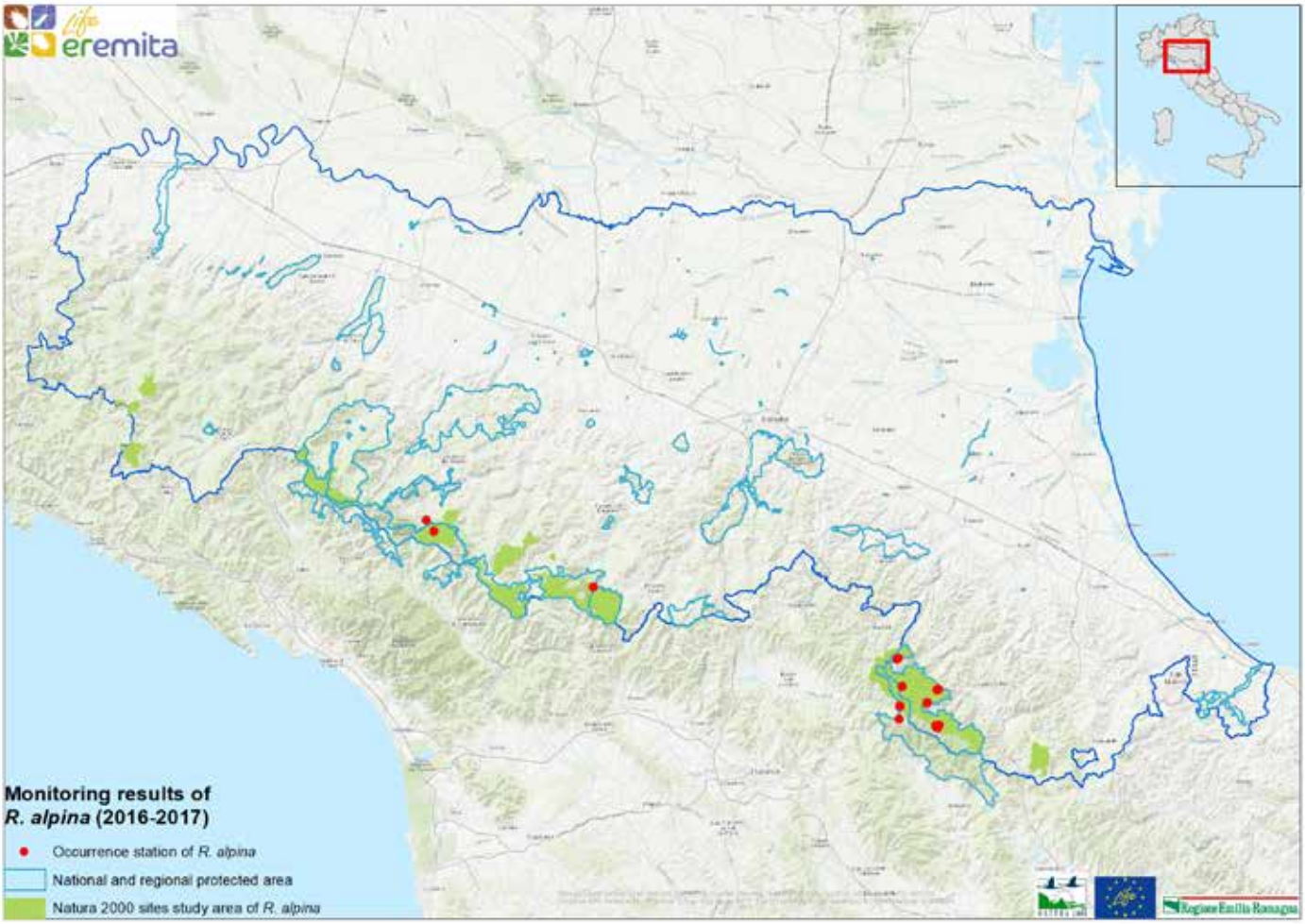

Figure 6. Distribution of new detections of *Rosalia alpina* in Emilia - Romagna as part of the Life Eremita project (2016-2017).

Of the n. 90 specimens captured or sighted within the Natura 2000 Network, 59 fall into two types of habitats of Community interest in Annex I of the Habitats Directive 42/93 / EEC, which correspond to two different types of beech woods: 9130 "*Asperulo-Fagetum* beech forests" and 9220 "Apennine beech forests with *Abies alba* and beech forests with *Abies nebrodensis*". No specimens have been found in the beech wood habitats 9110 "*Luzulo-Fagetum* beech forests" and 9210 * "Apennine beech forests with *Taxus* and *Ilex*", both present in the Natura 2000 sites in Emilia-Romagna (Table 13).

SCI	Habitat code	Number of specimens identified
IT4080003	9130	36
IT4080003	9220	22

Table 12. Number of individual specimens of *Rosalia alpina* captured or spotted within Natura 2000 habitats.

Discussion

Most of the places where *Osmoderma eremita* is found, extracted from previous data from the regional database (1891-2012), are located along the Via Emilia area. This distribution is probably due to the fact that in the past the species has not been the subject of in-depth specialist studies; in fact, the reports refer mostly to amateur entomologists who have carried out occasional searches. Therefore, the almost complete lack of reports in the Apennine belt probably does not correspond to a real absence of the species but to the lack of investigations in this territorial area where amateurism in the past was less widespread; in fact, the few reports in the Apennine area start from the year 2000, when with the development of the Natura 2000 network, greater emphasis was given to field studies.

During the Life Eremita monitoring (2016-2017), although it has not been possible to define estimates on the population abundance with more stringent methods, due to the absence of recaptures of the marked specimens, the most frequent sightings occurred in the Apennine belt, hilly and mountainous, where the presence of habitats suitable for the species is today more extensive. In fact, many specimens have been reported in habitat 9260 - *Castanea sativa* woods - where we still find centuries-old chestnut trees with deep cavities rich with boring dust. Finding the species in the sites that have been investigated in the Apennine belt is of considerable importance since in most of these areas it had never been previously reported. The survey has thus allowed proposing the inclusion of the species in 7 forms of the Natura 2000 Network (IT4030001, IT4030002, IT4030003, IT4030005, IT4050004, IT4070016, IT4090003). In the plains, on the other hand, the species has been confirmed in only one of the four monitored sites, where its presence was already known.

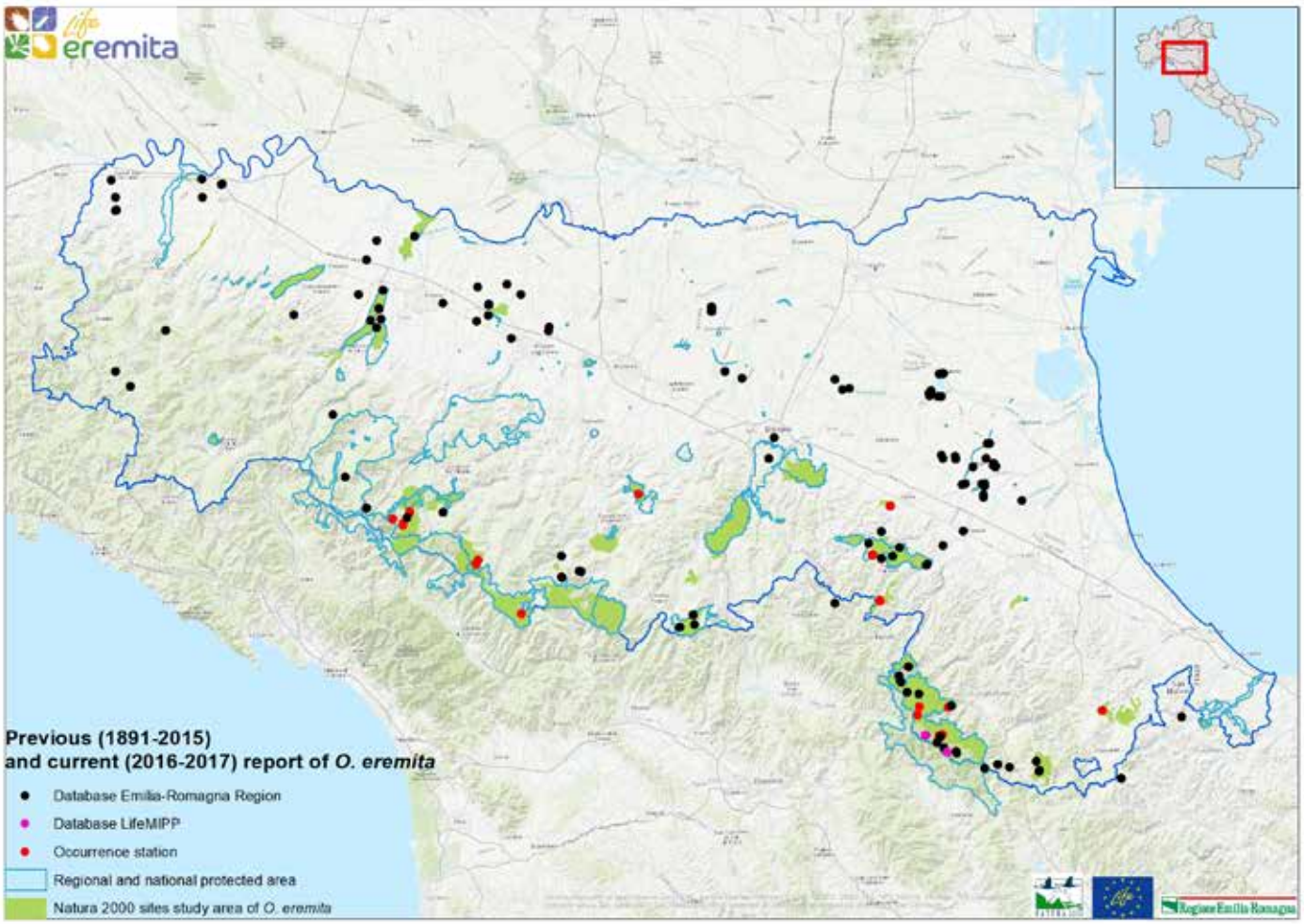

Figure 7. Presence of *Osmoderma eremita* in Emilia-Romagna: comparison between the historical data (1891- 1999) and the more recent ones, from 2000-2017, following the boost in the research in Emilia-Romagna prompted by the implementation of the Habitats Directive 92/43/ EEC with Presidential Decree 357/97.

Analysing overall all the series of available reports, those of previous data from the regional database and the data of the Life MIPP project, and new data on species of the Life Eremita project, it is noted that the distribution of data after year 2000 remains mostly confined to the Apennine sector. In the plains only very few Natura 2000 sites between the Bolognese, Ferrara and Ravenna plains, where the species has recently been detected on ancient willow trees or rows of mulberries (Fabbri R., 2013) or, with the monitoring of the Life Eremita project, on city avenues and old rows of planted cultivation. The picture that emerges, however, highlights a low presence of *O. eremita* in the plains, where the ancient trees present in the rows of vineyards have practically disappeared since the '70s and with them the preferential habitats for the species. Until the 70s of the last century, the planted vine cultivation was still widespread in the plain and in the very first hilly belt, therefore the historical reports are ascribable to the numerous and ancient hollow trees (called "stakes") that, once pollarded (Sebek *et al*., 2013), supported the vine. Since the end of the seventies of the last century the situation has changed rapidly, and most of the trees in the cultivated vineyards and along the watercourses have been eliminated. Fortunately, there are still several city avenues with pollarded and hollowed trees, able to act as substitutes for the old trees of the woods (Kadej *et al*., 2016). Therefore, many of the breeding sites of the species do not exist anymore and have left space, in the plains and in the first hill, to extensive agriculture where the only massively present trees generally are orchards and vineyards, mostly supported with concrete poles.

As regards *Rosalia alpina*, the historical series of presence data in the range of the Emilia-Romagna highland beech-woods shows few, dated and generic reports in some Natura 2000 sites in the western Emilia-Romagna area (Parma and Piacenza), never confirmed in the past. The reports for the provinces of Modena and Forlì-Cesena are more consistent over time. Here they are mainly concentrated in the National Park of the Casentinesi Forests.

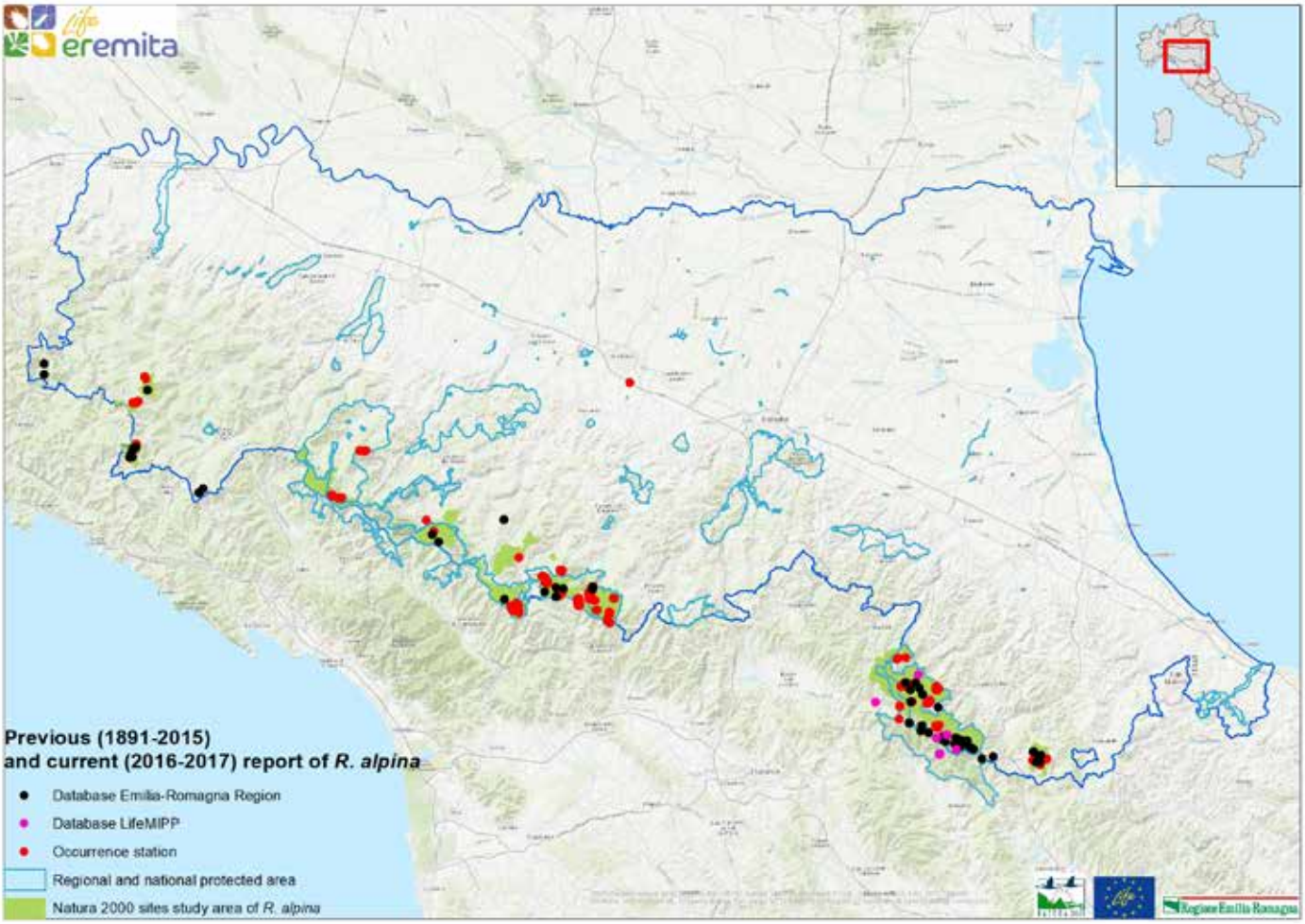

Figura 8. Presence of *Rosalia alpina* in Emilia-Romagna: comparison between the historical data (1891- 1999) and the more recent ones, from 2000-2017, following the boost in the research in Emilia-Romagna prompted by the implementation of the Habitats Directive 92/43/ EEC with Presidential Decree 357/97.

With the *ex-ante* monitoring of the Life Eremita project, the species was found in the mountain belt of the two National Parks present in the region. Most sightings are in the woods of the National Park of the Casentinesi Forests, where 101 specimens and 2 eclosion holes were counted. Inside the Park, the species has been found in almost all the inspected transects. Highly suitable habitat trees are mostly found along ridge areas and areas exposed to lightning, where the plants are more perishable and therefore more suited to the colonization of *R. alpina*. Although it has not been possible to produce a formal estimate of the population size, the interpretation of the results makes it possible to state that the species is distributed fairly evenly throughout the Park territory. Many habitat trees have been found suitable for its presence and this represents a strong point. Furthermore, the bibliographic research and the collection of previous data (even recent ones) showed a capillary distribution of the reports on the entire protected area, an indication of an even greater diffusion of the species compared to the findings in the present study. In fact, it should be recalled that the project area within the Park was conveniently limited to areas not subject to monitoring by the Life MIPP project, precisely in order to cover, with the Eremita project, the less investigated areas of the park. The results enabled the proposal to update the IT408002 SCI form.

As for the Tuscan-Emilian Apennine National Park, the presence of *R. alpina* has been ascertained in areas located in the central and southern part of the Park (Val d'Ozola, Cerreto Alpi and Civago), areas in which the species was not previously known. In the current state of knowledge, the species seems to be missing in the northern area of the Park, mainly due to the lack of habitat trees. With the VES method no individuals of *R. alpina* were detected in the transects, only remains of specimens.

The discovery of the eclosion holes within the boundaries of the SCI IT4050002 - Corno alle Scale (BO), a few meters from the border with the Modena SCI IT4040001, indicates the presence of the species in the Central-Eastern Emilia area. The few reports, both historical and recent, refer to sites in the Parco dell'Alto Appennino Modenese (*High Apennines of Modena Park*), but they are rather disconnected from one another and always concern single specimens. This leads to the supposition that the present population is now relict. In the remaining part of the territory investigated, despite the effort and the considerable number of areas and plants examined (over 71% of the examined habitat trees are found in Emilia), the species has not been spotted. Almost all the beech woods investigated do not have characteristics suitable for the species because of the predominant crop types that over time have led to very young coppice woods, with small trees, and the absence of noteworthy quantities of dead wood.

Conclusions

Thanks to the Life Eremita project, for the first time in Emilia-Romagna a monitoring of *Osmoderma eremita* was so broadly

organised that it simultaneously covered 34 sites of the Natura 2000 network, in environments potentially suitable for hosting the species, also detecting new presence stations. Finding of the species in the sites investigated in the Apennine belt is of considerable importance since in most of these areas it had never been previously reported. Given the previous knowledge and the results of the monitoring carried out, however, it is not possible to provide a complete picture of its dissemination on the regional territory. The knowledge of the current distribution of the species in the region is affected, in particular, by the scarcity of data due to the difficulty of observing this insect and the lack of specific research conducted by trained personnel and on a wider territorial scale. However, it can be confirmed that at present in the regional territory the species is in strong rarefaction, very localised and with a definitely fragmented distribution. The current diffusion of the species is a consequence of the generally inadequate management of forest environments and in particular of the decayed and hollowed trees that make up the reproductive sites and the only life environment. For these reasons, interventions that lead to a restoration of suitable environments, with an increase in habitat trees suitable for the species, turn out to be of fundamental importance for the conservation of the residual populations in the distribution area of the species. At the same time, it is essential to define and implement specific management measures for the management of forest environments, and in particular the habitat 9260 - *Castanea sativa* woods.

Also for *Rosalia alpina* for the first time in Emilia-Romagna a monitoring was organised that took place at the same time in all Natura 2000 sites with beech woods, from the Emilia to the Romagna Apennine area. The survey has outlined a regional distribution framework of the species, which is rather fragmented. In the beech woods of the Romagna Natura 2000 sites, the species has been confirmed only within the National Park of the Casentinesi Forests, where most of the reports are concentrated, mainly due to the availability of suitable habitat trees. As far as the Emilian mountain is concerned, for the first time the presence of the species has been documented in the central and southern part of the National Park of the Tuscan-Emilian Apennines, in areas where it was not previously known. Evidence was also found of the presence of *Rosalia alpina* in central-eastern Emilia, in an Apennine area where in the past it had already been reported (high Apennines of Modena). However, the scarce numbers obtained for the beech woods of the Emilia Apennines, despite the efforts made, suggest that these are relict and residual populations. The main critical issues for the conservation of *Rosalia alpina* are represented by extreme scarcity of dead wood and old trees, due to the aim at production in the management of the woods, especially those privately owned.

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ADDENDUM - Natura 2000 sites of the survey area with the indication for each of the extent, the forest habitats of Annex I of the Habitats Directive and related areas, the managing body, the province and the municipality on which the site is situated

Natura 2000 sites	Species	Area (ha)	Forest habitats	Area (ha)	Management bodies	Province	Municipality
IT4070011 Vena del Gesso Romagnola	Os	5.540	9180 91AA 91E0 9260 92A0 9340	14,2 92,48 29,58 51,24 49,12 25,48	Management body for the parks and biodiversity Romagna	BO-RA	Borgo Tossignano Fontanelice Imola Brisighella Casola Valsenio Riolo Terme
IT4090001 Onferno	Os	273	9180 91AA 92A0	1,04 14,7 3,2	Management body for the parks and biodiversity Romagna	RN	Gemmano
IT4050004 Bosco della Frattona	Os	392	91AA 91L0 92A0	17,78 5,81 5,82	Management body for the parks and biodiversity Romagna	BO	Imola Dozza
IT4070024 Podere Pantaleone	Os	9	Na	Na	Comune di Bagnacavallo Emilia-Romagna Region	RA	Bagnacavallo
IT4080004 Bosco di Scardavilla, Ravaldino	Os	454	91AA 91L0 92A0	6,41 7,92 17,59	Management body for the parks and biodiversity Romagna Emilia-Romagna Region	FC	Meldola Forlì
IT4070016 Alta Valle del Torrente Sintria	Os	1174	9180 91AA 91E0 9260 92A0	0,01 15,4 5,96 43,66 18,88	Management body for the parks and biodiversity Romagna	RA	Brisighella Casola Valsenio
IT4 080008 Balze di Verghereto, Monte Fumaiolo, Ripa della Moia	Os Ros	2461	9130 9180 9210 9220 9260	88,47 23,79 22,06 111,61 4,68	Emilia-Romagna Region	FC	Verghereto
IT4090003 Rupi e Gessi della Valmarecchia	Os	2526	9180 91AA 91E0 9260 92A0 9340	22,75 290,12 2,05 48,18 61,72 12,38	Management body for the parks and biodiversity Romagna Emilia-Romagna Region	FC-RN	Mercato Saraceno Maiolo Novafeltria San Leo Talamello
IT4030007 Fontanili Corte Valle Re	Os	877	91E0 91F0 92A0	0,17 1,93 17,22	Management body for the parks and biodiversity Central Emilia Comune di Reggio Emilia Emilia-Romagna Region	RE	Reggio nell'Emilia Campegine Cadelbosco di Sopra Sant'Ilario d'Enza
IT4040003 Sassi di Roccamalatina e di Sant'Andrea	Os	1198	91E0 9260 92A0	12,29 122,72 11,44	Management body for the parks and biodiversity Central Emilia Emilia-Romagna Region	MO	Guiglia Marano sul Panaro Zocca
IT4030011 Casse di espansione del Secchia	Os	277	92A0	105,57	Management body for the parks and biodiversity Central Emilia Emilia-Romagna Region	RE-MO	Rubiera Campogalliano Modena
IT4040004 Sassoguidano, Gaiato	Os	2419	9180 91AA 91E0 9260 92A0	3,04 10 0,34 160,14 47,39	Management body for the parks and biodiversity Central Emilia Emilia-Romagna Region	MO	Pavullo nel Frignano Montese Sestola
IT4040001 Monte Cimone, Libro Aperto, Lago di Pratignano	Os Ros	5174	9130 91E0 9260	147,48 9,26 57	Management body for the parks and biodiversity Central Emilia Emilia-Romagna Region	MO	Fanano Fiumalbo Montecreto Riolunato Sestola
IT4040002 Monte Rondinaio, Monte Giovo	Os Ros	4848	9130 91E0 9260	294,74 5,15 12,67	Management body for the parks and biodiversity Central Emilia Emilia-Romagna Region	MO	Fiumalbo Frassinoro Pievepelago
IT4020017 Aree delle risorgive di Viarolo, Bacini di Torrile, Fascia golenale del Po	Os	2622	91E0 91F0 92A0	0,06 3,57 69,8	Management body for the parks and biodiversity Western Emilia Emilia-Romagna Region	PR	Sissa Trecasali Torrile Colorno Parma
IT4020021 Medio Taro	Os	3810	91E0 91F0 92A0	16,32 1 384,7	Management body for the parks and biodiversity Western Emilia Emilia-Romagna Region	PR	Collecchio Fornovo di Taro Medesano Noceto Parma Fontevivo Solignano Varano de' Melegari

Natura 2000 sites	Species	Area (ha)	Forest habitats	Area (ha)	Management bodies		Province	Municipality
IT4020001 Boschi di Carrega	Os	1277	91L0 9260 92A0	329,02 171,97 6,1	Management body for the parks and biodiversity Western Emilia		PR	Collecchio Sala Baganza
IT4010017 Conoide del Nure e Bosco di Fornace Vecchia	Os	580	91L0 92A0	10,53 56,28	Emilia-Romagna Region		PC	Podenzano Ponte dell'Olio San Giorgio Piacentino Vigolzone
IT4010008 Castell'Arquato, Lugagnano Val d'Arda	Os	280	9260 92A0	2,9 3,46	Management body for the parks and biodiversity Western Emilia	Emilia-Romagna Region	PC	Castell'Arquato Lugagnano Val d'Arda
IT4020003 Torrente Stirone	Os	2747	91F0 91L0 9260 92A0	1,24 2,96 3,89 164,38	Management body for the parks and biodiversity Western Emilia	Emilia-Romagna Region	PC-PR	Alseno Fidenza Salsomaggiore Terme Vernasca Pellegrino Parmense
IT4020026 Boschi dei Ghirardi	Os	306	91E0 9260	0,15 9,21	Management body for the parks and biodiversity Western Emilia	Emilia-Romagna Region	PR	Albareto Borgo Val di Taro
IT4030005 Abetina Reale Alta Val Dolo	Os	3444	9110 9130 9180 91E0 9220 9260	382,99 109,81 1,74 10,97 303,5 63,47	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Villa Minozzo
IT4030009 Gessi Triassici	Os	1907	9180 91E0 9260 92A0	9,81 10,62 151,94 46,51	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Castelnovo ne' Monti Ventasso Villa Minozzo
IT4030002 Ventasso	Os	2909	9110 9130 9220 9260	32,13 77,46 12,16 278,51	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Ventasso
IT4030003 Monte la Nuda Cima Belfiore Passo Cerreto	Os	3462	9110 9130 91E0 9220 9260	106,54 379,56 24,9 58,17 98,81	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Ventasso
IT4030004 Val d'Ozola Monte Cusna	Os Ros	4878	9110 9130 91E0 9220 9260	1111,81 179,81 34,02 43,49 13,36	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Ventasso Villa Minozzo
IT4030009 Gessi Triassici	Os	1907	9180 91E0 9260 92°0	9,81 10,62 151,94 46,51	Tuscan Emilian Apennines National Park	Emilia-Romagna Region	RE	Ventasso Castelnovo ne' Monti Villa Minozzo
IT4080003 Monte Gemelli, Monte Guffone	Os Ros	13351	9130 9180 91E0 9210 9220 9260 92A0 9340	1192,92 582,38 23,26 69 371,44 204,69 11,45 4,03	Casentinesi Forests, MonteFalterona and Campigna National Park	Emilia-Romagna Region	FC	Bagno di Romagna Portico e San Benedetto Premilcuore Santa Sofia
IT4080002 Acquacheta	Os Ros	1656	9130 9180 91E0 9220 9260 92A0	106,01 18,76 13,07 4,55 56,6 0,32	Casentinesi Forests, MonteFalterona and Campigna National Park	Emilia-Romagna Region	FC	Portico e San Benedetto Tredozio
IT4050020 Laghi Suviana e Brasimone	Os	1902	9130 91E0 9210 9260 92A0	24,44 0,75 9,71 130,21 0,46	Management body for the parks and biodiversity Eastern Emilia		BO	Camugnano
IT4050001 Gessi Bolognesi, Calanchi dell'Abbadessa	Os	4296	9180 91AA 91L0 9260 92A0 9340	0,31 169,74 26,8 14,29 99,53 0,51	Management body for the parks and biodiversity Eastern Emilia	Emilia-Romagna Region	BO	Ozzano dell'Emilia Pianoro San Lazzaro di Savena

Natura 2000 sites	Species	Area (ha)	Forest habitats	Area (ha)	Management bodies		Province	Municipality
IT4050002 Corno alle scale	Os Ros	4578	9110 9130 9180 91E0 9210 9220 9260 92A0	337,95 195,21 5,17 3,88 129,65 208,67 463,13 1,46	Management body for the parks and biodiversity Eastern Emilia		BO	Lizzano in Belvedere
IT4050003 Monte Sole	Os	6476	9180 91AA 91E0 9260 92A0 9340	37,35 23,79 4,76 797,17 80,96 8,28	Management body for the parks and biodiversity Eastern Emilia	Emilia-Romagna Region	BO	Grizzana Morandi Marzabotto Monzuno Sasso Marconi
IT4050013 Monte Vigese	Os	618	91AA 9130 9180 9260 9340	37,84 1,87 5,29 158,34 12,26	Emilia-Romagna Region		BO	Camugnano Grizzana Morandi
IT4020020 Crinale dell'Appennino parmense	Ros	5280	9110 9130 91E0 9210 9220 9260	5 2671,51 16,16 3,74 13,52 16,61	Management body for the parks and biodiversity Western Emilia	Tuscan Emilian Apennines National Park	PR	Corniglio Monchio delle Corti
IT4010003 Monte Nero, Monte Maggiorasca, La Ciapa Liscia	Ros	852	9110 9130 9220 9430	547,95 3,73 31,99 36,18	Emilia-Romagna Region		PC-PR	Ferriere Bedonia
IT4020007 Monte Penna, Monte Trevine, Groppo, Groppetto	Ros	1689	91E0 9260	10,18 99,55	Emilia-Romagna Region		PR	Bedonia Tornolo
IT4020008 Monte Ragola, Lago Moo, Lago Bino	Ros	1398	9110 91E0 9210 9430	601,92 0,44 1,1 8,32	Emilia-Romagna Region		PC-PR	Ferriere Bardi Bedonia
IT4040005 Alpesigola, Sasso Tignoso e Monte Cantiere	Ros	3762	9130 91E0 9210 92A0	2257,96 23,04 1,41 20,06	Emilia-Romagna Region		MO	Frassinoro Lama Mocogno Palagano Pievepelago Riolunato
IT5140005 Muraglione - Acqua Cheta	Ros	4885	91L0 9210 9260	100 1000 100	Casentinesi Forests, MonteFalterona and Campigna National Park	Tuscany Region	FI	Marradi Dicomano San Godenzo

Implementation of a catalogue of the habitat trees of *Osmoderma eremita* (Scopoli, 1763) and *Rosalia alpina* (Linnaeus, 1758) in Emilia-Romagna

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Introduction

The LIFE Eremita project made it possible to carry out an extensive survey to identify the areas potentially suitable for the reproduction of the two target species *Osmoderma eremita* and *Rosalia alpina* in Emilia-Romagna region. While monitoring the species, the research and mapping of the trees with differing degrees of suitability for the life of the two species was carried on throughout the entire project area. The goal was to start by implementing a catalogue of the habitat trees, then, crosscheck the data with the verified presence of the species. This made it possible to identify the areas and plants on which to direct the implementation of the conservation actions envisaged by the Life project (creation of habitat trees for the target species, *in situ* reproduction and introduction into the natural environment of captive bred animals).

Monitoring methods

The study area concerns a total of 48 Natura 2000 sites in Emilia - Romagna, 38 survey sites for *O. eremita* and 18 sites for *R. alpina*. This extensive field operation involved simultaneously more than 10 entomologists in two annual growth seasons of 2016 and 2017.

The evaluation of the ecological functionality of the trees for the purpose of the survey was carried out following the protocols specifically set up during the initial phases of the LIFE Eremita project.

For *O. eremita* transects were walked and identified on the basis of the presence of habitats suitable for the species. These habitats are large and veteran broadleaved trees, even centenary, alive, with hollows and large cavities rich in wood boring dust and rotting wood. These include *Quercus* spp., *Castanea sativa*, *Tilia* spp., *Salix* spp., *Populus* spp., *Fagus sylvatica*, *Morus* spp., *Platanus* spp., *Aesculus hippocastanum*, as well as cultivated and wild plants of the Rosaceae, family, *Pyrus* spp., *Malus* spp. *and Prunus* spp. (Ranius & Nilsson, 1997; Ranius, 2002; Oleksa *et al*., 2007; Dubois, 2009; Dubois *et al*., 2009).

Along the transect, a metal plate bearing the identification code has been applied to each habitat tree; each tree was geo referenced via a GPS device (model Garmin 62s), photographed, and the specific survey card was compiled.

The main morphological and ecological factors used to identify habitat trees are the following:

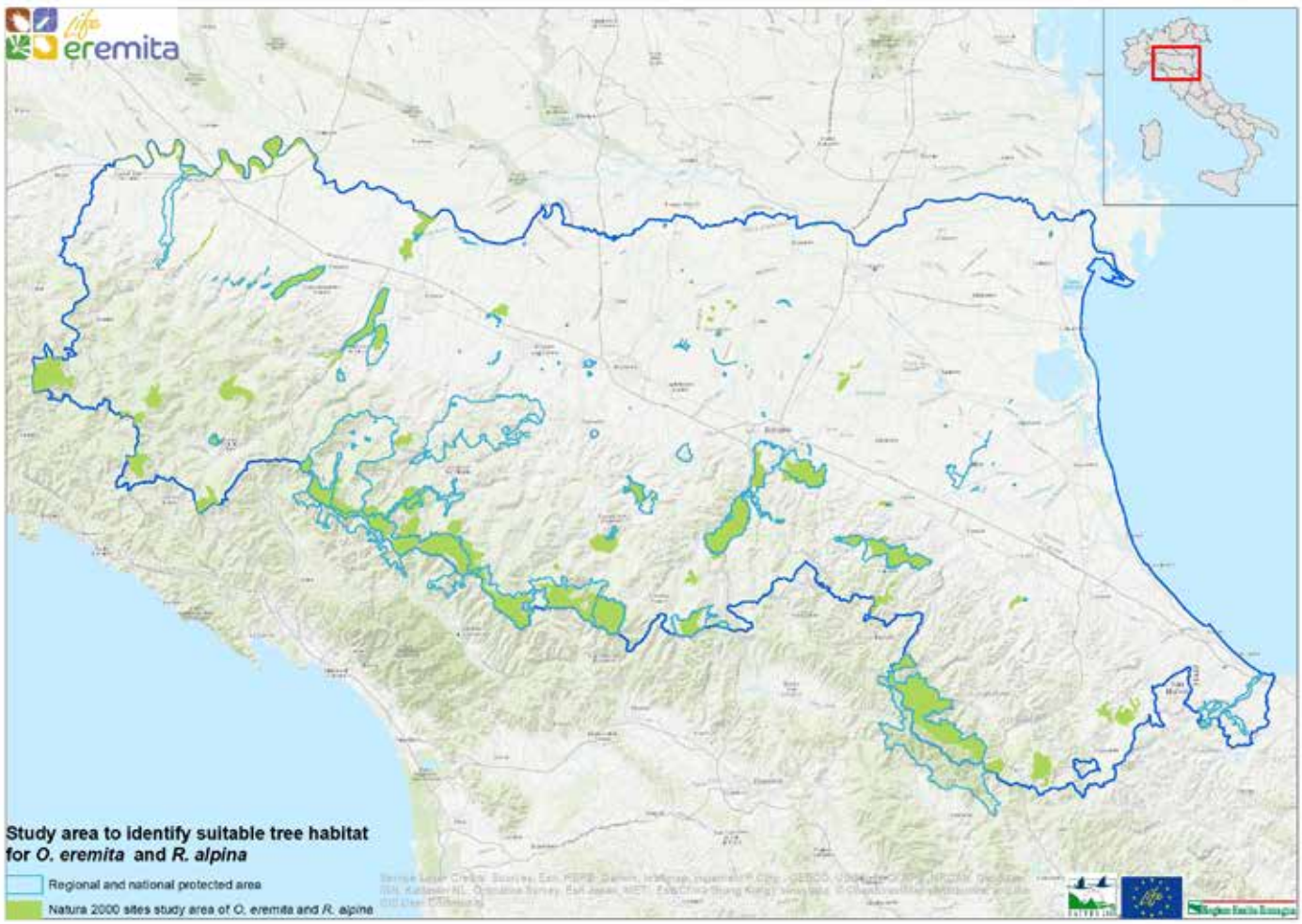

Figure 1. Survey area for the identification of habitat trees for *O. eremita* and *R. alpina*. The census was developed on 48 Natura 2000 sites in Emilia-Romagna in the two-year period 2016-2017.

- Diameter of the habitat plant at chest height (DBH -Diameter at Breast Height)
- Cavity height from the ground
- Cavity width
- Quantity of soil (in litres) present in the cavity
- Any disturbance factors
- Distance from other plants of the same size
- Distance from other hollow plants.

Figure 2. Monitoring operations of the habitat trees for *O. eremita*.

With regard to *R. alpina* the transects were identified with the same modalities used for *O. eremita*. The main morphological and ecological factors used to evaluate the functionality of the plant are:

- Presence and quantification of the dead wood parts of the plant
- Presence of fungal fructifications
- If the plant is dead, indication of the position: standing, on the ground, broken stump
- Distance between the habitat plants
- Presence of woodpiles
- Presence or absence of eclosion holes on the trunk
- Presence of larval galleries with boring dust and pupation cells.

For both species the measurements were made by an expert entomologist, covering the transects. The suitability assessment was carried out in the field on the basis of the morphological and ecological factors listed on the survey card, expressing them on a scale of values ranging from high, to medium, low and no suitability, according to the judgment of the surveyor.

All the data collected on the field survey cards were subsequently uploaded to Excel archives and inserted into the project databases.

Figure 3. Monitoring operations of the habitat trees for *R. alpina*.

Results and discussion

For *O. eremita* data was catalogued on the ecological function of 1,760 trees on 130 transects in the 38 Natura 2000 sites that were investigated. Each tree was assessed on the basis of its suitability to host the species and was mapped with a chromatic scale: green= high suitability; orange= medium suitability; yellow= low suitability; black=no suitability.

For *R. alpina* data was catalogued on the ecological function of 1,112 trees on 88 transects in the 18 Natura 2000 sites that were investigated. Each tree was assessed on the basis of its suitability to host the species and in this case also it was mapped using the same chromatic scale as for *O. eremita*.

Natura 2000 site (N)	Transects (N)	Habitat trees registered (N)	High suitability N)	Medium suitability (N)	Low suitability (N)	No suitability (N)
38	130	1760	283	605	125	747

Table 1. Number of habitat trees registered for *Osmoderma eremita*, divided according to their suitability, along the monitored transects in the investigated Natura 2000 sites.

Natura 2000 site (N)	Transects (N)	Habitat trees registered (N)	High suitability N)	Medium suitability (N)	Low suitability (N)	No suitability (N)
18	88	1112	318	198	491	105

Table 2. Number of habitat trees registered for *R. alpina*, divided according to their suitability, along the monitored transects in the investigated Natura 2000 sites.

This catalogue, superimposed on environment matrices, allows a full-scale picture (in the 38 Natura 2000 sites investigated) of the ecological function of the different environments, in order to assess the capacity of the various ecosystems to sustain the species in the long term. To this end, in the following table the results have been expressed by environment type, indicating, for each type detected in the field, the number of trees at high, medium, low and no suitability.

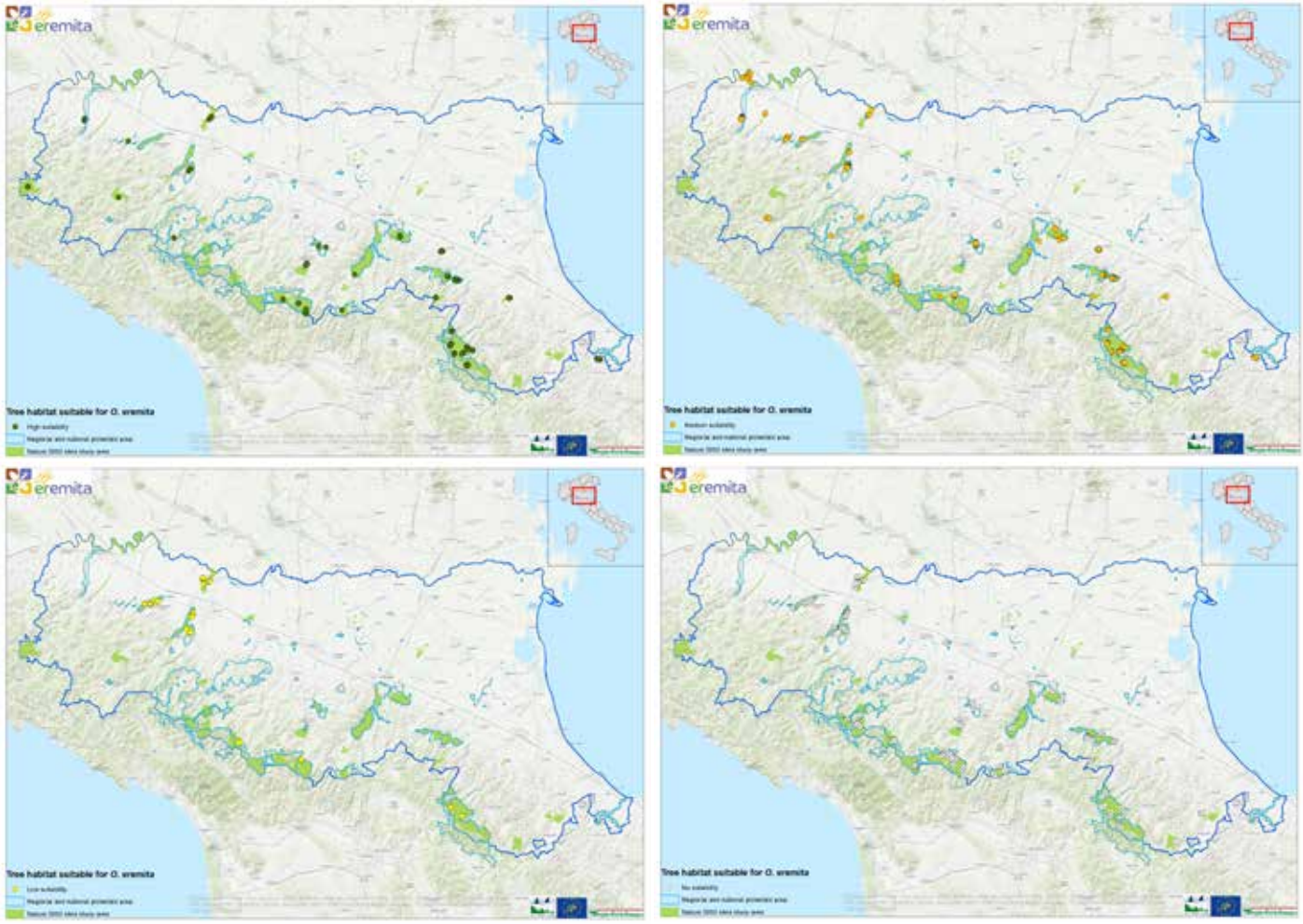

Figure 4. Mapping of the suitability of the habitat trees registered for *O. eremita* in 38 Natura 2000 sites in the survey area.

Type of environment	Transects (N)	Registered trees (N)	High suitability (N)	Medium suitability (N)	Low suitability (N)	No suitability (N)
River bank	1	5	4	1	0	0
Mesophilic broad-leaved wood	41	439	69	191	9	170
Hygrophilous wood	8	57	4	40	1	12
Mixed forest with conifers (*Pinus* spp.)	1	3	0	3	0	0
Chestnut grove for fruit production	15	76	2	0	3	71
Natural chestnut grove	14	103	11	38	2	52
Cultivation	24	492	0	32	102	358
Beech wood	16	303	24	69	0	210
Mixed beech forest	3	5	5	0	0	0
Row of trees	22	53	5	14	1	33
Walnut plantation	5	67	7	59	0	1
No information available	7	32	2	0	0	30
Public park	5	14	0	7	0	7
Oak forest	5	39	8	30	0	1
Mixed oak forest	2	69	20	49	0	0
Isolated oak tree	1	2	2	0	0	0
Pond	1	2	2	0	0	0
TOTAL	171*	1760	163	533	118	946

Table 3. Suitability of the habitat trees registered for *O. eremita*, subdivided by type of environment.
* Transects number is higher than the total number since in the same transect occur different type of environment

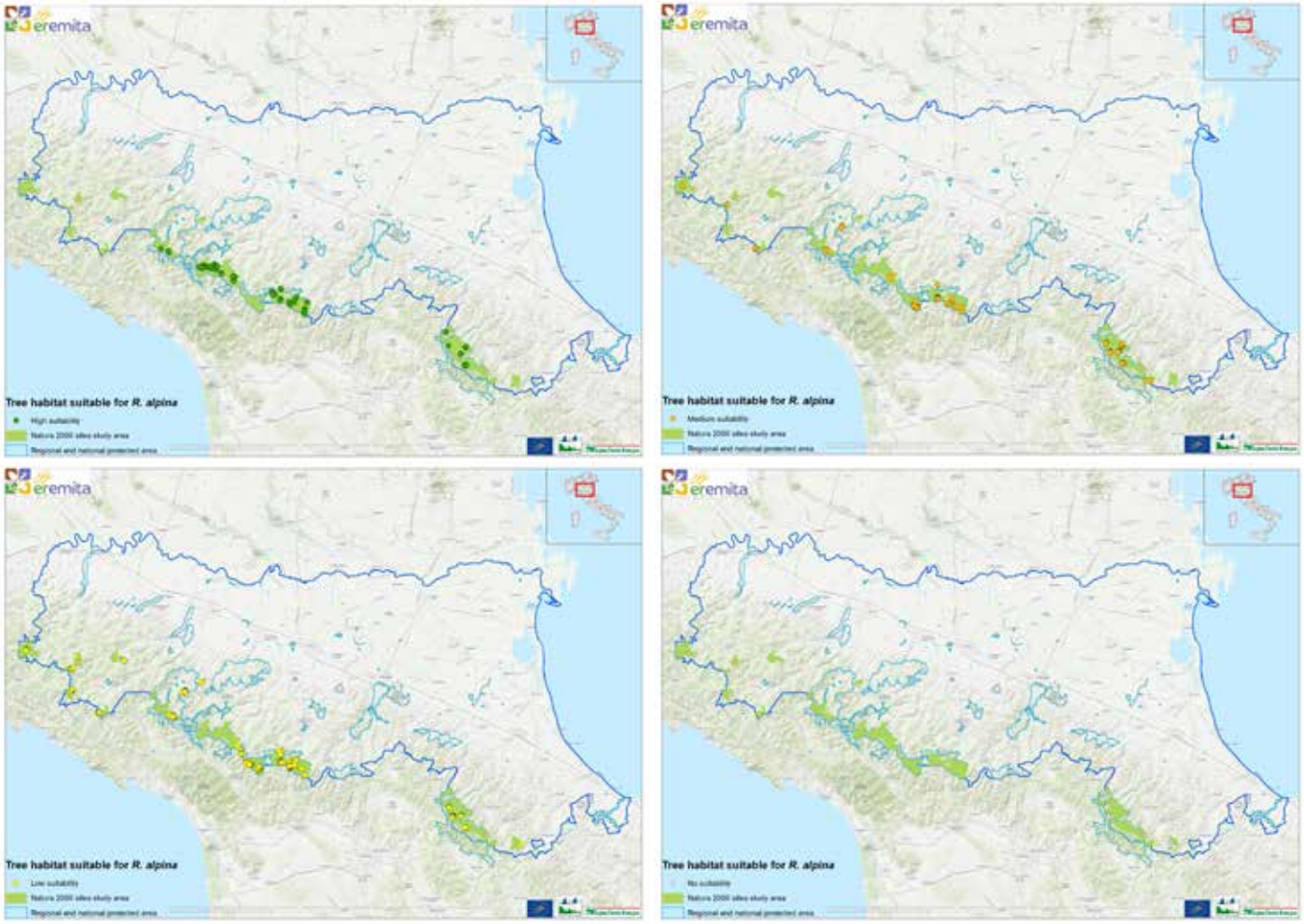

Figure 5. Mapping of the suitability of the habitat trees registered for *R. alpina* in 18 Natura 2000 sites in the survey area.

Type of environment	Transects (N)	Registered trees (N)	High suitability (N)	Medium suitability (N)	Low suitability (N)	No suitability (N)
Mixed fir and beech forest	3	23	0	0	21	2
Rock outcrops	3	9	1	0	0	8
Mesophilic broad-leaved wood	22	248	230	3	6	9
Thermophile broad-leaved wood	4	106	6	54	37	9
Hygrophilous wood	1	1	0	0	1	0
Beech wood	46	685	78	135	410	62
Ridge beech wood	3	8	1	1	6	0
Isolated beech tree	2	3	2	0	0	1
Row of trees	3	11	0	5	5	1
Margins of the pools of water	1	13	0	0	0	13
Meadow and prairie	2	5	0	0	5	0
TOTAL	88*	1112	318	198	491	105

Table 4. Suitability of the habitat trees registered for *R. alpina*, subdivided by type of environment.
* Transects number is higher than the total number since in the same transect occur different type of environment.

The picture that emerges shows that in most environmental typologies, the registered transects have habitat trees with low or zero suitability.

The situation is similar with regard to *R. alpina*, with the exception of the typology "Mesophilic broad-leaved wood" where the transects investigated have a high percentage of highly suitable plants.

Conclusions

Thanks to the extensive field operation that was carried out, it was possible to identify and map the habitat trees of the two target species of saproxylic beetles of the Life Eremita project on a large-scale.

The effort put forth in the 48 Natura sites of the region represents the first survey of this magnitude carried out in Emilia - Romagna, both in terms of territorial extension and in terms of precise return of technical results (number of trees individually assessed to determine their ecological functionality).

The results of this analysis confirm, for both species, the scarce presence in both primary and secondary habitats of conditions favourable to the expansion of their distribution area, due to the rarity of suitable habitat trees, which were found only in small and local environment matrices.

The survey has prepared a catalogue with a high number of evaluated trees (1,760 for *O. eremita* and 1,112 for *R. alpina*) in terms of ecological functionality, mapped on a GIS basis, which can be used to speed up processes of natural senescence or intervene ex-novo (e.g. with the production of cavities) to create conditions that can favour habitat expansion for the two species.

Superimposing the data of the species reports with those of the suitability of the trees makes it possible to provide a priority of intervention, applied with some of the actions of the LIFE Eremita project. The expansion of the available habitats favours the expansion of populations along the new ecological corridors thanks to the first interventions carried out. The cataloguing even of scarcely suitable habitat trees, for which it is necessary to increase the suitability, allows for the planning of new interventions in the medium and long term, also based upon the first evolution set off by the actions of the Life project. In fact, the trees with medium and low suitability on which interventions were carried out, once colonized by the species, can become themselves the barycentre for new expansion matrices.

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Actions in the habitats to widen the distribution area of *Osmoderma eremita* (Scopoli, 1763) and *Rosalia alpina* (Linnaeus, 1758)

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Introduction

After an extensive monitoring campaign aimed at verifying the distribution of *Osmoderma eremita* and *Rosalia alpina* and a parallel census of the areas and trees suitable to host the species, a plan of improvement and expansion of habitats has been designed as part of the Life Eremita project. The aim is to expand the distribution area of these two saproxylic beetles in Emilia - Romagna, guaranteeing adequate availability of reproductive sites and thus ensuring the survival over time of the two species. The interventions are also aimed at punctual reintroduction and/or reinforcement actions of the populations present, through the introduction of specimens obtained from captive and *in situ* breeding operations.

The main causes of the decline of the two species are to be ascribed to the local disappearance and/or extreme rarefaction of their preferential environments: mature broadleaved trees with large cavities for *Osmoderma eremita* and large beeches with portions of trunk or dry limbs for *Rosalia alpina*. The life cycle of both these species depends on the availability of dead or rotting wood.

The ecological role of dead wood in forest ecosystems and its importance for biodiversity has been known for a long time. However, it is only following the results of studies carried out since the late 80s that today the scientific community agrees in considering the ecological, functional and structural importance of dead wood as central for the integrity and, consequently, the stability of forest ecosystems. In fact, dead wood is not only a key factor in the forest nutrient cycle (N, P, Ca and Mg), but is also a fundamental element in the ecological, geomorphological and hydrological processes of the soil. It is very important in the calculation of the carbon pool in the forests and, above all, it is a habitat of extraordinary importance for the conservation of numerous species. The dead wood present in the forests is in fact a dynamic group of micro-habitats which, going through different levels of decomposition, is able to provide a large number of ecological niches that can host a very high number of living species. The main taxonomic groups that inhabit these habitats, with a high number of extremely specialized species, are above all mushrooms, bryophytes, lichens, but also amphibians, birds and of course beetles. Other taxonomic groups, among which mammals, molluscs, syrphidae and dipterans are present in smaller numbers, but with organisms highly specialised in living in this particular habitat and of great conservation interest.

The history of the Apennine woods of the last century, unfortunately, consists mainly of indiscriminate cutting, mostly along the Emilia-Romagna ridge, since the early decades of the 20th century. Because of the great use of wood during the First World War and in the twenty years of fascism, despite the policy of intense reforestation, there was the need for large quantities of railway sleepers; furthermore, the Second World War imposed extraordinary expenditure of timber.

Today, most of the Emilia-Romagna beech woods, consisting essentially of coppices, have mediocre or moderate growing conditions, and are very poor in terms of biodiversity. The best biological-structural conditions are found in the state forests (Rovelli, 2013).

The traditional management of forest stands has led to conditions of strong structural, compositional and age simplification of the Apennine beeches that today are monospecific, monoplane and coeval, with inevitable negative repercussions in terms of availability of dead wood in the forest and therefore biodiversity. Not only species such as white fir (*Abies alba*), english yew (*Taxus baccata*) and holly (*Ilex aquifolium*), for example, have been systematically disfavoured, also in relation to their limited ability to compete with beech, for their excessive use in the past, or because of their poor commercial value, but also the systematic elimination of large or senescent plants has negatively affected the conservation possibilities of a large number of species linked to dead wood or to specific microhabitats. This, above all, reduces the taxa biodiversity of lichens, epiphytes, birds, fungi and - obviously - saproxylic beetles. In any case, similar problems concern the use of all the Apennine woods, not to mention the lowland forests that have almost disappeared. In the case of *Osmoderma eremita*, which has a good resilience in relation to the modification of its primary habitat (mature broad-leaved woods), it can still be found in isolated groups of pollarded and hollow trees in the Po valley (e.g. rows of mulberry and willow trees), or even in urban environments, in eco-poor contexts with now-residual populations, due to the limited extension of these secondary habitats.

Despite the fact that most of the studies conducted in the last twenty years have widely demonstrated that the highest biodiversity is found in forest environments characterised by large amounts of necromass in different states of decomposition, the current utilizations of the Apennine forest topsoil have not changed at all compared to the past. They are still completely devoid of specific and diversified measures that might be able to guarantee over time a higher and higher degree of naturalness of the forests. Actually, they remain in situations of general simplification.

This type of forest management has led to a considerable habitat reduction for many species of invertebrates and vertebrates with the consequent local extinction of many populations and the isolation of small residual populations.

In this context, in the specific case of *Osmoderma eremita* and *Rosalia alpina*, which are saproxyilic beetles whose life cycle depends totally on dead or rotting wood, the improvement of the conservation status of the populations present in the project area is closely linked to the rapid restoration of adequate volumes of dead wood in the forest, on the ground or still standing. Therefore, to emulate the natural processes it is necessary to carry out interventions that simulate biological processes, in order to increase the biological and structural complexity of the forest habitats.

Indications for the definition of the intervention plan

The main bio-ecological factors to consider for the realisation of the actions are the scarce dispersion capacity of the two species and the need to have mature broad-leaved trees with large cavities for *Osmoderma eremita* and large beeches with stumps or dry limbs for *Rosalia alpina*. Therefore, the actions have the purpose of creating opportunities for connection between sites that might otherwise be too distant, and to recreate habitat trees suitable for the species. However, to start a process that allows designing and then enacting effective interventions aimed at increasing the availability of useful necromass volumes in the Apennine beech forests for *Osmoderma eremita* and *Rosalia alpina*, it is necessary to preliminarily achieve: 1) a precise inventory of the wood necromass within the target forests (habitat trees); 2) a picture of the presence, distribution and abundance of the two saproxylic species; 3) sharing the objectives between the owners of the forest topsoil, and consequent simultaneous authorisation to proceed with the silvicultural interventions.

We deem that with these premises it is possible to implement effective actions - hereinafter generically described - aimed at improving the quality of forest environments and increasing the availability of habitats for saproxylic beetles.

Types of actions identified

Actions in favour of *Rosalia alpina*

The actions for the creation of necromass are aimed at speeding up the ordinary evolutionary processes of a forest that lead, normally over a long period, to the formation of dead trees standing or dead trees on the ground. Therefore, the interventions were articulated according to these two types of dead trees, both present in nature.

Standing dead trees

This type of tree can be obtained through girdling or partial ring-barking, uprooting, cutting of the top at a height of at least 3-4 meters from the collar, or even resorting to basal cuts of trees, controlling the direction of fall in such a way that the tree does not fall to the ground but leans on another tree.

Girdling e partial ring-barking

Girdling is made using only the chainsaw, carving the entire outer circumference of the trunk with two oblique and converging cuts, about 5 cm deep. The double girdling is preferably carried out in the basal part of the stems with a diameter greater than 25 cm. The maximum height of the execution of the intervention must not however exceed the metre from the collar of the plant. The distance between the two cuts is between 10 and 15 cm. In the case of partial ring-barking the cut is made by carving half of the outer circumference of the trunk with two oblique and converging cuts with a depth of about 5 cm. As with girdling, the distance between the two cuts is between 10 and 15 cm. This type of action is used to create trees that are dead or dying, but still standing for long periods of time.

Figure 1. Girdling e partial ring-barking.

Leaning dead trees

Leaning dead trees, with a minimum diameter of 25 cm, are preferably made with the winch by only partially uprooting the trunk, which is laid against one or more neighbouring trees. In this case, the tree must be devitalised by performing double girdling in the basal part of the trunk. Alternatively, and more rapidly, it is possible to obtain leaning dead trees using basal cuts, controlling the fall direction so that the tree does not fall to the ground but leans instead on one or more neighbouring trees. In this case it is preferable to use trees with a diameter of at least 30 cm.

Figure 2. Leaning dead trees.

Broken stems standing

Standing broken stems can be made by breaking the stem at a height of 3-4 metres from the ground, thus obtaining a standing stump and leaving the remaining portion on the ground.

In order to make the intervention as natural as possible (simulating a natural event) it is preferable to proceed with the chain saw for the sole purpose of making a directional notch and the felling cut at the predetermined height, leaving a hinge 4-5 cm thick. At this point one proceeds to break the stem using a winch. It is preferable to perform a double girdling or partial ring-barking on the stump that remains standing, to prevent the plant from growing again. The part of the stem on the ground must be delimbed but not sectioned.

Figure 3. Broken stems standing.

Dead trees on the ground

Dead trees on the ground can be obtained with the types of intervention described below.

Uprooted trees

They are obtained with the aid of a winch by positioning the choker chain on the stem at a height of about 8-9 m and using a block and tackle with its choker. These trees, with a minimum stem diameter of at least 25 cm, once fallen on the ground should not be delimbed and sectioned. However, to speed up the decomposition processes of the wood, they can be sectioned in 2-3 sections and carved with the chainsaw in several points.

Broken stems on the ground

They are preferably obtained with the aid of a winch by positioning the choker chain on the stem at a height of about 8-9 m and using ablock and tackle with its choker after having made with the chainsaw a directional notch and the felling cut at the pre-established height, leaving a hinge 4-5 cm thick. As a rule, logs fallen to the ground should be delimbed but should not be sectioned so as to delay decomposition. However, even these trunks once fallen on the ground can be sectioned in 2-3 sections and carved with the chainsaw at several points if it is necessary to speed up the decomposition processes of the wood and therefore depending on the degree of decomposition of the wood that you intend to obtain in the short term.

Figure 4. Uprooted trees.

Basal slits

For the purpose of triggering rotting processes and the consequent creation of rotting areas at the base of the stems of trees with a diameter greater than 40 cm, useful to trigger processes of senescence of the tree, it is possible to perform small basal slits. Their creation can take place through cuts at the base of the stem to create a series of pockets, generally three, arranged in vertical succession and tilted in order to promote water stagnation. These pockets are made with a chainsaw, first carving the vertical walls and then making horizontal cuts at the top and bottom margins. Once the dowels have been extracted, you can carve the stem with the chainsaw to facilitate the entry of water into the pockets. The dimensions of the slits must be proportionate to the tapering of the stem. Alternatively, cavities can be made at the base of the stems by making cuts with a chainsaw to remove sections of about 15x15 cm of wood and bark.

Figure 5. Basal slits.

Waste piles

In order to increase the necromass on the ground, it is possible to resort to the formation of small waste stacks made of logs no less than 2 m long and with a diameter greater than 25 cm. The piles can be given a pyramid shape, composed of at least 3-4 rows of logs, or a cubic shape, composed of 4-5 rows of logs. In both cases it is advisable that the stack is raised fat least 20 cm from the ground. The stacks should preferably be placed in sunny places and always in full compliance with the provisions of the current fire prevention plan and regional forest regulation.

Figure 6. Waste piles.

Tripods

Tripods are made using three beech logs over 30 cm in diameter and at least 2 m long. The trunks should be placed inclined to form a pyramid, at a distance of about 1.3 m at the base, and at the top they must be fixed by metal staples and wire. At the base the three logs must be made stable by placing them in the ground for at least 10-15 cm. Tripods should be placed on flat land and in small clearings or beech woods clearing, well exposed to the sun for most of the day.

Figure 7. Tripods.

Actions in favour of *Osmoderma eremita*

Creation of cavities on live trees

Small cavities can be made on the stems of standing trees with a diameter of at least 30 cm at chest height (= 100 cm girth or, even better, trees with a girth>150 cm).

Figura 8. Creating hollows.

Creating hollows

This operation is carried out using a cordless drill or a chainsaw with carving bar and possibly a rasp. Cavities of different sizes and different heights can be created. As part of the Life EREMITA project, three different types of cavities have been identified based on their size (max volume 18 l), to be implemented depending on the size of the habitat tree on which one intervenes, and in reference to the possible presence and extension of wood decay in the stem or branch of the tree chosen for the intervention.

- Grade I: cavities with an opening of about 10x10 cm in width and depth of about 12/15 cm;
- Grade II: cavities with an opening of about 10/15x10/15 cm of width and depth of about 15/25 cm;
- Grade III: cavities with an opening of about 15/20x15/20 cm of width and depth of about 25/30 cm

In the case of mixed broadleaf and conifers forests, it is advisable to remove the conifers in a surrounding area of 10/15 meters from the broadleaved trees subject to cavity creation.

Pollarding

Pollarding is done with a chain saw clean cutting the tree trunk parallel to the ground and at about 2 metres in height or, in the case of trees already cropped, cutting all the branches or the main branches just above their insertion point with the stem, thus resulting in simple stumps.

In order to obtain suitable habitats for the species in question (cavities of at least 5 l) it is advisable to proceed with the pollarding of trees with a diameter of more than 60 cm. In all cases of *ex novo* pollarding, in order to speed up the formation and deepening of the cavity, it is preferable to proceed with the creation of a top basin to make the water stagnate and thus favour the inoculation of the fungi.

It might be necessary to intervene again, some time after the pollarding intervention, to deepen the apical cavity using a chain saw or of mills on drill.

Installation of wood mould boxes

Installation of wood mould boxes (Jansson *et al*., 2009) i.e. artificial wood environments that simulate the cavity of a tree and contain the soil suitable for the reproduction of the species. These are structures similar to nest boxes for birds, rectangular in shape, 70 cm deep and 30 cm wide to ensure a capacity of at least 60 l of soil. This intervention is aimed at increasing the size of *Osmoderma eremita* populations and testing their capacity for expansion and colonisation

Figura 9. Installation of wood mould boxes.

Felling or thinning of the suckers

In order to increase the effectiveness of the actions described above, besides intervening on individual tree habitats, it might be necessary to also fell or thin the suckers in the surrounding area, even 10-15 m from the habitat tree, or to remove all the suckers at the base, or eliminate shadowing branches around the habitat tree. These operations are necessary to provide light to the tree on which the main intervention has been carried out.

Figura 10. Felling or thinning of the suckers.

Choice of the areas of intervention

The intervention areas for *Osmoderma eremita* were identified starting from an accurate analysis and interpretation of the data on distribution and abundance of the species, and of the distribution of potential habitat trees, deriving from the results of the *ex-ante* monitoring, which was part of the project. Starting from the centres of verified species presence and with priority given to civic or state properties, the choice of the habitat trees on which to intervene, to improve their suitability to host the species, was carried out by identifying - on a GIS basis - macro-areas. These were circular in shape due to the dispersal capacity of the species (Ranius & Hedin 2001; Hedin & Ranius, 2002; Dubois & Vignon, 2008; Dubois *et al*., 2010; Svensson *et al*., 2011; Chiari *et al*., 2013). In particular, in the case of *O. eremita*, three concentric macro-areas of increasing amplitude (radius of 500 m, 1500 m and 2500 m) were identified, starting from the localisation of each ascertained presence of the species; subsequently the trees were selected on which to realise actions, giving priority to those located within the first circle. In all cases in which it was impossible to identify a sufficient number of potential habitat trees in the first circle, we went on choosing trees located in the second and/or third circle, until reaching the number of habitat trees expressly set in the plan of actions.

WMBs were installed on the side either shaded or exposed to the sun for only a few hours, of trees at least 50 cm in diameter and not less than 30-40 m apart (maximum 100 m).

In cases where *O. eremita* was already present in the WMB installation area, the same were placed less than 200 m from the trees occupied by the species.

WMBs have been placed on habitat trees that were already hollow in their natural state (but nonetheless stable) or on plants located near - about 10-20 m - trees subject to previous hollowing operations.

The boxes were placed at a height of no less than 4 m to avoid possible damage by grazing animals or wild animals, or as a consequence of vandalism; the WMB base has always been placed at least 3 m from the ground.

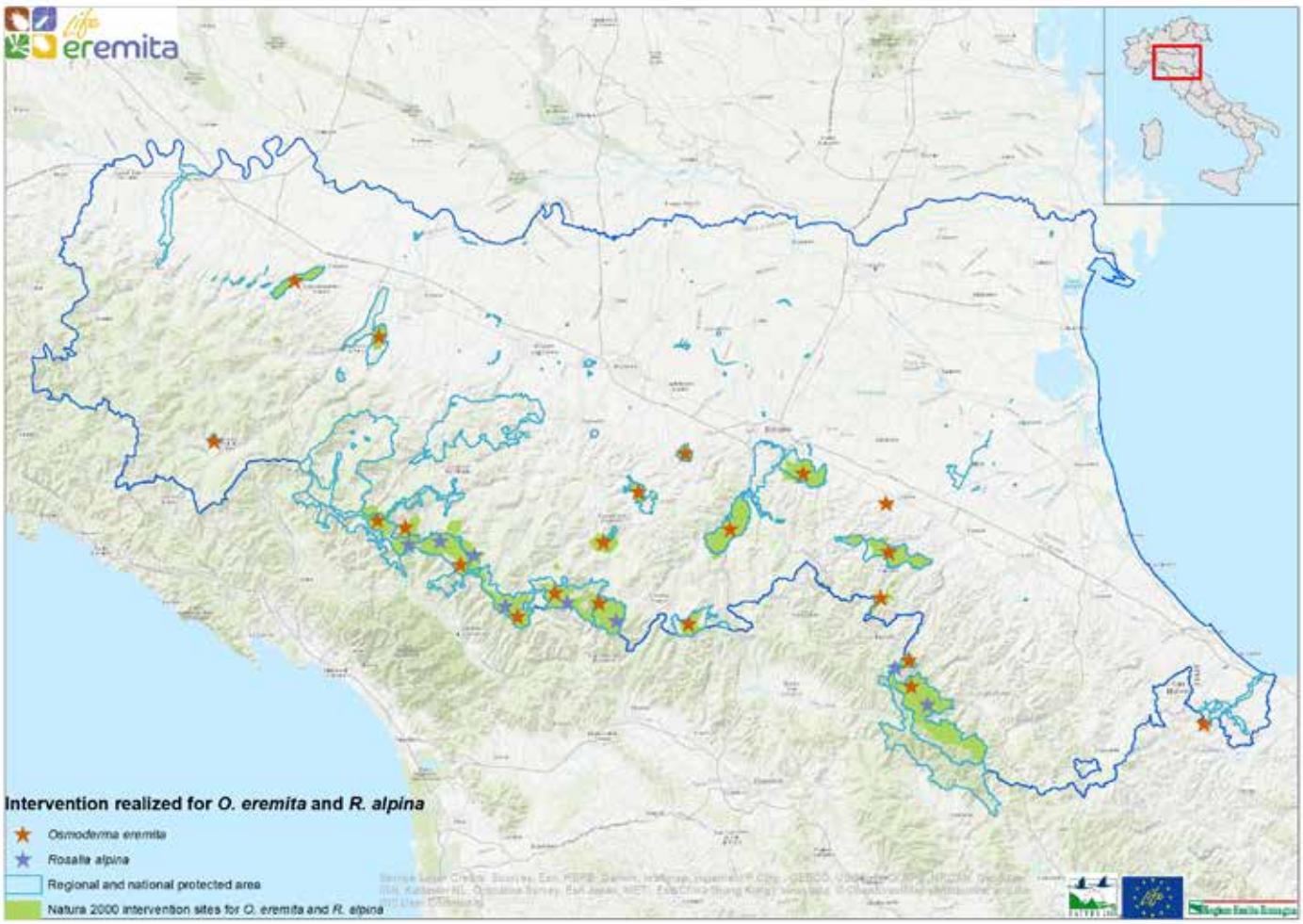

Figure 13. Location of the interventions carried out in Emilia-Romagna in favour of *O. eremita and R. alpina*.

As far as *Rosalia alpina* is concerned, the trees subjected to intervention to favour the species were chosen from those that presented characteristics of potential suitability, necessarily located within beech woods, and at a distance (3000 m). These were compatible with the known biology of the species, in terms of flight dispersion (Drag *et al*., 2011; Bosso *et al*., 2013), starting from the three ascertained presence centres.

During the Life Eremita project these methods of operation have been fully or partially implemented in relation to the possibility of totally or partially applying the criteria for choosing habitat trees.

All the areas of intervention, identified according to the methodology described above, fall entirely within the sites of the Natura 2000 network.

Conclusions

In total, the interventions for the improvement of the living environments of *O. eremita and R. alpina* involved 23 sites of the Natura 2000 network, from Parma to Rimini. In particular, in 8 of these sites actions have been implemented in favour of *R. alpina,* and in 22 sites in favour of *O. eremita*.

It is expected that the implemented actions will favour the creation of ecological corridors, or rather of stepping zones, between the different locations of presence of the two target species of saproxylic beetles, in order to connect populations presumably isolated from each other, and therefore create a network of habitat trees suitable for the species.

A brief description of the implemented interventions is shown in the Appendix.

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ADDENDUM - Data sheets of the actions carried out on the habitats of Osmoderma eremita and Rosalia alpina as part of the Life Hermit project

SCI: IT4020001 Boschi di Carrega

Municipality: Sala Baganza, Collecchio (PR)

Habitat: oak and chestnut grove

Actions: in the Boschi di Carrega site, the actions carried out were exclusively aimed at expanding the habitat of *O. eremita*. In particular, 35 interventions involved the creation of new cavities inside the trunks and the deepening of existing cavities. The plant hollowing interventions mainly concerned chestnut trees of medium and large size, but also pubescent oaks, oaks, and black walnuts deemed suitable for actions aimed at the settlement of the species. Furthermore, 10 areas within a radius of 5 m around as many plants involved in the interventions, have been trimmed and cleaned up, and 7 wood mould boxes were installed.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Occidentale (*Management body for the parks and biodiversity Western Emilia*)

SCI: IT4020003 Torrente Stirone

Municipality: Vernasca (PC)

Habitat: Bosco Lame oak forest

Actions: in the Torrente Stirone site 15 interventions were carried out to create new cavities inside the trunks in order to expand the habitat suitable for the establishment of *O. eremita*. The plant hollowing interventions involved medium-sized pubescent oaks. Furthermore, some white poplars have been pruned and 5 areas around the plants involved in the interventions have been trimmed. 3 wood mould boxes have been installed.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Occidentale (*Management body for the parks and biodiversity Western Emilia*)

SCI: IT4020026 Boschi dei Ghirardi

Municipality: Collecchio (PR)

Habitat: oak forest at Case Ghirardi

Actions: in the Boschi dei Ghirardi site, 17 actions were carried out to create cavities on medium and large pubescent oaks in favour of *O. eremita*. Furthermore, 10 areas of the radius of 5 m around as many plants involved have been trimmed and cleaned up, and 3 wood mould boxes were installed.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Occidentale (*Management body for the parks and biodiversity Western Emilia*)

SCI: IT4030001 Monte Acuto Alpe di Succiso

Municipality: Ventasso (RE)

Habitat: beech woods and chestnut groves

Actions: in the Monte Acuto Alpe di Succiso site, actions were carried out to promote the availability of habitats for *O. eremita*. For this purpose, 17 cavities were created *ex novo* on beech trees with a diameter between 32 and 63 cm, removing suckers (11) and cleaning 9 areas, up to a distance of 5 metres from the hollow plants to favour the penetration of light. The dimensions of an existing cavity have been increased. Six WMBs have been placed in this SCI.

Agency in charge: Parco Nazionale dell'Appennino Tosco Emiliano (*Tuscan Emilian Apennines National Park*)

SCI: IT4030002 Monte Ventasso

Municipality: Ventasso (RE)

Habitat: chestnut grove

Actions: in the Monte Ventasso site the actions carried out were exclusively aimed at increasing the availability of habitats for *O. eremita*. For this purpose, cavities have been created *ex novo* on 18 beech trees with a diameter between 30 and 110 cm, and the volume of the cavities already present on secular chestnut trees has been increased up to obtain 6 cavities of a size suitable for hosting larvae of the species. In 12 cases it was necessary to remove suckers at the base of the hollow trees and in 8 cases it was necessary to prune some large branches. It was necessary to trim the vegetation present in the area around 8 hollow trees (up to 5 m in diameter) to favour the penetration of light and consequently increase the level of suitability of the cavities created ex novo. A WMB has also been placed in the site.

Agency in charge: Parco Nazionale dell'Appennino Tosco Emiliano (*Tuscan Emilian Apennines National Park*)

SCI: IT4030003 Monte la Nuda Cima Belfiore Passo Cerreto

Municipality: Ventasso (RE)

Habitat: beech and chestnut trees

Actions: in the Monte la Nuda Cima Belfiore Passo del Cerreto site, in order to favour the presence of *O. eremita*, 26 hollowing interventions on as many beech and chestnut trees have been carried out, and the dimensions of 4 cavities already existing on as many chestnut trees have been increased. To maximise the effectiveness of the hollowing interventions, it was also necessary to provide for the removal of the basal suckers of 18 potential habitat trees and the removal of the arboreal and shrubby vegetation in an area of 5-10 m surrounding 12 hollow plants. Furthermore, 13 WMBs have been installed. To encourage the presence of *R. alpina*, a total of 52 beech trees have been girdled (28 partial ring-barking and 24 girdling interventions), 8 standing stumps have been created, and beech trees with a diameter greater than 25 cm have been felled, to achieve a total of 45 stacks. On this site also, it was necessary to proceed with the removal of suckers on 14 plants and to thin and clean 29 circular areas, up to a maximum of 10 m radius, in the surroundings of girdled plants. 3 plants have been pruned.

Agency in charge: Parco Nazionale dell'Appennino Tosco Emiliano (*Tuscan Emilian Apennines National Park*)

SCI: IT4030004 Val D'Ozola Monte Cusna

Municipality: Ventasso (RE)

Habitat: beech forest

Actions: in the Val d'Ozola Monte Cusna site, the actions carried out were exclusively aimed at encouraging the presence of *R. alpina*. In particular, 44 girdling and 29 partial ring-barking interventions have been carried out on as many beech trees, with a diameter between 25 and 80 cm. 8 standing stumps have been created and some plants with a diameter exceeding 25 cm were felled and used to make 20 stacks. In addition, suckers were removed from the base of 12 plants, 52 cleanings and 1 thinning were performed (up to a maximum of 10 metres from the plant subject of the main intervention), and 7 trees have been pruned to increase the availability of light.

Agency in charge: Parco Nazionale dell'Appennino Tosco Emiliano (*Tuscan Emilian Apennines National Park*)

SCI: IT4030005 Abetina Reale Alta Val Dolo

Municipality: Villa Minozzo (RE)

Habitat: chestnut grove mixed with beech and beech forest

Actions: in the Abetina Reale Alta val Dolo site, actions aimed at creating a greater availability of suitable habitats for the *O. eremita* species were performed on old chestnut trees and beech trees. In particular, 22 new cavities have been created on beech trees, and the volume of the existing ones (7) on chestnut trees was increased, because they were deemed inadequate in terms of size. Furthermore, suckers were removed from the base of 8 large chestnut trees that had cavities suitable to accommodate the species, and the shrubbery was trimmed in the area (5-10 m) surrounding 18 hollow trees, to encourage solar radiation. It was also necessary to prune 18 trees. Furthermore, 7 WMBs were placed on trees of adequate size. In order to increase the availability of suitable habitat for *R. alpina*, girdling and partial ring-barking interventions have been carried out on 11 beech trees, and a standing stump has been created. Two thinning and 8 cleaning operations of areas of a 10 m radius around as many plants have been carried out. Finally, 13 stacks of beech logs with a diameter greater than 25 cm were created.

Agency in charge: Parco Nazionale dell'Appennino Tosco Emiliano (*Tuscan Emilian Apennines National Park*)

SCI: IT4040001 Monte Cimone, Libro Aperto, Lago di Pratignano

Municipality: Fanano (MO)

Habitat: beech forest

Actions: in the Monte Cimone, Libro Aperto, Lago di Pratignano site actions for both *R. alpina* and *O. eremita* were carried out. For *O. eremita* 21 hollowing interventions were performed, including the creation of new cavities inside medium and large beech trees (over 30-40 cm in diameter), and the deepening of cavities already present. Furthermore, 10 areas of 5 m radius around the plants involved in the interventions have been trimmed, and 6 wood mould boxes have been installed. To encourage the establishment of *R. alpina*, 139 complete or partial girdling interventions have been carried out in order to favour and accelerate the natural decay of some beech trees, and encourage the establishment of the species. Furthermore, 10 stacks of wood were created.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Centrale (*Management body for the parks and biodiversity Central Emilia*)

SCI: IT4040002 Monte Rondinaio, Monte Giovo

Municipality: Pievepelago (MO)

Habitat: Beech forest

Actions: In the Monte Rondinaio, Monte Giovo site, new cavities have been created in medium and large beech woods to favour the establishment of *O. eremita*. The hollowing interventions were in total 29 and 6 wood mould boxes were put in place. These interventions are very important to connect the populations present in the nearby Tuscan-Emilian Apennines National Park with the local ones. Furthermore, 82 girdling or partial ring-barking interventions were carried out on beeches with a diameter greater than 30-40 cm, in order to favour and accelerate the natural decay of some beech trees and thus encourage the establishment of *R. alpina*. Shrubs have been trimmed in 22 areas of 5 m radius around the plants subject to the interventions to create 14 stacks of wood *for R. alpina*.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Centrale (*Management body for the parks and biodiversity Central Emilia*)

SCI: IT4040003 Sassi di Roccamalatina and Sant'Andrea

Municipality: Guiglia (MO)

Habitat: riparian forest and chestnut grove

Actions: In the Sassi di Roccamalatina and Sant'Andrea site, 47 hollowing interventions of plants were carried out, to expand the habitat suitable for *O. eremita*. The interventions mainly concerned chestnut trees of medium and large size in an old chestnut grove located at the foot of the Sassi, but also oaks, willows, alders and poplars deemed as suitable, in a riparian forest that stretches along the banks of a river. Interventions to create new cavities inside the trunks, and to deepen existing cavities were carried out, in order to make them suitable for the settlement of the species. Moreover, 15 areas of the radius of 5 m, around as many plants subject to the interventions, have been trimmed and cleaned, where necessary. Nine wood mould boxes have been installed in the site.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Centrale *(Management body for the parks and biodiversity Central Emilia*)

SCI: IT4040004 Sassoguidano, Gaiato

Municipality: Pavullo (MO)

Habitat: chestnut grove

Actions: in the Sassoguidano, Gaiato site, in the area consisting of an old chestnut grove, 10 new cavities inside medium and large chestnut trees have been created to favour the establishment of *O. eremita*. In addition, 3 bush-trimming interventions were carried out in areas of 5 m radius around the plants subject to the interventions and 4 wood mould boxes were placed.

Agency in charge: Ente per la gestione dei parchi e della

biodiversità Emilia Centrale (*Management body for the parks and biodiversity Central Emilia*)

SCI: IT4050001 Gessi Bolognesi, Calanchi dell'Abbadessa

Municipality: San Lazzaro di Savena (BO), Castel San Pietro Terme (BO)

Habitat: riparian forest, thermophile oak wood.

Actions: in the Gessi Bolognesi site, the actions carried out were in favour of *O. eremita,* and they included 35 hollowing interventions, the creation of cavities *ex novo* or the deepening of small pre-existing cavities on various broadleaved trees, including pubescent oaks and poplars of large dimensions, located in thermophile oak woods and along riparian woods. Furthermore, 4 delimbing intervention were carried out and 5 wood mould boxes were placed to allow the insertion of specimens coming from the *ex situ* breeding centres.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Orientale (*Management body for the parks and biodiversity Eastern Emilia*)

SCI: IT4050002 Corno alle Scale

Municipality: Lizzano in Belvedere (BO)

Habitat: beech forest

Actions: the actions carried out in the vast Corno alle Scale site were in favour of *O. eremita and R. alpina*, mainly in beech-woods located near the ridges, in order to promote the diffusion of the two species in the area. As regards *O.eremita*, cavities with varying degrees of depth and size have been dug in large beeches and cavities already present have been deepened, for a total of 31 hollowing interventions. In addition, felling for the purpose of thinning was performed around the habitat plants and 4 wood mould boxes were placed in each area of intervention. As far as *Rosalia alpina* is concerned, a total of 210 interventions were carried out on beeches with a diameter of more than 30 cm. These interventions included girdling, partial ring barking, thinning, felling and creation of stumps either standing or on the ground, leaning dead trees and the creation of 10 stacks of disposable beech logs. Furthermore, smaller plants and branches that impede the penetration of the sun have been eliminated.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Orientale (*Management body for the parks and biodiversity Eastern Emilia*)

SCI: IT4050003 Monte Sole

Municipality: Marzabotto (BO)

Habitat: oak wood, chestnut grove

Actions: in the Monte Sole site actions were carried out in favour of the target species *Osmoderma eremita*, in the chestnut groves and in the mixed thermophile oak woods. In the chestnut groves with trees of considerable size that already have cavities, 16 delimbing and pruning interventions of the dry and perishing branches were carried out, in addition to thinning and trimming operations. In the thermophile woods 18 hollowing interventions were performed on large broadleaved trees, including the creation of cavities with varying degrees of depth and size, and the deepening of existing cavities; moreover, felling for the purpose of thinning was carried out around the habitat plants. In all the areas of intervention, 6 wood mould boxes were placed to allow the insertion of the species.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Orientale (*Management body for the parks and biodiversity Eastern Emilia*)

SCI: SIC IT4050004 Bosco della Frattona

Municipality: Imola (BO)

Habitat: wood with a prevalence of oak

Actions: in the Bosco della Frattona site the actions carried out were in favour of *O. eremita*. Forty-eight hollowing interventions were performed in the highest portion of the area, characterised by woods dominated by Mediterranean pubescent oak, including the creation of new cavities and the extension and deepening of existing ones, in order to obtain suitable habitat trees for the species.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Romagna (*Management body for the parks and biodiversity Romagna*)

SCI: IT4050016 Abbazia di Monteveglio

Municipality: Monteveglio (BO)

Habitat: row of mulberries and woods

Actions: in the Abbazia di Monteveglio site the interventions carried out were in favour of *O.eremita*. There are old mulberry trees in the area, some already decaying. Six hollowing interventions were carried out for the deepening of existing cavities. Furthermore, 6 limbing interventions and the pruning of part of the branches were performed. Finally, 2 wood mould boxes where placed for the insertion of specimens coming from the *ex situ* breeding of the project.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Orientale (*Management body for the parks and biodiversity Eastern Emilia*)

SCI: IT4050020 Laghi di Suviana e Brasimone

Municipality: Camugnano (BO)

Habitat: chestnut grove

Actions: at the Laghi di Suviana e Brasimone the actions carried out were in favour of *O. eremita*. In a public property area there are several very large fruit chestnut trees, which are no longer cultivated, are in a state of decay and are likely to wither in the short term. The majority of chestnut trees already have large cavities suitable for the species *O. eremita* but the state of health of the plants is not good and the solar radiation inside the foliage is very low. Thus, 16 delimbing operations and pruning of the dry and perishing branches have been performed, in order to thin out the crowns; in addition, some small hollows and cavities were deepened (a total of 18 hollowing interventions), trimming, removal of suckers and the thinning of young plants shading the old chestnut trees were carried out. Six wood mould boxes were installed to allow the stabilisation of the population of the target species.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Emilia Orientale (*Management body for the*

parks and biodiversity Eastern Emilia)

SCI: IT4070011 Vena del Gesso Romagnola

Municipality: Brisighella (RA)

Habitat: wood arboriculture plants

Actions: in the Vena del Gesso Romagnola site actions in favour of *O. eremita* were carried out. In the area characterised by karst morphologies around Monte Mauro, the formation of environments suitable for the diffusion of the species is obtained mainly with the pollarding of 97 plants of black walnut and common walnut of medium size and by hollowing some more developed plants (a total of 140 hollowing interventions). In addition, smaller plants have been eliminated and extensive trimming has been carried out.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Romagna (*Management body for the parks and biodiversity Romagna*)

SCI: IT4070016 Alta Valle del Torrente Sintria

Municipality: Brisighella (RA)

Habitat: chestnut grove.

Actions: In the Alta Valle del Torrente Sintria site, actions in favour of *O. eremita* were carried out. In an area of public ownership, where fruit chestnut trees are widespread, partly granted to private individuals for cultivation, 28 pruning interventions were carried out to eliminate dry parts and release the crown of old, medium and large-sized trees, no longer cultivated and provided with adequate cavities. In addition, extensive trimming of shrubs were performed around the hollowed trees. The goal is to promote the establishment of *O.eremita* by increasing the solar irradiation and longevity of the habitat trees.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Romagna (*Management body for the parks and biodiversity Romagna*)

SCI: IT4080002 Acquacheta

Municipality: Portico and San Benedetto (FC)

Habitat: beech forest

Actions: in the Acquacheta site the actions in favour of *O. eremita* included the *ex novo* hollowing or the deepening of small pre-existing cavities in beeches of important dimensions, placed in the best sun-exposed ridge beech-woods. In total, 25 hollowing interventions were carried out. Also, 6 wood mould boxes were installed to allow the introduction or strengthening of the species population, through the establishment of the specimens coming from the *ex situ* breeding centre. As regards *R. alpina*, the following interventions were carried out: 41 girdling and partial ring-barking on beech trees with a diameter over 30-40 cm, 3 thinning and felling interventions for the creation of stumps either standing or on the ground, dead leaning trees and waste wood piles. Smaller plants and branches that impeded the penetration of the sun were eliminated. These interventions are functional to connect the subpopulations of the two species of saproxylic beetles present in the site of community importance.

Agency in charge: Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna (*Casentinesi Forests, Monte Falterona and Campigna National Park*)

SCI: IT4080003 Monte Gemelli, Monte Guffone

Municipality: Santa Sofia (FC), Premilcuore (FC), Portico and San Benedetto (FC)

Habitat: beech wood and chestnut grove

Actions: In the vast site of Monte Gemelli and Monte Guffone, 7 different areas of intervention for both saproxylic species - *O. eremita* and *R. alpina* -, have been envisaged, mainly in beech-woods near the ridges, in order to create a connection between the different subpopulations identified during field monitoring, and to promote the diffusion of the two species in secondary areas. With regard to *O. eremita* the formation and recovery of environments suitable for the species was carried out through the creation of cavities with varying degrees of depth and size in large beeches. Other activities included deepening cavities already present (in total 136 hollowing interventions), felling plants around the habitat tree to give it light, brush thinning, and placement of wood mould boxes in each area of intervention (29 WMB in total). Also, some conifers were felled and thinning interventions were carried out close to plants that were either hollowed or to be hollowed. The interventions also involved a portion of chestnut grove in the area of public property, with several large chestnut trees that have large cavities, where the purpose was to favour the target species *Osmoderma eremita* by increasing the solar irradiation and at the same time the longevity of the habitat trees, improving and recovering the chestnut grove overall. In favour of *Rosalia alpina*. 328 interventions on beeches included girdling, partial ring-barking, thinning, felling with the creation of stumps standing or on the ground, dead leaning trees, uprooted trees, and the creation of 20 stacks of beech logs and tripods; moreover, smaller-sized plants and branches that impeded the penetration of the sun have been eliminated (18 delimbing interventions).

Agency in charge: Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna (*Casentinesi Forests, Monte Falterona and Campigna National Park*)

SCI: IT4090001 Onferno

Municipality: Gemmano (RN)

Habitat: wood with a prevalence of oak

Actions: In the Onferno site, in the portion characterised by woods dominated by Mediterranean pubescent oak, new cavities have been created and the existing ones have been enlarged and deepened, in order to set up suitable habitat trees for the settlement of *O. eremita*. In total, 10 hollowing interventions were carried out.

Agency in charge: Ente per la gestione dei parchi e della biodiversità Romagna (*Management body for the parks and biodiversity Romagna*)

Conservation of *Osmoderma eremita* (Scopoli, 1763) in Emilia-Romagna: *in situ* and *ex situ* breeding

Roberto Fabbri, Marco Uliana, Massimiliano Costa, Davide Alberti, Giovanni Carotti, Raffaele Gattelli, Francesca Moretti

Introduction

The breeding of *Osmoderma eremita*, envisaged by the Life Eremita project, is aimed at obtaining a series of specimens through *ex situ* and *in situ* reproduction, starting from founding individuals taken from natural populations. The breeding makes it possible to have animals - larvae and adults - for reintroduction and repopulation activities during the Life project. This permits a wide action of reinforcement of the populations and the expansion of the species distribution area in Emilia-Romagna in the medium and long term.

The breeding of *Osmoderma eremita* has been subjected to checks by the Ministry of the Environment and by ISPRA (*Higher Institute for Environmental Protection and Research*), as prescribed by Presidential Decree 357/97. To this end, the feasibility study was drawn up (Fabbri *et al*., 2017) in compliance with national and European guidelines (AA.VV, 2007; IUCN / SSC, 2013; IUCN, 2014).

Before the Life Eremita project was activated, these conservation activities for the *Osmoderma eremita* species had not yet been applied in European projects (Dubois, 2009; Silva *et al*., 2012). A previous *ex situ* larval breeding experiment was recently conducted in Finland on the *Osmoderma barnabita* species (Motschulsky, 1845) in order to examine the role played by the main substrates present in tree cavities on adult female preferences and larval growth (Landvik *et al*., 2016).

Figure 1. Rooms set up for the breeding of *Osmoderma eremita* in Russi (RA.)

The *ex situ* breeding

In Emilia-Romagna, 3 *ex situ* breeding centres were set up and located in the National Park of the Tuscan-Emilian Apennines, in Ligonchio (RE), the National Park of the Casentinesi Forests, Monte Falterona and Campigna, in Santa Sofia (FC), and the Management body for the Parks and Biodiversity Romagna, in Russi (RA). The facilities are also used for educational purposes, for informational activities, and for raising awareness as to the importance of insects and their ecological role. Each breeding facility has some vertical shelves, a table with a lamp and a sink.

Figure 2. Rooms set up for the breeding of *Osmoderma eremita* in Santa Sofia (FC).

The three breeding facilities were created from an appropriate number of adult specimens that were withdrawn from natural habitats. As an alternative or in combination with the extraction of adults, one could also proceed to collect larvae in nature. The withdrawal of the founding individuals was carried out only where the ex-ante monitoring has verified the presence of sufficiently large populations, able to guarantee an exemplary ratio captured/census population of about 1/10, in order not to damage the population of origin.

The capture of the founders has been achieved by employing passive fall traps (pitfall traps) and also the intercepting by attraction traps (black cross window traps), or by direct research of adults and larvae in the colonised trees (wood mould sampling) (Ranius & Jansson, 2002; Chiari *et al*., 2013). The black cross window traps are triggered with a racemic mixture of γ-decalactone, the pheromone emitted in nature by the males of *Osmoderma eremita* to attract females (Larsson *et al*., 2003).

In order to breed *O. eremita*, the technique employed was based on a set of containers, simulating as many cavities, and containing the *pabulum* (nutrient substrate). The method is effective for obtaining a high number of third-instar larvae and adults to be introduced into the wild. Specimens bred with

Figure 3. Centre for the breeding of *Osmoderma eremita* in Ligonchio (RE).

Figure 4. *Osmoderma eremita* eggs in the soil.

this system do not show significant differences in average size compared to wild individuals. The containers used for breeding are made of transparent plastic and have the shape of a parallelepiped (about 30 containers per breeding facilities, measuring 39x28x28 cm and with capacity of 22 l), equipped with a lid and kept in the dark, to simulate the natural conditions of life inside the cavities of the trees. On the top, on the lid, a large window was opened and covered with a fine plastic net (like a mosquito net) fixed with hot glue. The height of these containers allows an adequate thickness of the alimentary *pabulum* in order to guarantee a more differentiated gradient of humidity and oxygenation between the bottom and the surface, thus allowing the larvae to move to the most suitable depth. For the preparation of the substrate, a certain amount of litter, which corresponds to about 30% of the finished product, was collected in nature from deciduous forests; subsequently, other materials have been added with the following proportions: 50% of beech wood sawdust, 25% of manure, 25% of soil improver (for example peat), all free of chemical residues and glues. The mixture has been matured in large crates (even 100 l) for at least 4 months, moistened and stirred every 7 days.

The founding adults were placed at the beginning of the summer (2018) in the containers already filled 2/3 full of soil, whose surface was covered with pieces of trunk or branches (to facilitate the walking of adults), and closed at the top with the lid and a fine-knit net. To prevent the larvae from being attacked by pests or predators, in fact, every single container with soil has been covered with a fine plastic net. The animals are fed with fresh fruit, fruit purees and also with artificial jellies produced for beetles' sustenance. Following the reproduction and hatching of the eggs, one waits for the larvae to reach the second growth stage (L2), which is generally achieved in a few

Figure 5. The three larval stages of *Osmoderma eremita* in real dimensions: L1 (left), L2 (centre) and L3 (right).

weeks. At this stage of development, to avoid cannibalism, the larvae are moved to other containers of the same capacity, about 20 larvae per container. These containers are filled for about 3/4 of soil and litter (2/4 of soil at the bottom and above it 1/4 litter). Here they are bred until complete development.

The sets of containers are checked every seven days; in particular, the checks focus on the structure, the soil production, and the containers that host insects in the extra-reproductive period. In the period of reproduction and oviposition, however, it is necessary to inspect the containers in which the adults are housed daily and, subsequently, the larvae. During any operation, disposable gloves are used to minimise the risk of entry of any diseases through contact with the operators.

Through manual inspection, any larvae of elateridae or other potential predators are removed.

Fig.6. Weighing *Osmoderma eremita* L3 larvae.

During the inspections the correct degree of humidity of the soil and the possible need to replace a part of it are verified. The food is renewed in the containers of adults, and the larvae containers are sample checked to verify the good health of the larvae themselves and any signs of disease (e.g. presence of dark necrotic lesions on the tegument, finding dead larvae, etc.) or stress and suffering (e.g.: observation of larvae emerging and remaining on the surface). During the checks and the change of the soil, the contents of the containers are emptied into large plastic basins (30-40 l type). A 2 l pressure sprayer is used to humidify the breeding containers. The soil is replaced approximately once a month from the end of the second year of breeding or, anyway, when faecal pellets exceed 50% of the volume. Whenever the soil is changed, the counting and weighing of the specimens and their division by stages are also carried out. The weighing is done using a digital precision scale. The checking and emptying of the terrariums are strictly suspended from the beginning of October until the end of December, to avoid the risk of damaging the newly formed cocoons.

Before each release action, a check is planned on the health status of the specimens and to divide the larvae by sex (the determination of the sex of the specimens is done by looking for the organ of Herold that distinguishes the male). Before the release, all adults will be measured with a digital or analogue precision calliper, weighed and provided with a numbered label (e.g. numbers to mark queen bees).

The breeding facilities are also equipped with some

Figure 7. Measurement of an adult by using a digital calliper.

transparent plastic containers that are smaller than those used for breeding (28x19x14 cm and 5 l volume), for various uses such as the isolation of individuals for various reasons, the temporary accumulation of individuals during counting and measuring operations, educational purposes, transportation, etc.

The breeding facilities are equipped with a paper register. All visits and checks carried out by operators and other employees are recorded in the register. Also recorded are the environmental conditions detected by multifunction probe or data-logger, the number of specimen after the count, the number of active containers (with live specimens), the dates of movements, the dates of eclosions and hatches, the number of eggs observable against the light, the operations performed (soil humidification, substitution of litter and/or soil, etc.), the annotation of any particular phenomena (presence and elimination of pests, development of fungi and other organisms, formation of excessive condensation, ice formation in winter, and death of larvae or pupae, etc.).

All phases of *ex situ* breeding are documented and photographed.

Since in Italy the natural cycle of *Osmoderma eremita* is above all biennial, at the end of the first year of breeding it is possible to obtain young third-stage larvae (L3) and, in the second year of breeding, mature third-stage larvae (L3), ready to cocoon in the soil and remain in the pre-pupa stage until the spring of the following year (third year of breeding), when the adults hatch. The cycle can be synchronised to the different picking altitudes of the founders, by adjusting the temperature inside the breeding and therefore speeding up or delaying it.

The management of each breeding facilities is carried out by an expert entomologist assisted by an expert operator and involves a work commitment of about 4 hours daily for 70 days/ year.

In situ breeding

In situ breeding is carried out at sites where the presence of the species is certain and the populations are not large enough, and in sites where the species is not present but which have the potential for its establishment, always within the distribution area of the species.

In situ breeding plans for the reproduction of *Osmoderma eremita* in semi-natural conditions makes it possible to have larvae and adults to reinforce the existing population and introduce the species. It is mainly executed in sites where tree interventions are carried out to increase the availability of the habitat of the species. In order to decide where to carry out *in situ* breeding, a preparatory survey was executed, which provided an updated picture of the presence and size of the *O. eremita* population (*ex ante* monitoring).

Artificial environments, suitable for reproducing the species, were then created through the installation of "wood mould boxes" - WMB (Jansson *et al*., 2009; Hilszczański *et al*., 2014; Carlsson *et al*., 2016). WMBs are artificial wooden boxes that simulate the cavity of a tree and contain the soil suitable for the reproduction of the species. These structures are similar to bird boxes, with parallelepiped shape, 70 cm high, 40 cm wide and 30 cm deep, 3 cm thick, to ensure a capacity of over 50 l, 80% of which will be occupied by soil and litter (about 40 l). WMBs are made of oak wood and assembled with nails or screws or joints, without the use of glues. The dimensions described above must be intended as minimal as it would be desirable to use boxes of larger dimensions, but in our case it was necessary to reach a compromise between the optimal size and the handling of the boxes. On the front there is an entrance hole with a diameter of 50 mm (80 mm according to Jansson et al., 2009 and Carlsson et al., 2016 and 30 mm according to Hilszczański *et al*., 2014). The upper side of the box (roof) can be opened in order to carry out checks and is protruding on all sides for at least 1 cm. It is currently being assessed whether there is the possibility to insert, on an experimental level, a transparent plastic side (polypropylene) to allow for the observation of the activity inside. An external wooden wall that can be opened outwards will in any case cover this transparent panel. On the wooden roof one or more holes (10 mm diameter) are made to allow rain to enter. To facilitate moisture retention, a plastic tray, 13 cm high - the exact size of the cavity – is placed inside the box, on the bottom. The inner contents of the box are made up of the same soil as for the *ex situ* breeding (50% beech sawdust, 25% soil improver and 25% manure) up to about 3/4 of the internal height. Foliage taken from deciduous trees forests is added for about 1/4 of the height.

WMBs must be installed on trees at a height of about 4 m, to avoid possible damage by grazing or wild animals, and possible vandalism. They have been installed on the shaded side or on a side exposed to the sun for only a few hours (East or West side or, on less exposed slopes, also South East or South West sides), of trees at least 50 cm in diameter and distant from each other not less than 30-40 m (maximum 200 m). The boxes were placed in groups of at least 2-3 boxes, depending on the number of surrounding hollow trees. If *Osmoderma eremita* is present in nature in the WMBs installation area, these must be located less than 200 m from trees hosting the target species (Hilszczański *et al*., 2014).

The installation requires about 4 m of galvanised steel rope (4 mm thick) depending on the diameter of the tree trunk, 4 galvanised rope clamps (3 mm thick), two 8cm long galvanised turnbuckles with two eye bolts, 8 steel screws for wood with eyelet (eyelet diameter 15 mm), about 2 m of rubber tube (10 mm diameter) in which to insert the steel rope.

WMBs must be placed next to existing hollow trees or those hollowed on purpose, at a distance of about 10-20 m. In the case that the WMBs are placed next to trees with artificially created habitats, in sites where the species is not reported and has not been detected with the *ex ante* monitoring (despite being in the distribution area of the species), some third-instar

larvae (up to 10) and adults (2-4) can be inserted in the boxes. This can be done in order to allow for the temporary

Figure 8. Wood mould box (WMB) filled with soil almost up to the entry hole.

establishment of a colony of the species, before the artificially created cavities become suitable. A part of the WMBs (10%) can be installed without inserting specimens, in order to have a comparison sample.

During the Life Eremita project, 150 WMBs were made and installed on the territory of the Emilia-Romagna Region, in the sites identified on the basis of the monitoring results and planned conservation interventions to restore habitats suitable for the species.

After installation, during the first and the second year inspections are planned with a frequency of 3 to 4 times per year. The inspections always detect the temperature (at 20 cm depth in the substrate) and the humidity inside the boxes, to make sure that the microclimate remains as constant as possible, and thus similar to the natural cavities. Successful colonisation is verified by monitoring the presence of larvae of the target species, using the wood mould sampling method (WMS), by emptying the box and checking all the soil present, then putting it back at the end, inside the box.

In addition to the larval count, the detection of the developmental stage and the weight measurement, the state of health will be assessed, based on the larvae's mobility and body turgidity. The larvae are categorised as follows: healthy, when the larva has good turgidity; not healthy, when it has limited movements and has weak bodily turgidity; dead, when it has no movement and the body is decomposing. The state of health of the pupae is not assessed because breaking the cocoon usually involves the death of the pupa or its abandonment by the larva, which is incapable of producing a second cocoon. In case of accidental breakage, it is advisable to note down if the pupa was alive or not.

For comparison purposes, temperature and humidity must also be measured in cavities of neighbouring trees. In the event that the amount of soil should decrease, it must be reintegrated; the obviously worn-out soil must instead be replaced. In this way the disturbance is reduced and the settlement process by *Osmoderma eremita* is facilitated.

From the second year of the installation, adults will also be monitored; for this reason, in addition to the wood mould sampling method, pitfall traps are placed in vivo within the WMBs (one per box) to verify that the species' stable colonisation has taken place. The pit fall trap consists of a transparent plastic glass (about 8 cm in diameter), buried in the boring dust inside the tree cavity, and with the upper edge at the level of the surface (Ranius & Jansson, 2002; Chiari *et al*., 2013). Wet moss should be placed on the bottom of the trap, which is perforated. Daily checks are planned for at least 3 consecutive days.

Adult monitoring takes place between the beginning of June and the middle of August (depending on the altitude), on a daily basis for 3 consecutive days every 2 weeks; larvae monitoring takes place between June and late September. The *Osmoderma eremita* specimens in the boxes are counted, weighed and measured with a precision calliper and, if appropriate, also marked. The marking must be made before release with a small numbered label (e.g. numbers to mark the queen bees).

During the *in situ* breeding all data are recorded. This includes during the WMB installation phase, in the larval insertion phase and during the periodical checks by the entomologists, recording dates, geo-referenced location, environmental conditions, type of tree, height of installation, number of adult specimens and larvae, including their stage when inserted, number of specimens after each check, measurement of size, weight and evaluation of health status, annotations of found remains, other species present, etc.

Also all phases of *in situ* breeding are documented and photographed.

For each area of intervention the management of *in situ* breeding is cared for by an expert entomologist and an expert operator, and involves a work commitment of about 2 hours per day for 10 days for each WMB.

ADDENDUM

Indicators for *ex situ* breeding

1. Number of specimens reproduced in the different years as third-instar larvae and as adults;
2. Absence of various diseases and predators in the breeding facilities;
3. Very low percentages (overall below 20%) of the rate of early mortality in larval, pupal and adult stages;
4. Good adaptation of the founding individuals to the conditions recreated in the breeding (e.g. through verification of the oviposition and the feeding);
5. Reproductive rate that falls within the expectations or values stated in the literature;
6. Production of bred specimens with average size and average weight falling within the parameters of the species.

Indicators for *in situ* breeding

1. Number of specimens reproduced in the different years as third-instar larvae and as adults in the different boxes;
2. Number WMBs colonised in the first, second and third year after installation; then calculation of the colonisation rate over several years;
3. Absence of diseases of various kinds and predators in the breeding, as well as very low percentages of early mortality rate in larval, pupal and immature adult stages;
4. Good adaptation of the founding individuals to the conditions recreated in *in situ* breeding, and reproduction rate ranging within the expectations or within values declared in the literature;
5. Production of specimens with average size and average weight, ranging within the parameters of the specie;s
6. Numerical increase of *Osmoderma eremita* population settled in the different WMB boxes, calculated both per single box and overall per placement site;
7. Settlement of other saproxylic species, including valuable ones, in the WMBs.

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Aspects of the biology, ecology and conservation status of *Coenagrion mercuriale castellanii* (Roberts, 1948) and *Graphoderus bilineatus* (De Geer, 1774)

Roberto Fabbri

Coenagrion mercuriale castellanii (Roberts, 1948)

The Southern damselfly is a small dragonfly with a length between 2.7 and 3.1 cm and wingspan between 2.5 and 4.0 cm. The body is slim and coloured in a bright light blue with black patterns. The specific term "mercuriale" derives from the Mercury (Greek-Roman mythological god) helm shape, of the black pattern on its second abdominal segment.

The *C. mercuriale* species' geonemy is predominantly West Mediterranean; it is present from the United Kingdom and Germany to Spain and North Africa. The species is generally subdivided into three subspecies: *C. mercuriale* present in Central Europe, *C. mercuriale hermeticum* present in North Africa and *C. mercuriale castellanii* present in peninsular Italy and in Sicily (Ferreira, 2016). In light of the knowledge gathered so far on the distribution of the species, the taxon *C. mercuriale castellanii* (Roberts 1948) present in Italy, is considered by various authors an endemic subspecies, while others elevate it to the rank of a proper species (*C. castellanii*) (Roberts, 1948; Dijkstra & Lewington, 2006; Riservato *et al*., 2014b; Ferreira, 2016).

In Italy, it is reported in all peninsular regions from Emilia-Romagna to Calabria, with the exception of Abruzzo and Molise; in the continental sector populations are known to be located in Piedmont and Liguria; it is also present in Sicily, while it is not reported in Sardinia (Riservato *et al*., 2014b).

From the historical and recent picture of the species presence data in Emilia-Romagna it is possible to delineate a fairly continuous original distribution of the species in the foothill area, at least between Imola and the Rimini area. Currently the species is known to be present only in three stations included in two SCI: IT4070001 "Vena del Gesso Romagnola" and IT4090001 "Torriana, Montebello, Fiume Marecchia".

Figure 1 Streams in the Rimini area with settlements of *Coenagrion mercuriale castellanii*.

Figure 2. Male specimen of *Coenagrion mercuriale castellanii*.

The species is extremely selective in its choice of reproductive habitat and is ecologically demanding. *C. mercuriale* is associated with running waters, slow, even cold, in particular of streams, spring streams and resurgences, often of karstic nature, up to 750 m altitude. The environmental characteristics that influence the presence and density of populations the most are the direct exposure to sunlight of the watercourse, the constant presence of water, the presence of perennial aquatic plants for spawning, foraging and shelter, the width and depth of the watercourse and the presence of a predominantly silty substrate (Harris, 2000; Strange, 1999; Purse, 2001; Purse, 2002; Rouquette & Thompson, 2006; Rouquette & Thompson, 2007; Purse & Thompson, 2009).

The adult is rather sedentary and flies from April to August. During the breeding season the male does not show a territorial behaviour; it hooks on the female in flight, then the couple settles on the vegetation. At the end of the mating, the female looks for a suitable place for spawning, often in the company of the male, and releases the eggs in the floating or partially emerged vegetation, sometimes submerging itself in water completely; the eggs take two to six weeks to hatch and the development is completed in about a year (Thompson *et al*., 2003).

Generally, the species is monovoltine, semivoltine in Great Britain (Purse & Thompson, 2003), but sometimes, in the southern part of its range as in southern Italy and Algeria, behaves like a bivoltine species, with two annual generations and adult activities also in September (Conci & Nielsen 1956; Dijkstra & Lewington, 2006; Mahdjoub *et al*., 2015).,

This behaviour was confirmed during the field activities of the Life Eremita project in the Rimini area site IT4090001 "Torriana, Montebello, Fiume Marecchia", where the species is present. From the first week of September and until the first week of October, the species has had a second generation, however with a small number of adult individuals, about 10% compared to the number of active specimens in spring-summer, therefore with an only partial late summer extension. A possible explanation could refer to the prolongation of the summer season also in the first part of autumn, with mild temperatures that last until late October (Mahdjoub *et al*., 2015). This has

also been observed in other easily identifiable species such as *Gryllus campestris*, which in recent years has an autumn activity (Fabbri, 2015). On the northern Romagna site IT4070001 "Vena del Gesso Romagnola", where the species is present but with small populations, a second generation has not been verified.

The species is included in Annex II of the Habitats Directive 92/43 / EEC. In Europe, according to IUCN *C. mercuriale* is near to being threatened (NT) and presents decreasing populations (Kalkman *et al.*, 2010; Lorenzo-Carballa *et al.*, 2015). Also in the Italian IUCN red list of Odonates the species is considered almost threatened (NT) (Riservato *et al.*, 2014c). The conservation status of the species, in fact, is considered favourable at national level (Riservato *et al.*, 2014a), while in the northern sector of northern Italy it is considered inadequate as a whole, due to population trends, worsening for several parameters, such as range, population, habitat and future prospects (Riservato *et al.*, 2014a, 2014b, 2014c).

Also in Emilia-Romagna, the taxon conservation status is inadequate and has been assessed as at critical risk (CR) in the regional red list (Agnelli *et al.*, 2010). There is a concrete risk that the species will disappear from the region within a few years. In fact, there are numerous threats that press on regional metapopulation; among the main ones we can list:

- water abstractions and excessive water withdrawals from the sources for various purposes,
- evolution of the vegetation succession with a dense arboreal-shrub mantle that causes the closing and shading of the water courses,
- practices of intensive agriculture causing water pollution due to the percolation of pesticides and agricultural fertilizers,
- reorganisation of small watercourses,
- decrease in rainfall to be ascribed to climate change (Hassall & Thompson, 2008),
- presence of exotic animals that profoundly alter hydrophyte vegetation (e.g. *Myocastor coypus* and *Procambarus clarkii*),
- disturbance by pets in the riverbed (ducks, geese, poultry, etc.)
- the general isolation of the present subpopulations, often characterised by a low number of individuals, combined with the limited dispersal capacity of the species (Riservato *et al.*, 2014a).

Figure 3. Female specimen of *Coenagrion mercuriale castellanii*.

Figure 4. Spawning of *Coenagrion mercuriale castellanii* on stream vegetation.

Graphoderus bilineatus (De Geer, 1774)

The Water beetle is a medium-sized dytiscidae beetle with a length of 14-16 mm. The body is wide and oval. The main colour of the dorsal surface is pale yellow and black, the ventral side is pale yellow. *G. bilineatus* is the only species of the genus *Graphoderus* in which the black band of the pronotum's posterior rim is less than half as wide as the central yellow band (Nilsson & Holmen 1995).

Graphoderus bilineatus is a species with a W-Palearctic chorotype; present from Western Siberia to Europe (Nilsson & Hájek, 2015), it is also reported in Turkmenistan (Forster, 1996). In Europe it is more frequent in the northern regions of its distribution area (Trizzino *et al.*, 2013), while it is absent in the Iberian Peninsula (Franciscolo, 1979; Nilsson, 2004) and is very rare in the Netherlands. In Europe, the Water beetle is only present in some regions of Germany (Hendrich & Balke, 2000), in France (Bameul, 1994; Foster 1996; Queney, 2004) and in Belgium (Bosmans & Van Stalle, 1983; Dopagne, 1995). In England the species has not been recorded for almost a century and therefore can be considered extinct (Foster, 1996). *G. bilineatus* is rare in Denmark, but widespread in Sweden and Finland (Holmen, 1993; Nilsson & Holmen, 1995). Stable and more numerous populations are known in the south of Scandinavia, in Russia, in Belarus and in Ukraine (Hendrich & Balke, 2000). In western and central Europe, the species appears to have been on a declining trend since the mid-20th century (Nilsson & Holmen, 1995; Foster, 1996; Hendrich & Balke, 2000; Huijbregts, 2003). Despite this decline new populations have recently been discovered, for example in France (Bameul, 1994), Germany (Haesloop, 2001; Hendrich & Balke, 2000; Hendrich *et al.*, 2012) and in various nature reserves in the Netherlands (Huijbregts, 2003; Cuppen, 2005; Cuppen & Koese, 2005). In Eastern Europe, several stations have recently been found in countries such as Slovenia (Ambrožič *et al.*, 2015), Croatia (Temunovic *et al.*, 2012a; Temunovic *et al.*, 2012b), Serbia (Mesaroš, 2012), and Hungary (Csabai *et al.*, 2015).

In Italy it was known only in some parts of Piedmont, Lombardy, Trentino, Emilia-Romagna and Tuscany, but in many of them it was no longer reported in the last thirty years (Rocchi 2005; Mazzoldi, 2009; Mazzoldi *et al.*, 2009; Nardi *et al.*, 2015). In particular, in Emilia-Romagna, in the lowland

Figure 5. Patrignano Lake, Modena Apennines, presence station of *Graphoderus bilineatus*.

stations mentioned in the last century (near Bologna, Mezzolara and Buda (BO), along the Romea road and valleys in Ravenna), the species has not been confirmed anymore (Mazzoldi *et al*., 2009; Fabbri R., 2013; Nardi *et al*., 2015).

The *G. bilineatus* species is bound to fresh stagnant mesotrophic or oligotrophic fresh water, which is cool, limpid and permanent. Depending on the area of presence in its distribution area these waters are more or less rich in aquatic plants, in particular of *Phragmites*, but also *Nupher*, *Nymphaea*, *Menyanthes*, *Myriophyllum*, *Utricularia*, and differ in the degree of sun exposure. The basins are generally of large size, such as lakes, large ponds, and large peat bogs. However, the species can also live in small pools (less than 5 m wide) and in channels with adequate depth (Franciscolo, 1979; Hájek, 2004; Cuppen *et al*., 2006; Hendrich & Spitzenberg, 2006; Koese *et al*., 2008; Mazzoldi *et al*., 2009; Hendrich *et al*., 2012; Trizzino *et al*., 2013). The other species of *Graphoderus* (*austriacus, cinereus, zonatus*), on the other hand, prefer small bodies of water, even with temporary waters. *G. bilineatus* generally prefers basins with slightly acid pH (between 5.5 and 7) and low conductivity, less than 90 μS/cm (Cuppen *et al*., 2006).

G. bilineatus is an adult stage predator and necrophage. The larva is specialised in hunting small planktonic organisms. Both stages are excellent swimmers and can also be found in very deep waters (over one metre) but generally they are at a depth between 75 and 125 cm (Cuppen *et al*., 2006). The larvae probably feed in open waters on Cladocera Crustaceans, and on macroinvertebrates such as Ephemeroptera and Diptera Chironomidae (Galewski, 1975; Nilsson & Holmen, 1995). In the last larval phase, they move towards the bottom where they often hunt also in a dense, submerged vegetation (Cuppen *et al*., 2006). The mating takes place in water; the eggs are spawned between late spring and early summer. The development of egg, larva and pupa takes about 60-70 days altogether (Nilsson & Holmen, 1995; Hendrich & Balke, 2000). Wintering takes place in water during the adult phase and adults are found throughout the year (Franciscolo 1979; Nilsson & Holmen 1995; Trizzino *et al*., 2013). As in all *Dytiscidae*, the nymphosis takes place on the ground inside underground cells along the banks of ponds and lakes. The adult is able to remain underwater for several minutes, thanks to the ability to keep an air bubble under the elytra. Recently, a low colonisation rate has been observed due to poor ability to fly (Iversen *et al*., 2017).

The monitoring carried out during the Life Eremita project in 2016-2017 and the data collected in previous years highlighted that the species is still present in Lake Pratignano in the high Emilian Apennines, an evolving peat bog with acid waters and low conductivity. Here the species has an annual generation (monovoltine cycle), with the presence of larvae at least from June and until the beginning of August, and activity of the new generation of adults from September to past mid-October, and then wintering until the following spring. The mating already takes place in the autumn. The adults resume activity around May and they have been observed feeding on a wide range of aquatic invertebrates (larvae of Ephemeroptera, Coleoptera and Diptera, Annelids, Nematodes), but also on adult insects accidentally fallen into water (Trichoptera, Diptera, Gryllidae).

The species is included in Annexes II and IV of the Habitats Directive 92/43 / EEC. It is considered by the IUCN in the European Red List as a vulnerable species (VU) (Forster, 1996) (this ranking is to be considered not updated, IUCN 2018 note) and in Italy, according to IUCN criteria, as at Critical Risk (CR) (Nardi *et al*., 2015). Also in Emilia-Romagna, the taxon conservation status is classified as inadequate and has been evaluated as critically endangered ("criticamente minacciato" - CR) in the regional red list (Agnelli *et al*., 2010).

Among the main threats are eutrophication of aquatic environments (for example due to cattle drinking and grazing around the basins), the progressive increase in temperatures, the introduction of alien species, such as the Louisiana shrimp (*Procambarus clarkii*), and exotic predatory fish species, which can cause the disappearance of local populations of Dytiscidae Coleopterans (Bamuel, 2013; Trizzino *et al*., 2013; Nardi *et al*., 2015).

Figure 6. Male specimen of *Graphoderus bilineatus*.

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Update of the distribution of *Coenagrion mercuriale castellanii* (Roberts, 1948) in Emilia-Romagna

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Introduction

As part of the Life Eremita project (LIFE14 NAT / IT / 000209), monitoring was carried out on the dragonfly *Coenagrion mercuriale castellanii* (Roberts, 1948), an Italian endemic subspecies undergoing strong rarefaction, which in Emilia-Romagna is extremely localised. The monitoring was aimed at defining the presence and distribution of the species in the project area (*ex ante* monitoring), in order to plan the projected interventions for the improvement of its conservation status. To this end, the Life Eremita project includes interventions for habitat improvement and translocation actions for the purpose of restocking or reintroduction, with the ultimate goal of strengthening existing populations. The new data, compared to previous data, already available on the regional database, permitted an update on the distribution of the species in the intervention area of the Life Eremita project.

Study area

The study area was identified by analysing the previous data of species presence. The investigations were concentrated in the Natura 2000 sites falling between eastern Emilia and Romagna, where the bibliography reports verified observations of the species presence, both recent and in the past. The investigative effort was also enlarged by investigating the species also in central-western Emilia, in territories where specific studies had never been carried out. Once having chosen the area, the water bodies potentially suitable for the ecological needs of the *taxon* have been identified on a cartographic basis. Subsequently the environmental characteristics suitable to host the species were verified in the field. In total, 24 water courses or other types of wet environments (e.g. springs) have been monitored; of these, 17 fall within the Natura 2000 network (in 7 sites), while the remaining ones fall outside, often in areas that are however adjacent.

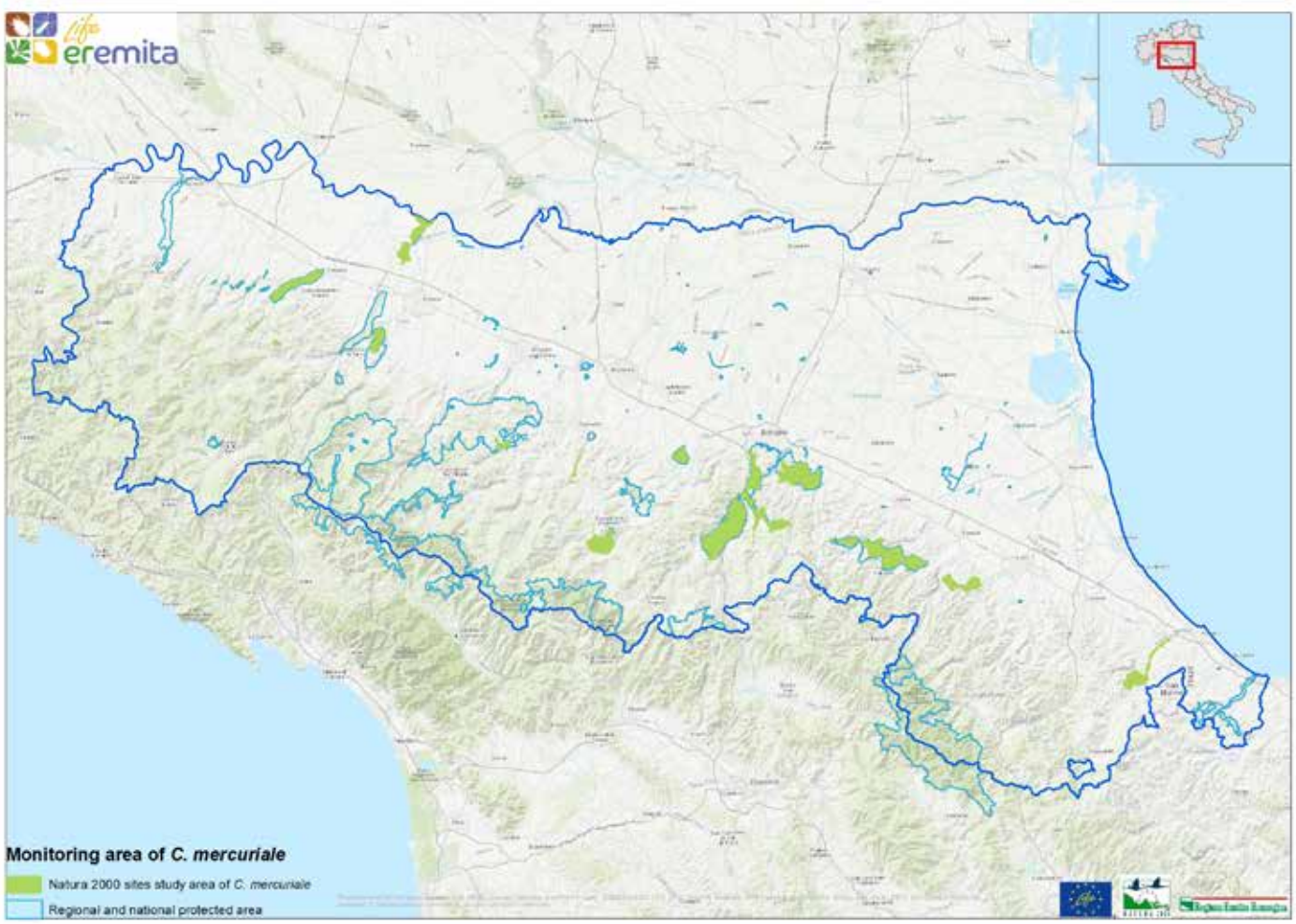

Figure 1. Study area of the *ex ante* monitoring of *C. mercuriale castellanii* as part of the Life Eremita project (2016-2017). Monitoring has been carried out on 7 Natura 2000 sites in Emilia-Romagna.

Rete Natura 2000 site	Area (ha)	Managing bodies	Province	Municipali
SCI-SPA IT4020017 - Area delle risorgive di Viarolo, Bacini di Torrile, Fascia golenale del Po	2.622	Management body for the parks and biodiversity Western Emilia	PR	Colorno, Parma, Sissa Trecasali, Torrile
SIC IT4030018 - Media Val Tresinaro, Val Dorgola	514	Management body for the parks and biodiversity Central Emilia	RE	Baiso, Carpineti, Casina, Viano
SCI IT4040013 - Faeto, Varana, Torrente Fossa	391	Management body for the parks and biodiversity Central Emilia	MO	Prignano sulla Secchia, Serramazzoni
SCI IT4050003 Monte Sole	6.476	Management body for the parks and biodiversity Eastern Emilia	BO	Grizzana Morandi, Marzabotto, Monzuno, Sasso Marconi
SCI-ZPS IT4050012 Contrafforte Pliocenico	2.628	Management body for the parks and biodiversity Eastern Emilia	BO	Loiano, Monterenzio, Monzuno, Pianoro, Sasso Marconi
SCI-ZPS IT4070011 - Vena del Gesso Romagnola	5.540	Management body for the parks and biodiversity Romagna	BO-RA	Borgo Tossignano, Casalfiumanese, Fontanelice, Imola, Brisighella, Casola Valsenio, Riolo Terme
SCI IT4090002 - Torriana, Montebello, Fiume Marecchia	2.472	Management body for the parks and biodiversity Romagna	RN-FC	Poggio Torriana, Rimini, Santarcangelo di Romagna, San Leo, Verucchio, Sogliano al Rubicone
Outside SCI (2 transects)	---	Management body for the parks and biodiversity Romagna	RA	Riolo Terme
Outside SCI (1 transect)	---	Management body for the parks and biodiversity Central Emilia	MO	Serramazzoni
Outside SCI (4 transects)	---	Management body for the parks and biodiversity Eastern Emilia	BO	Pianoro, Castel San Pietro Terme, Sasso Marconi
Outside SCI (2 transects)	---	Casentinesi Forests, MonteFalterona and Campigna National Park	FC	Tredozio

Table 1. Natura 2000 sites of the study area for *C. mercuriale*.

Figure 2. *Coenagrion mercuriale castellanii* occurrence station, in the Casola Valsenio municipality, in the Vena del Gesso Romagnola site.

Data source

The previous data have been extracted from the database of the species reports, available at the Protected Areas, Forests and Mountain Development Service of the Emilia-Romagna Region. The database, updated in 2014, is the result of a substantial effort started in 2010 with funds from the 2007-2013 PSR ("Piano Sviluppo Rurale", that is, *Rural Development Plan*), which has set up systematic reporting of species of conservation interest in the regional territory, through a thorough analysis of the existing bibliography, contacts with the managing bodies of Natura 2000 sites, research institutions, naturalistic groups, specialist scholars, and the study of findings kept in museums and other public institutions or in private collections. Overall, the previous data on the presence of the species covers a period of more than a century, from 1877 to 2010. Added to these were the new data coming from the *ex ante* monitoring of the Life Eremita project.

Monitoring methods

Two monitoring campaigns were carried out in 2016 and 2017, during the adult activity period, in relation to meteorological conditions: from April to September in 2016 and from March to July in 2017. The monitoring methods used were the count (VES) and capture-mark-recapture (CMR) of adult specimens (Thompson *et al*., 2003; Watts *et al*., 2007; Hassall & Thompson, 2012; Rovelli *et al*., 2016), following a monitoring protocol specifically written within the Life project.

Linear transects of at least 100 m in length have been outlined, appropriately identified with an identification code (ID) composed of the site code of the Natura 2000 site, the acronym of the Park or Macro-area, the transect code composed of the identification code of the species (Coe = *Coenagrion mercuriale castellanii*), the letter L (linear) and a sequential number (e.g. IT4090002_MAR_Coe_L2).

Figure 3. Habitat highly suitable for the life of *Coenagrion mercuriale castellanii* in the San Leo (RN) municipality, in the Torriana, Montebello, Fiume Marecchia site.

In relation to the extension of the body of water, the sampling was carried out walking slowly on several transects, chosen randomly or on the basis of some variables (e.g. plant cover) and of the possibility to walk along the bank. In the counting method, notes were taken of all the adult specimens spotted along the transects. During the counting of the specimens a mechanical or digital counter was also used, and only specimens necessary for the identification of the species were captured with a dragonfly net and immediately released. To verify the suitability for the species of each watercourse, its main morphological and ecological characteristics have been described in the field sheet, and also the factors deemed as limiting have been taken note of. All monitoring phases are documented and photographed. The gathered data were collected on special field sheets, subsequently uploaded to the project information system.

Figures 4. Monitoring of *C. mercuriale castellanii* using the capture-mark-recapture method.

Results

The following table lists the previous reports in chronological order (from 1899 to 2012) of *C. mercuriale* in Emilia-Romagna, extracted from the database of species reporting, available from the Emilia-Romagna Region archives.

In the *ex ante* monitoring campaigns of the LIFE Eremita project in the 2016 and 2017 biennium, *C. mercuriale castellanii* resulted as present in SCI-SPA IT4070011 "Vena del Gesso Romagnola", in the province of Ravenna, and in SCI IT4090002 "Torriana, Montebello, Fiume Marecchia ", in the province of Rimini.

A male specimen has been identified, probably a wandering individual off-site Natura 2000, near SCI IT4070011, on the same stream on whose stretch flowing inside the site the population is well established.

Report source	Date	Location	Municipality and Province
Bentivoglio, 1899	[1877]	Valli di Sant'Anna	San Cesario sul Panaro (MO)
Conci & Galvagni, 1948	1941-42	Ladino	Forlì (FC)
Conci, 1949	1934	Torrente Ravone	Casalecchio di Reno (BO)
Terzani *et al.*, 1994	1973	Quartiere Pedagna	Imola (BO)
Terzani, 1978	1974	Madonna dei Fornelli	San Benedetto Val di Sambro (BO)
Fabbri, 2012	1997	Ponte Fantella	Premilcuore (FC)
Fabbri, 2012	1999	Rio Basino	Riolo Terme (RA)
Fabbri, 2012	2008	Rio delle Zolfatare	Brisighella (RA)
Fabbri, 2012	2009-10	Rii di Pietracuta	San Leo (RN)

Table 2. List of previous reports (1899-2010) of *Coenagrion mercuriale* extracted from the regional database.

Natura 2000 site	Transect ID	Municipality	Transect length (m)	Description	Specimens 2016 (N)	Specimens 2016 (N)
Fuori SIC	Fuori_SIC_MAR_Coe_L5-1	Borgo Tossignano (BO)	842	Watercourse fed by resurgent. Arboreal-shrub shading of 60-90% of the watercourse. Medium suitability for the species.	1	0
Vena del Gesso Romagnola	IT4070011_MAR_Coe_L5-3	Casola Valsenio (RA)	772	Watercourse fed by resurgent. Arboreal-shrub shading of 60-90% of the watercourse. Medium suitability for the species.	22	52
Vena del Gesso Romagnola	IT4070011_MAR_Coe_L4	Brisighella (RA)	206	Watercourse fed by resurgent. Arboreal-shrub shading of 70-90% of the first part of the watercourse, the second part threatened by breeding farm of domestic animals. Low-medium suitability for the species.	1	0
Vena del Gesso Romagnola	IT4070011_MAR_Coe_L8	Casola Valsenio (RA)	348	Watercourse fed by resurgent. Arboreal-shrub shading of 30-40% of the watercourse. Medium suitability for the species.	0	4
Torriana, Montebello, Fiume Marecchia	IT4090002_MAR_Coe_L1	San Leo (RN)	578	Watercourse fed by groundwater. Arboreal-shrub shading of 50-80% of the watercourse. Medium suitability for the species.	1482	1068
Torriana, Montebello, Fiume Marecchia	IT4090002_MAR_Coe_L2	San Leo (RN)	588	Watercourse fed by groundwater. Arboreal-shrub shading of 50-80% of the watercourse. Medium suitability for the species.	272	344
Torriana, Montebello, Fiume Marecchia	IT4090002_MAR_Coe_L3	San Leo (RN)	508	Watercourse fed by groundwater. Arboreal-shrub shading of 50-90% of the watercourse. Medium suitability for the species on the right bank only.	134	227

Table 3. Sites of presence of *Coenagrion mercuriale castellanii* observed in the 2016-2017 biennium.

The highest number of individuals counted was registered on site IT4090002 - Torriana, Montebello, Fiume Marecchia in three streams in the municipality of San Leo (RN). With the VES method, on the Rimini site 1513 adults were counted in 2016 and 1639 adults in 2017. With the CMR method, applied only in 2016, 375 males were captured and marked, 8 of which were recaptured (Fabbri *et al*., 2017). In the IT4070011 Vena del Gesso Romagnola site, with the VES method, 24 adults were found in 2016 and 56 adults in 2017.

Discussion

During the *ex ante* monitoring the current presence of *C. mercuriale castellanii* was ascertained only in the easternmost area, despite the effort to extend the study area also in the Natura 2000 sites part of the project, which are located in the central and western sector of the regional territory. The species was found only in the foothill area. In particular, *C. mercuriale castellanii* was only found in two Natura 2000 sites (IT4070011 and IT4090002) located in the province of Ravenna and in the province of Rimini.

In the IT4070011 Vena del Gesso Romagnola site the species contact consisted of only a few specimens in two transects corresponding to two streams (IT4070011_MAR_Coe_L8, IT4070011_MAR_Coe_L5-3) in the municipality of Casola Val Senio (RA). Pending a quantitative analysis of the populations surveyed, the few individuals observed suggest the presence of numerically very small populations, probably due to the widespread covering of watercourses by trees and bushes that reduces the extension of the suitable habitat. These stations are new sites for the presence of the species in Emilia-Romagna. At the same site - IT4070011 – the species was not found in three other transects monitored, corresponding to three different streams, but after appropriate and precise interventions on the habitat (thinning and elimination of the arboreal-shrubby vegetation) the watercourses will become suitable to host *C. mercuriale castellanii*. The station IT4070011_MAR_Coe_L4 in the Brisighella municipality, previously known, is today not suitable to the species anymore, or to the greater part of the Odonates. This is a consequence of the powerful interference of the manager of the adjacent lands who started a breeding farm of domestic animals (poultry and more) close to the stream; therefore, it was not possible to reconfirm the presence of the species in this station.

In the IT4090002 Torriana, Montebello, Fiume Marecchia site the population seems to be conspicuous and stable, even though it is located near the northern margins of the distribution area of the sub-species in Italy. The population is distributed discontinuously in three parallel, ecologically connected channels located in the Municipality of San Leo (RN). The first stream, corresponding to transect IT4090002_MAR_Coe_L1, has the longest section with minor shading and on its south-eastern side has a spring pool that generates a small marshy area well frequented by the adults of *C. mercuriale* and other species of Odonates. The third stream, corresponding to transect IT4090002_MAR_Coe_L3, is substantially shaded by tall trees and bushes, and is also characterised, along with the second stream (IT4090002_MAR_Coe_L2), by banks with numerous hedges of shrubs and brambles. At any rate, the three streams are particularly suitable for the species because they retain large stretches of their course not completely covered by arboreal-shrubby vegetation. It can be assumed that, as a result of habitat improvement interventions in favour of the species in the more shaded sections, the population could rapidly spread in the new suitable stretches (Poloni, 2017).

The framework provided by the previous data of species reports in Emilia-Romagna (Table 2) makes it possible to delineate a fairly continuous original distribution in the foothills area, at least between Imola and the Rimini area, however no longer confirmed by the results of the 2016-2017 monitoring. The Bologna and Modena stations, to which the reports of the last century refer, no longer support the characteristics of suitability for the target species. Also, other previous reports in the territory of Romagna have been checked but the result was negative.-

The reasons for this result, despite the sampling effort made, can be traced back to the fact that there are few watercourses in Emilia-Romagna that have morphometric characteristics, water quality and aquatic and riparian vegetation, suitable to accommodate *C. mercuriale castellanii*. Several streams and watercourses are subject to pressure factors that reduce their suitability to host the species. Examples of this, in order of importance, are: water abstraction from the springs resulting in a reduction in the flow or drainage of the river, vegetation left to freely evolve, with consequent too much shadowing of the river by shrubs and trees, the presence of civil, agricultural and industrial waste that reduce the quality of water, the presence of domestic animals inside or near the watercourses, the management of riparian vegetation mowed to ground level.

Conclusions

The presence of *C. mercuriale castellanii* in Emilia-Romagna is limited to the foothill area and located in only three stations, in disjointed areas of the Romagna territory. All known populations fall within sites of the Natura 2000 network, two particularly small ones within SCI-SPA IT4070011 Vena del Gesso Romagnola and one, more stable and abundant, within SCI IT4090002 Torriana, Montebello, Fiume Marecchia. The populations of the IT4070011 site are disjoined from that of the IT4090002 site as they are more than 50 km apart.

The results confirm the regressive trend of the species in Emilia-Romagna, also because several reports dated between the 40s and 70s of the last century have not been confirmed recently, due to the gradual disappearance of the small sunny streams with clear and permanent waters, suitable to host the species. The monitoring carried out enabled the planning of habitat improvement interventions and translocation actions aimed at reinforcing the populations present. The monitoring of C. mercuriale castellanii was the first application in Italy of the methods adopted by ISPRA (Higher Institute for Environmental Protection and Research) and the Ministry of the Environment for the monitoring of species of Community interest (Stoch & Genovesi, 2016). By using this method also in the ex post monitoring planned by the Life Eremita project, for the first time a temporal series of abundance values from which to obtain quantitative information on the population trends in Emilia-Romagna will be available.

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Coordinated actions to preserve *Coenagrion mercuriale castellanii* (Roberts, 1948) in Emilia-Romagna

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Introduction

As part of the Life Eremita project, after a preliminary monitoring campaign aimed at verifying the distribution of *Coenagrion mercuriale castellanii* in Emilia-Romagna and a parallel investigation to identify the areas potentially suitable for hosting the species, a conservation program has been defined with the goal of expanding the species distribution area in Emilia-Romagna and reinforcing the populations present.

The species presents an inadequate conservation status in northern Italy with the worsening of all the reference parameters: range, population, habitat and future prospects (Riservato *et al*., 2014a, 2014b, 2014c). The results of the *ex-ante* monitoring confirm the regression trend of the species In Emilia-Romagna, due to the progressive disappearance of its habitat of choice.

To counter this process, the Life Eremita project has identified conservation actions toward the restoration of the ecological conditions of the aquatic lotic habitats where the species lives to favour the expansion of its distribution area, and the subsequent translocation of specimens coming from a source population, for the purpose of numerically reinforcing reduced populations.

Habitat improvement actions

To select the watercourses on which to intervene, a wide census of the environments potentially suitable for hosting the species was carried out on a regional scale. The suitability of the watercourses to the species was determined using the following criteria:

- Previous presence data;
- Water present all year;
- Chemical-physical data of the water (pH, conductivity, temperature, depth, clarity);
- Riverbed not excessively shaded;
- Distance (not more than 3 km) from the sites of ascertained presence of the species during the *ex ante* monitoring;
- Properties with preference for the public, as a guarantee of the sustainability of the interventions in the medium and long term and of a greater ease and speed of operation.

Based on these criteria, a suitability value for each watercourse surveyed has been assigned in the field, expressing it in the following scale of values: high, medium, low, and none, according to the expert judgment of the detector.

In SCI IT4070001 "Vena del Gesso Romagnola" six watercourses were found to be suitable for hosting the species. In two of these streams the species is present, but with numerically very small populations and with a limited extension of the suitable habitat; in four other streams the species has not been found, but the environments show characteristics suitable to host it, following the implementation of habitat improvement interventions.

In SCI IT4090002 "Torriana, Montebello, Fiume Marecchia", the three streams where the highest number of individuals has been counted are the ones involved in the interventions. In the other Romagna and Emilia areas of the project where the species has been researched, the watercourses have turned out to not be sufficiently suitable to be subjected to concrete actions to improve the habitat. In fact, there are numerous factors of threat to the species in Emilia -Romagna: water abstraction and excessive water withdrawals from the sources for various purposes, reorganisation of the small watercourses, decrease in rainfall (to be ascribed to climate change; Hassall & Thompson, 2008), evolution of the vegetation succession that causes the closing and shading of the water courses, presence of exotic animals that profoundly alter hydrophyte vegetation (e.g. coypu), disturbance of the riverbed by domestic animals (ducks, geese, poultry, etc.) and, last but not least, the general isolation of the present subpopulations, often characterised by a low number of individuals.

Table 1 shows the features of the streams identified by the conservation intervention plan for the species. The interventions concern n. 10 transects in total n. 9 water courses, all within the Natura 2000 network (Figure 1).

Within each Natura 2000 site, the action plan is aimed at promoting the creation of ecological corridors between the various sites of presence in order to allow greater diffusion of the species in its distribution area.

Natura 2000 site	Transect ID of the stream-watercourse	Municipality	Length	Suitability for actions
IT4090002 "Torriana, Montebello, Fiume Marecchia"	[IT4090002_MAR_Coe_L1]	San Leo	578 m	high
	IT4090002_MAR_Coe_L2	San Leo	588 m	high
	IT4090002_MAR_Coe_L3	San Leo	508 m	high
IT4070001 "Vena del Gesso Romagnola"	IT4070011_MAR_Coe_L4	Brisighella	206 m	medium-high
	IT4070011_MAR_Coe_L5-2*	Borgo Tossignano	340 m	medium
	IT4070011_MAR_Coe_L5-3*	Casola Valsenio	772 m	high
	IT4070011_MAR_Coe_L6-2	Brisighella	255 m	high
	IT4070011_MAR_Coe_L7	Riolo Terme	199 m	medium
	IT4070011_MAR_Coe_L8	Casola Valsenio	348 m	high
	IT4070011_MAR_Coe_L9	Casola Valsenio	206 m	medium

Table 1. List of transects subject to concrete actions for *Coenagrion mercuriale*. The interventions involve 9 watercourses corresponding to 10 transects. * Two transects, IT4070011_MAR_Coe_L5-2 and IT4070011_MAR_Coe_L5-3, belong to a single watercourse.

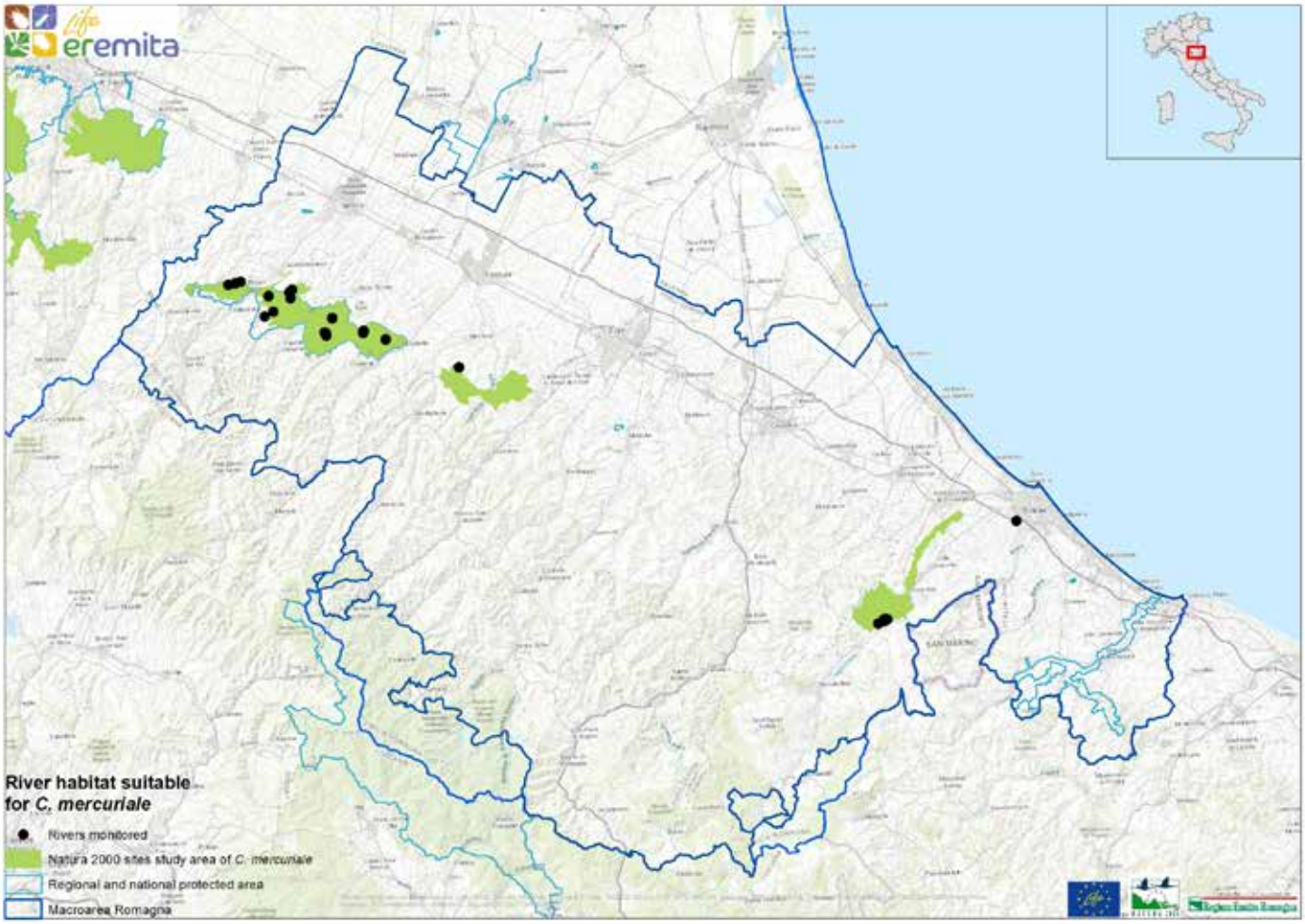

Figure 1. General localisation of the areas for the interventions on *Coenagrion mercuriale* in the Macro-area Romagna.

The main limiting factor for the species found in the watercourses identified for the interventions concerns the excessive development of riparian vegetation and the consequent shadowing that prevents the growth of submerged and semi-submerged aquatic plants (e.g. sp. *Mentha, Veronica, Carex, Juncus, Equisetum, Rorippa*, etc.), whose presence is necessary for spawning.

The control of the vegetation along the banks in the past was carried out naturally and continuously by the flocks and by the cattle that went watering in the rivers, and in the distant past by the herds of wild grazing animals. Now the practice of sheep farming and cattle grazing in the majority of the foothills area of the Emilia-Romagna region is either lost or has been greatly reduced.

Therefore, to allow a greater insolation on the riverbed, and thus allow a conspicuous development of the aquatic plants necessary for the biological cycle of the species, interventions have been planned and implemented, such as trimming along the banks with elimination of shrubs, bushes and brambles, delimbing and pruning of large trees, felling of trees placed within the streams and on the banks.

These types of interventions were carried out alternately on short sections of about 80 m with intervals of 30-50 m, so as to maintain a certain degree of naturalness of the watercourse and not adversely affect the flora and fauna present (e.g. amphibians and birds). The wood material and the obtained waste have been moved away from the watercourses so as not to release vegetable debris inside the riverbed. These interventions will be repeated in the following years to control the regrowth of trees and shrubs.

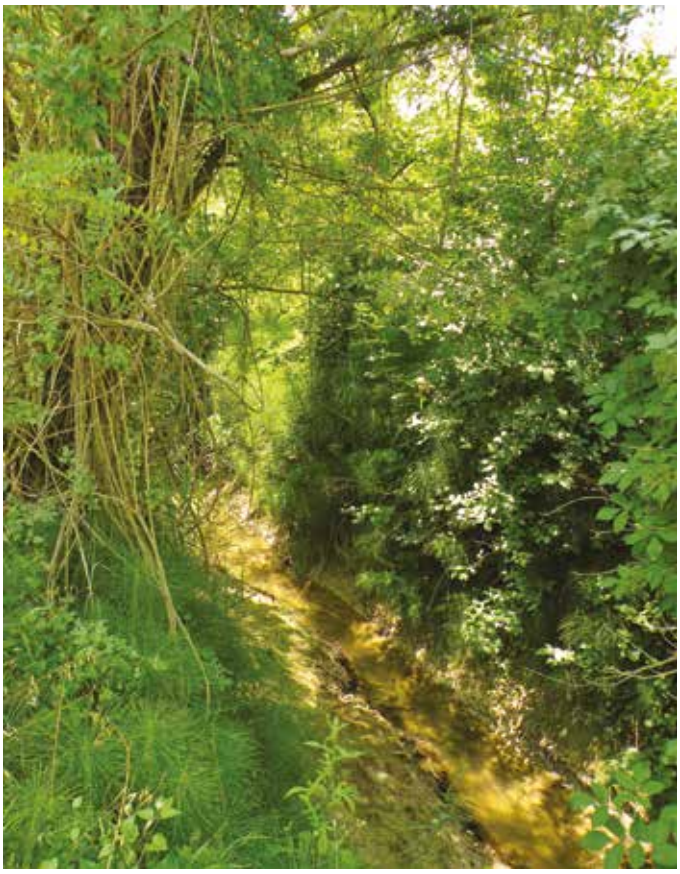

Figure 2. Stream in the Vena del Gesso Romagnola before and after the works to partially control the arboreal-shrubby vegetation had taken place.

The translocation program

During the *ex ante* monitoring of the LIFE Eremita project, the highest number of individuals counted (1513 adults in 2016, 1639 adults in 2017) was recorded on the Natura 2000 site IT4090002 - Torriana, Montebello, Fiume Marecchia in three streams in the municipality of San Leo (RN). On the site IT4070011 - Vena del Gesso Romagnola - the species was found in two streams with a few dozen individuals (24 adults in 2016, 56 adults in 2017). The extensive habitat monitoring campaign carried out during the LIFE Eremita project to detect the suitability of watercourses to host the species highlighted that the only watercourses with ecological characteristics suitable for *C. mercuriale* remain located in the two IT4070011 and IT4090002 sites.

The Rimini area population present in the waterways of the IT4090002 site is fundamentally isolated with respect to the other one in Romagna that is present in the streams of the IT4070011 site; they are at a distance between 50 and 65 km from each other. Again, it is known that *C. mercuriale castellanii* has a low dispersion capacity and is typically a sedentary species, generally with 75% of adults who do not move over 100 m, and 95% over 300 m (Watts *et al*., 2004; Watts *et al*., 2007b). Some studies show that the species can also move significantly, over 2 km, in the open agricultural landscape (Keller *et al*., 2010; Keller *et al*., 2012; Lorenzo-Carballa *et al*., 2015), on the contrary, when there are obstacles, like woods and substantial changes in altitude, its dispersion capacity decreases.

In light of the historical and current distribution described, which is extremely reduced compared to the past, and of the low dispersal capacity of the species (Watts *et al*., 2007a and 2007b; Keller *et al*., 2012), a translocation plan was set up with the aim of expanding the distribution area of the species and strengthening the population on site IT4070011. The operational area will involve the two Natura 2000 sites IT4070011 and IT4090002, in the waterways suitable for hosting the species, especially after the improvement and restoration of the habitats. Some adult specimens of *C. mercuriale castellanii* will be moved from the source site of Rimini (IT4090002) towards 6 streams on the site IT4070011-Vena del Gesso Romagnola.

Transfers that will take place in the Romagna area with the translocation plan, with distances from 50 to 65 km, are considered transfers within the same metapopulation. It is considered possible to exclude any risk of genetic pollution between different phyletic lines. In fact, from recent genetic analysis (study of the mitochondrial genes COI and 16S rRNA and of the nuclear genes PRMT, MLC and AgT) conducted throughout the European-North African area of species distribution (with materials also coming from various Italian locations from Piedmont to Basilicata, including specimens collected in Romagna in the Vena del Gesso Romagnola) it is possible to affirm that the Italian populations belong to a single phyletic line (Ferreira, 2016). Also the operational territory is ecologically similar for both the sites (of source and repopulation), located in low hills with withdrawal and release heights between 110-250 m, and therefore with negligible variations in terms of altitude and consequent phenology.

The translocation plan provides for the withdrawal from the source site of about 170 specimens per year (with a sex ratio of 1:1) for a period of three years to be divided, during the release phase, among the various suitable streams on SCI-SPA IT4070011. The quantity of specimens to be released on the individual sites is assessed on a case-by-case basis according to the environmental characteristics, the size of the stream, the result of *ex-ante* monitoring activities, the size of the population and the suitability of the habitat.

The translocation operations will begin in spring 2019, at the beginning of the period of activity of the species and will be repeated (2 -3 times) until June of the same year. Although the species is bivoltine, in Romagna operations will take place between April and June, when the majority of adult individuals emerge. The operations will include the capture of adults in the Rimini stations of the IT4090002 site, which will be marked on the wings as described in the species monitoring protocol (Fabbri; 2017). For each specimen the dimensions, the state of health and the vitality will be checked. Subsequently, the captured individuals will be placed inside a "fauna box" container with some plant stems and food (e.g. *Drosophila* sp.). The transport and release operations will take place within an estimated time interval of two hours. Approximately 10 adults will be placed inside each fauna box, and temperature checks will be performed during the journey. The specimens will be released at the edges of the streams, in a partially shaded area and not exposed to the wind. The data of the release operations (date, time, environmental conditions of the release watercourse, number of specimens, etc.) will be written on data sheets and all steps of the translocation activity will be documented with photos and films.

The program drawn up until now will be preliminarily submitted for verification by the Ministry of the Environment and ISPRA, as mandated by the Decree of the President of the Republic 357/97.

Overall, it is expected that when the species conservation plan is fully implemented, in the two Natura 2000 sites in Romagna, the species will occupy (linear estimation) about 4 km of smaller watercourses compared to the current 1.7 km.

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Update on the presence of *Graphoderus bilineatus* (De Geer, 1774) in Emilia-Romagna

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Introduction

As part of the Life Eremita project (LIFE14 NAT / IT / 000209), monitoring was carried out on the two-striped dytiscid beetle *Graphoderus bilineatus* (De Geer, 1774), an extremely localised entity that is at risk of extinction in Italy and Emilia-Romagna. The monitoring was aimed at updating the presence of the species in the project area, in order to plan the envisaged conservation actions (*ex ante* monitoring). In particular, for this target species the Life Eremita project plans habitat improvement interventions and reintroduction to the wild of *ex situ* bred individuals, with the ultimate goal of strengthening existing populations and / or widening the distribution area of the *taxon*. Starting in 2010, the Emilia-Romagna Region had expanded its knowledge on regional biodiversity, implementing a database of species reporting through the funding of Measure 323 of the 2007-2013 PRSR (*Rural Development Plan*). From the database, it emerged that *Graphoderus bilineatus* was reported in Emilia-Romagna only in three Natura 2000 sites, in the provinces of Bologna, Ravenna and Modena (Agnelli *et al*., 2010). With the project Life Eremita it was possible to perform a coordinated monitoring of the species on a large territory on a regional scale for the first time in Emilia-Romagna. This permitted verification and an update of the previous data, updating also the distribution of the species in Emilia-Romagna. Acquiring this information made it possible to assess the feasibility of setting out an *ex situ* breeding starting from existing populations.

Materials and methods

Study area

The study area covered 38 basins inside 14 sites of the Natura 2000 network, 13 of which in Emilia-Romagna. Considering that in the Life Eremita project area are also included the territories of the two national parks that fall between Emilia-Romagna and Tuscany, the survey area also included a Natura 2000 site in Tuscany. The choice of the water bodies to be monitored was carried out by analysing the previous data for the presence of the species and by searching for further possible suitable sites. These have been preliminarily identified on a cartographic basis and subsequently verified in the field, taking into account the following ecological factors: basin size, constant presence of deep water, water characteristics (mesotrophic or oligotrophic) with low conductivity and pH, presence of aquatic plants,

Figure 1. Study area for the *ex-ante* monitoring of *G. bilineatus* within the Life Eremita project in 2016-2017. The monitoring was carried out on 13 Natura 2000 sites in Emilia-Romagna and 1 site in Tuscany.

Rete Natura 2000 site	Area (ha)	Managing bodies	Province	Municipalitues
IT4010003 Monte Nero, Monte Maggiorasca, La Ciapa Liscia	852 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia occidentale	PC-PR	Ferriere, Bedonia
IT4020008 Monte Ragola, Lago Moo, Lago Bino	1396 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia occidentale	PC-PR	Ferriere, Bedonia
IT4020020 Crinale dell'Appennino Parmense	5280 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia occidentale; Parco Nazionale Appennino Tosco-Emiliano	PR	Monchio delle Corti
IT4030001 Monte Acuto, Alpe Succiso	3524 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale; Parco Nazionale Appennino Tosco-Emiliano	RE	Ventasso
IT4030002 Ventasso	2909 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale; Parco Nazionale Appennino Tosco-Emiliano	RE	Ventasso
IT4030003 Monte La Nuda, Cima Belfiore, Passo Cerreto	3462 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale; Parco Nazionale Appennino Tosco-Emiliano	RE	Ventasso
IT4030006 Monte Prado	618 ha	Parco Nazionale Appennino Tosco-Emiliano	RE	Ventasso, Villa Minozzo
IT4040001 Monte Cimone, Libro Aperto, Lago Pratignano	5173 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale	MO	Fanano, Fiumalbo, Montecreto, Riolunato, Sestola
IT4040002 Monte Rondinaio, Monte Giovo	4849 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale	MO	Fiumalbo, Frassinoro, Pievepelago
IT4040005 Alpesigola, Sasso Tignoso e Monte Cantiere	3761 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia centrale	MO	Frassinoro, Lama Mocogno, Palagano, Pievepelago, Riolunato
IT4050015 La Martina, Monte Gurlano	1107 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia orientale	BO	Monghidoro, Monterenzio
IT4050029 Boschi di San Luca e Destra Reno	1951 ha	Ente di Gestione per i Parchi e la Biodiversità – Emilia orientale	BO	Bologna, Casalecchio di Reno, Pianoro, Sasso Marconi
IT4070011 Vena del Gesso Romagnola	5540 ha	Ente di Gestione per i Parchi e la Biodiversità - Romagna	RA-BO	Brisighella, Casola Valsenio, Riolo Terme, Borgo Tossignano, Casalfiumanese, Fontanelice, Imola
IT4080002 Acquacheta	1656 ha	Ente di Gestione per i Parchi e la Biodiversità – Romagna	FC	Portico, San Benedetto, Tredozio
IT5180018 Foresta di Camaldoli, Badia Prataglia	2937 ha	Parco Nazionale Foreste Casentinesi, Monte Falterona, Campigna	AR	Camaldoli

Table 1. Natura 2000 sites investigated for *G. bilineatus* monitoring.

absence of shrimp and exotic predatory fish and other limiting factors. All monitored wetlands are distributed in the Apennine belt, since the only *G. bilineatus* populations historically known in the plain territory have not been confirmed, and the sites resulted to be not suitable to host the species.

Data source

The previous data have been extracted from the database of the species reports, available at the Protected Areas, Forests and Mountain Development Service of the Emilia-Romagna Region. The database, updated in 2014, is the result of a work started in 2010 with funds from the 2007-2013 PSR (*Rural Development Plan*) which has set up systematic reporting of species of conservation interest in the region. This was carried out through a thorough analysis of the existing bibliography, contacts with the managing bodies of Natura 2000 sites, research institutions, naturalistic groups, specialist scholars, and the study of findings held in museums and other public institutions or in private collections. Following the extraction of the data, as part of the Life project all the locations of the reports were geo-referenced, thus making it possible to represent them graphically on the geographical map of the Emilia-Romagna Region. Overall, the previous data on the presence of the species cover a period from the early decades of 1800 to 2012. Added to these were the new data coming from the *ex ante* monitoring of the Life Eremita project.

Monitoring methods

The monitoring was carried out according to a protocol prepared in the initial phase of the LIFE Eremita project (Fabbri, 2017). The research of *G. bilineatus*, in the 2016 and 2017 biennium, took place using standard capture techniques, already used for the species (Koese & Cuppen, 2006; Koese *et al*., 2008; Hendrich *et al*., 2012; Volkova *et al*., 2013).

Different techniques have been used in relation to the depth and vegetation cover of the investigated basin. In basins with shallow water (less than 40 cm), monitoring was carried out using a D-Frame net for aquatic macroinvertebrates, with a side of 25 cm and a net with meshes of 1 mm on the side and a net depth of 30 cm. In basins with deeper waters and with aquatic vegetation the technique of the triggered, floating Bottle Traps (BT) was used for live capture. The traps were installed at points at least 5 m away from the points where sampling was performed using nets. The traps were checked on the day of placement and withdrawn the next day. The Bottle Trap technique was used only in 2016 in the Lake of Pratignano and then abandoned because it was too risky due to the possible death of the individuals captured. A third method of capture used non-floating traps equipped with bait and air bubbles for live capture. From the spring of 2017, fishing nets were also used, with larger meshes (5 mm side mesh) for free and deep waters. For Lake Pratignano, from October 2017 a further and specific monitoring protocol has been developed and adopted, and sampling was carried out both with nets for macroinvertebrates and with fish nets (5 mm side mesh), employing a minimum of 2 operators (Malavasi & Stefanelli, 2017). All specimens sampled were released after identification and the data were collected on special field sheets, subsequently uploaded to the project information system.

Report source	Date	Location	Municipality and Province
Nardi *et al.*, 2015	Primi decenni XIX sec.	Bologna surroundings	Bologna (BO)
Angelini, 1984	10/05/1957 e 1958	Mezzolara	Molinella (BO)
Banca Dati Regione ER	1978	Buda	Medicina (BO)
Pederzani, 1976; Banca Dati Regione ER	1970, 1980	Punte Alberete	Ravenna (RA)
Banca Dati Regione ER	1981	Valle Mandriole	Ravenna (RA)
Pederzani, 1976; Angelini, 1984	25/04/1963	Fossi lungo strada Romea, Pineta San Vitale	Ravenna (RA)
Ansaloni et al., 2007 (citato come *Graphoderus sp.*, da riferire a *G. bilineatus* dopo verifica del materiale)	2000	Lago di Pratignano	Fanano (MO)
Ansaloni et al., 2016 (citato come *Graphoderus sp.*, da riferire a *G. bilineatus* dopo verifica del materiale)	1999 e 2006	Lago di Pratignano	Fanano (MO)
Pacchioni O., 2010 - Forum degli entomologi italiani (http://www.entomologiitaliani.net)	2001 - 2009	Lago di Pratignano	Fanano (MO)
Mazzoldi *et al.*, 2009	3/09/2009	Lago di Pratignano	Fanano (MO)
Banca Dati Regione ER	17/10/2012	Lago di Pratignano	Fanano (MO)

Table 2. List of previous reports of *G. bilineatus* extracted from the Emilia-Romagna Region database.

Results and discussion

The Table 2 shows the previous reports chronologically (from the first decades of 1800 to 2012) of *G. bilineatus* in Emilia-Romagna, extracted from the database of species reporting available at the Emilia-Romagna Region, verified and updated with new bibliographic references. It is specified that the data of Ansaloni *et al*., 2007 and 2016 cited as *Graphoderus* sp., have been reported by the author as *G. bilineatus* as it was possible to verify the specimens.

G. bilineatus is reported in stations of the Bolognese plains known until the '70s of last century (loc. Mezzolara in the municipality of Molinella and loc. Buda in the municipality of Medicina), within the SCI IT4050022 "Biotopi e ripristini ambientali di Medicina e Molinella". It is also reported in stations of the coastal area of Ravenna, known until the 80s (Punte Alberete, Valle Mandriole and some ditches on the edge of the San Vitale Pine forest, in the municipality of Ravenna), respectively in the IT4070001 SCI, "Alberte, Valle Mandriole" and SCI IT4070003 "Pineta San Vitale, Bassa del Pirottolo". In these stations, as well as others in Italy, the species has not been found again in the last thirty years (Mazzoldi *et al*., 2009; Nardi *et al*., 2015) and has not been reconfirmed even during the monitoring carried out to update the knowledge framework of the Natura 2000 sites, as part of the 2007/2013 PSR (*Rural Development Plan*) Measure 323. The conservation measures and the management plans of the Natura 2000 sites found and described several limiting factors: diffusion of highly invasive alien species such as *Procambarus clarkii*, excessive eutrophication and water pollution, salt-wedge ingression in coastal areas. These limiting factors were further verified in the field inspections preliminary to this work, therefore the presence stations were deemed not currently suitable to host the species.

With reference to the monitoring campaigns carried out in the two-year period 2016 and 2017, the species was found only in Lake Pratignano (Fanano, MO) on the IT4040001 "Monte Cimone, Libro Aperto, Lago Pratignano" site. The number of individuals found is 4 adults and 1 larva in 2016, and 1 adult in 2017.

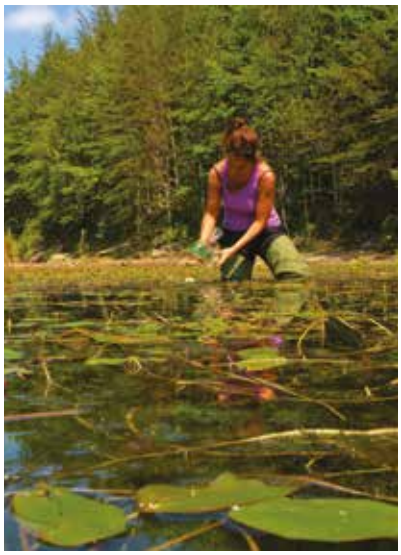
Captures dates in Lake Pratignano	Type of Monitoring	Captured individuals (N)
28/06/2016	Net	1 larva
28/09/2016	Net	1 adult
29/09/2016	Bottle Trap	1 adult
07/10/2016	Net	2 adults
05/09/2017	Net	1 adult

Table 3. Number of sampled individuals in Lake Patrignano in site IT4040001.

During the monitoring of the lake numerous other species of insects have been found (larvae of Anisoptera, Zygoptera, Trichoptera and Ephemeroptera; Hydrophilidae, Notonectidae, Gerridae) and over 10 species of Dytiscidae, which confirm the

Figure 2. Sampling of *Graphoderus bilineatus* with net for aquatic insects.

Figure 3. Use of the fishing net to capture *Graphoderus bilineatus*.



Figures 4. Checking the Bottle Traps.

good suitability of the site to host different taxa of aquatic invertebrates.

In the remaining 37 Apennine basins monitored, the species was not found, although in many cases other large and medium-sized Ditiscidae species (*Dytiscus marginalis, Cybister lateromarginalis, Acilius sulcatus*) were discovered.

Considering the great effort applied and the various sampling techniques adopted, and also considering the ecology and the elusive habits of the species, this species was difficult to find. Despite the considerable effort and the development of protocols expressly studied for this purpose, the low number of individuals captured (6) in Pratignano does not allow a numerical estimate and an analysis of the population structure. Considering that already in the recent past the species had been evaluated as not abundant (although with an approximate evaluation of the abundance estimated "at sight"), even in the absence of quantitative researches (Mazzoldi *et al*., 2009) the results of the monitoring conducted suggest a possible negative trend for the only population confirmed in the region.

Conclusions

The presence of *G. bilineatus* in Emilia-Romagna is reduced to a single mountain basin, an evolving peat bog within the SCI IT44040001 in the Modena Apennines, with a population that is likely in a negative trend. In the other sites of the Natura 2000 Network in Emilia-Romagna where the species was known (IT4050022 in the province of Bologna, IT4070001 and IT4070003 in the province of Ravenna), it has not been reported for more than twenty years and it is believed that historical stations do not have the proper environmental characteristics currently, due to the pressure factors in the Emilia-Romagna plain. The search for new stations of presence has not provided positive results and it is therefore possible to confirm the contraction of the historical distribution area of the species. Based on the results obtained so far on the only population present on the territory today, and not having sufficient guarantees that it might support the removal of possible founders for an *ex situ* breeding, it is deemed as prudent to exclude this work hypothesis, thus avoiding any risk of further worsening of the species state of conservation.

Figura 5. The Life Eremita project's entomologists at Lake Pratignano.

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Genetic analysis aimed at comparing the relict population of *Graphoderus bilineatus* of Lake Pratignano and other potential European source populations

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Introduction

Among the objectives of the Life Eremita project is the conservation in the best conditions of the residual and severely threatened populations of the aquatic beetle *Graphoderus bilineatus* (Insecta, Coleoptera, Dytiscidae) in the territory of the Emilia-Romagna region. The species is strictly protected throughout Europe being included in Annexes II and IV of the Habitats Directive 92/43 / EEC. The project envisaged captive breeding actions and subsequent releases to the wild starting from founders coming from the populations, based on the results of *ex ante* monitoring. The monitoring campaigns carried out in 2016 and 2017, however, confirmed the presence in Emilia-Romagna of a single population of *G. bilineatus*, whose distribution is limited to a single station in the Modena area high Apennines (Lake Pratignano). This is, to date, also the only station of presence for the species confirmed in Italy. Moreover, the extremely low numbers of individuals discovered during the monitoring did not make it possible to determine the size of the population, hence the possibility of withdrawing the founders for *ex situ* breeding from the population of Lake Pratignano was excluded. It is therefore necessary to promptly adopt an alternative emergency strategy, which takes into consideration the possibility of obtaining the founders from other European populations of *G. bilineatus* that are in a good state of conservation. To this end, a genetic characterisation was carried out aimed at comparing the relict population of *Graphoderus bilineatus*, originally present in the Italian sites of Lake Pratignano, and other potential European source populations. The ultimate goal was to acquire useful indications for the choice of the source population from which to take individuals for restocking or reintroduction, and criteria to be taken into account for the recovery of the Italian population of *G. bilineatus*, in consideration of the Ispra (*Higher Institute for Environmental Protection and Research*) "guidelines for the introduction of fauna species "(2007) and the IUCN "Guidelines for Reintroduction and Other Conservation Translocations "(2013).

Materials and methods

To compare populations of the same species it is necessary to use genetic markers with a high degree of variability. Preliminarily, molecular investigations have been carried out on markers already available in the literature, which have led to the identification of potentially informative regions for the purpose: the mitochondrial gene that encodes the first subunit of Cytochrome Oxidase (COI).

The characterisation of COI, generally used to compare different species in studies of phylogeny and barcoding, is undoubtedly a valid starting point also for analysis intended for repopulation and reintroduction. This is because it has the dual utility of verifying the given species and, in the case of definite and prolonged reproductive isolation, of highlighting phylogeographic discontinuities between populations. This could be the case of *G. bilineatus* for which a low rate of colonisation, due to poor flight behaviour, has recently been observed (Iversen et al., 2017). Furthermore, mitochondrial DNA has the advantage of being much more represented than nuclear DNA, making it a potential tool for analysis even on ancient or highly degraded samples. However, mitochondrial DNA has some features - such as matrilineal transmission and the absence of recombination -, which, while being advantageous in phylogenetic studies, may constitute a limitation when comparing populations within species, where gene flow is best described by biparental inheritance markers.

The samples of *G. bilineatus* came from different locations distributed in 4 countries: Hungary (UNG), Lithuania (LIT), Croatia (CRO) and Italy (ITA). In most cases, the samples provided were whole specimens stored in 70% or 95% ethanol, while for some individuals a single leg, or part thereof, was provided, either dry or stored in 95% ethanol. Table 1 details the sampling.

Country of origin (acronym)	Locality	N	Biological sample	Conservazione
Hungary (UNG)	Drava	5	Whole specimens	Eth70
	Bodrog	5	Whole specimens	Eth70
	Danube	1	2 legs	Eth95
Lithuania (LIT)	Klimbales durpynas	1	Whole specimens	Eth75
	Nevežio senvages	1	Whole specimens	Eth75
Croatia (CRO)	National park Kopacki	5	Whole specimens	Eth75
	River Drava, Dsijek	5	Whole specimens	Eth75
	Lonnjsko Poye Natual Park	6	Whole specimens	Eth75
Italy (ITA)	Lago Pratignano	2	Whole specimens	Eth75
		3	1 leg	Eth95
		4	1 leg	Dry

Table 1. *G. bilineatus* samples used in the project.

Analysis of the mitochondrial COI gene

Considering the poor quality of most of the extracts obtained, the analysis of a mitochondrial gene remained the only viable strategy. This choice is dictated by the fact that since the mitochondrial genome is over-represented and more stable compared to the nuclear one, the chances of having integral copies of a mitochondrial gene are much higher even in strongly degraded samples. We therefore proceeded with the characterisation of the mitochondrial gene COI.

In insects, the rate of nucleotide variability of COI is different in different regions of the gene (Lunt et al., 1996). In order to exploit all the polymorphisms, we tried sequencing the gene in its entirety using initially the COI_LCO1490_For and TL2-N-3014-Rev primers already described in the literature (Table 2). However, the amplification of the expected PCR product of about 1500

base pairs in a single reaction was possible only for the 4 samples for which the extract was of good quality: the Lithuanians Gb10 and Gb11 and the Italians Gb28 and Gb29 (Fig. 1, left).

It was therefore necessary to reduce the size of PCR amplified both by using other primers available in the literature and by conceiving new ones suitably designed to cover the entire region to be sequenced (Table 2). We started by bringing to two the number of amplifications necessary for each individual, using primer pairs COI_LCO1490_For- COI_HCO2198_Rev and COI_C1-J-2183_For- TL2-N-3014-Rev. However, the expected bands of about 800 base pairs were obtained and successfully sequenced only from 6 individuals. For all the others it was necessary to further reduce the size of PCR products up to about 200 base pairs, increasing the number of PCR reactions per individual up to 6 (Fig. 1, right) and the number of sequences up to 8 (for some low-quality amplified individuals sequencing in both directions was necessary). Moreover, considering the low concentration of many of the extracts it was necessary to use Taq polymerase with high sensitivity, able to amplify starting from very few template molecules. Fig. 2 shows a schematic representation of the number of portions that have been analysed for each individual to obtain the complete sequence of COI.

Namre of the primer	Primer sequence	Source
COI_LCO1490_For	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
COI_C1-J-1718_For	GGAGGATTTGGTAATTGATTAGTTCC	Simon *et al.*, 1994
COI_Gb_C1-J-1859_For	GGGACAGGATGAACAGTTTATCCTCC	*
COI_C1-J-2183_For	CAACATTTATTTTGATTTTTTGG	Simon *et al.*, 1994
COI_Gb_2269_For	TGCCATATTAGCTATTGGAC	*
COI_Gb_2634_For	CAATAGGGGCTGTATTCGCA	*
COI_Gb_1918_Rev	TACAGAAGCTCCTCCATGGG	*
COI_C1-N-2191_Rev	CCTGGTAAAATTAAAATATAAACTTC	Simon *et al.*, 1994
COI_C1-N-2329_Rev	ACTGTAAATATATGATGTGCTCA	Simon *et al.*, 1994
COI_HCO2198_Rev	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
COI_Gb_2700_Rev	GTGATTCCTGTAAATAAAGGAAATC	*
TL2-N-3014-Rev	TCCAATGCACTAATCTGCCATATTA	Simon *et al.*, 1994

Table 2. Sequences of primers used to amplify and sequence the COI gene.
*Primers designed within this work.

Results

All five individuals of the Italian population of Pratignano presented the same mitochondrial haplotype, never observed in populations sampled in other areas (Figures 3 and 4). In contrast, all other populations showed a greater haplotypic difference compared to the Italian population with: 5 haplotypes in Hungary, 8 in Croatia and 2 in Lithuania obtained respectively from 7, 12 and 2 individuals analysed for a total of 13 different mitochondrial haplotypes.

Considering the small number of samples that could be analysed, the results obtained were integrated by the joint analysis of our dataset with other COI sequences of *G. bilineatus*. The latter are 28 partial COI sequences (only the second half of the gene sequence was available) obtained from Koese and colleagues (unpublished data) starting from DNA extracts from other 5 populations sampled at different sites (Germany, Holland, Austria, Russia and Sweden). Although the analyses were conducted considering only the portion of COI common to the two datasets, and therefore with a reduced number of variable sites, the uniqueness of the haplotype of Pratignano was confirmed as represented by the haplotype networks in Figure 4.

The following box summarises the results of the analysis of the mitochondrial COI gene.

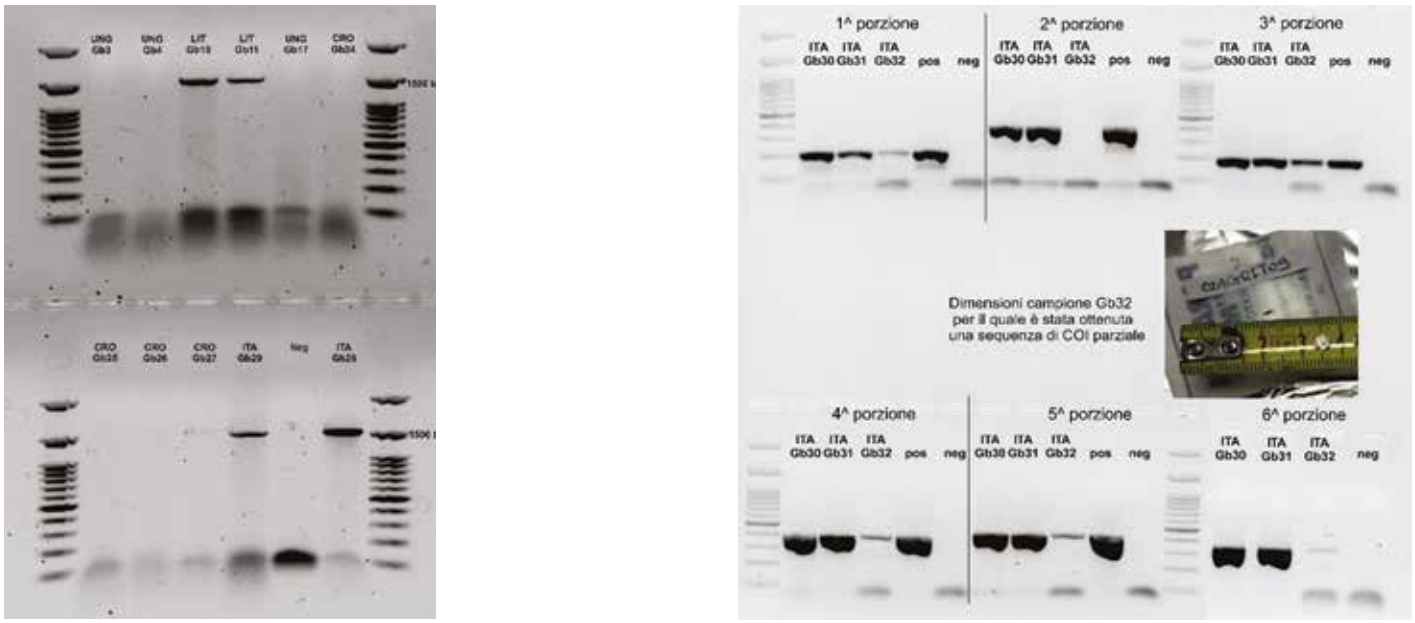

Figure 1. *On the left*, examples of electrophoretic runs of PCR products obtained with the primers COI_LCO1490_For and TL2-N-3014-Rev. The expected band of about 1500 base pairs is only visible for the 4 samples with good quality DNA extracts. *On the right*, results of the amplification of the COI for the Italian leg samples Gb30, Gb31 and Gb32 for which it was necessary to carry out the amplification of 6 shorter fragments to obtain the entire sequence. For the Gb32 sample in particular it was not possible to obtain a complete sequence due to the small amount of material received for DNA extraction, as shown in the image.

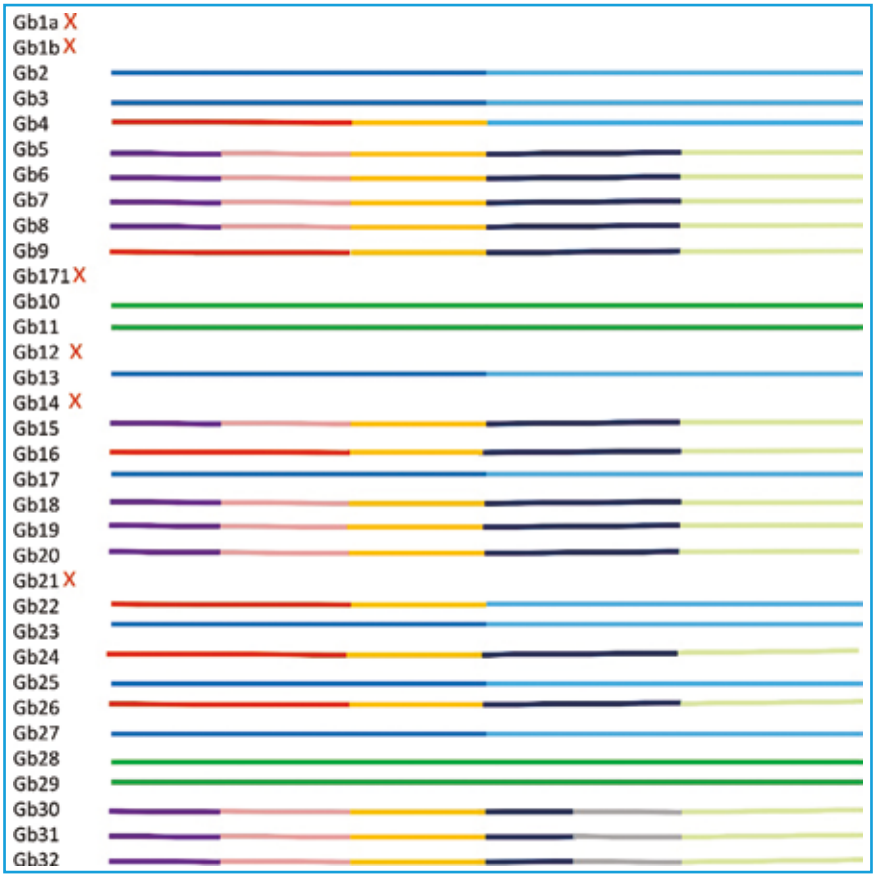

Figure 2. Schematic representation of the number of amplification reactions performed for each individual in order to obtain the sequencing of the COI gene along its entire length. The lines represent the entire region analysed in the different individuals; the segments of different colours that compose them correspond to the sub-fragments amplified independently.

Conclusions

The national and international guidelines (ISPRA, 2007, IUCN / SSC, 2013) agree in recommending in the case of a restocking that the source population should not be significantly differentiated from the native population. On the contrary, in the case of a reintroduction, the scruples for safeguarding the integrity of the original genetic patrimony are less stringent, since there is no longer an autochthonous gene pool to be safeguarded, and the criteria for choosing the source population must be based both on the results of the genetic investigations and on ecological considerations. Taking into account the different guidelines on the subject, and considering the difficulty encountered in obtaining data that can only be analysed on a single mitochondrial gene, we would like to clarify that each type of consideration proposed here is closely linked to what emerged from this work and will have to be debated to define an action plan based not only on the genetic data.

The most interesting result is certainly the uniqueness of the population of *G. bilineatus* in the site of Pratignano found by the analysis of the mitochondrial gene COI. In Pratignano only one mitochondrial haplotype was found, different from any other haplotype found in other European populations. This suggests that the population of Patrignano suffered a strong bottleneck and reproductive isolation that led to the reduction of its genetic

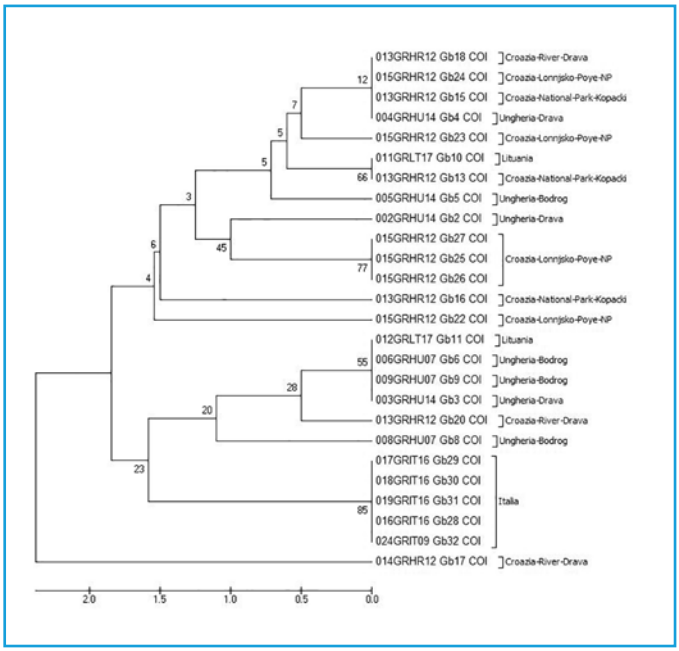

Figure 3. Dendogram built with the MEGAX software (Kumar *et al*, 2018) on the basis of the number of nucleotide differences between the 26 sequences of 1386 pairs of bases of COI characterised in the present work.

Outcomes characterisation of the mitochondrial COI gene

- A total of 26 sequences of COI were obtained with a maximum of 6 amplifications and 8 sequences per individual.
- The COI sequences had 19 variable sites out of 1386 pairs of sequenced bases.
- A total of 13 mitochondrial haplotypes have been characterised, some of which are shared between different geographical regions (Figure 4).
- All individuals in the Italian population present the same haplotype that has never been observed in populations of other areas. The data were confirmed by analysing also other sequences of COI of the same species for a total of 54 sequences.

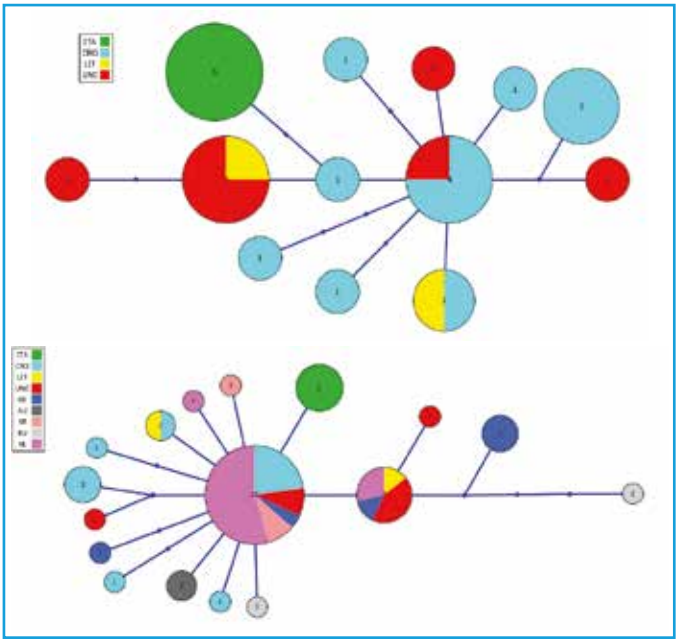

Figure 4. Haplotype network obtained with Haplotype Viewer (http://www.cibiv.at/~greg/haploviewer) where each circle represents a different haplotype separated from the others by a number of nodes corresponding to the number of nucleotide differences between sequences. The dimensions of the circles are directly proportional to the total number of individuals that have that certain haplotype (shown in the centre of the circle). The colouring is proportionally corresponding to the number of individuals of a certain population presenting that particular haplotype. Above, haplotype network obtained starting from the 26 sequences of COI of 1386 base pairs characterised in the present work. Below, haplotype network obtained with the dataset integrated with the Koese and colleagues data for a total of 54 sequences. In this second graph the second half of the COI gene was analysed for a length equal to 741 base pairs. In the legends are reported the acronyms identifying the country of origin of the samples: Italy (ITA), Croatia (CRO), Lithuania (LIT), Hungary (UNG), Germany (GE), Austria (AU), Sweden (SE), Russia (RU), Netherlands (NL).

variability. This makes it difficult to choose a source population due to the lack of another European population genetically similar to the one in Patrignano. Based on this result, the selection of the source population from which to transfer the animals destined to a possible reintroduction on the Italian site should be based mainly on ecological considerations, sustainability in the chosen habitats, and on a study of the existing communities. Moreover, from the genetic point of view it might be appropriate to withdraw the founding individuals taking care to ensure a good genetic diversity, avoiding at the same time to introduce animals from genetically too different populations, so as not to generate outbreeding problems. The best source population in this case could be the one where the degree of variability is higher and the population size is such as to allow a withdrawal of all the necessary animals from the same site.

Considering the difficulty in highlighting the presence of this species, the possibility that the target population is not completely extinct cannot be excluded. In this regard, in order to further minimise the risk of outbreeding, we suggest a preliminary reintroduction campaign in confined and controlled sites. This would avoid the hybridisation of possible survivors of the Italian relic population of Pratignano with individuals genetically too different and/or poorly adapted to the new introduction habitat. Only following the completion of the entire life cycle of the animals transferred will it be possible to proceed with new translocations of individuals.

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