

## S1 Appendix: Barcoding

To ensure the specific identity of the two species of genus *Protaetia*, we used molecular identification through DNA Barcoding which is based on the divergence from part of the COI gene sequence (Hebert et al. 2003). Note that a similarity of 100% of this gene sequence does not guarantee to being in the same taxon, but it reinforces the membership of the same operational taxonomic unit (OTU). A total of eight *Protaetia* individuals from Cortes and Montán (close to Andilla fire) were selected, preserve on absolute ethanol and deposited in the collection of the Universitat de Valencia).

### *DNA extraction and sequence analysis*

For each sample, the medium left leg was extirpated and placed on a 1,5 ml Eppendorf tube with absolute ethanol. Samples were sent for molecular process to AllGenetics & Biology, SL (A Coruña, Spain), where they were analysed as follows: DNA isolations were carried out using the RealPure Microspin Kit (Durviz) following the manufacturer's instructions, and resuspended in a final volume of 30 µL. A negative control that contained no sample was included in every isolation round to check for contamination during the experiments. The primers used for polymerase chain reactions (PCRs) were LCO1490 (5' GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer et al. 1994). PCRs were carried out in a final volume of 25 µL, containing 6.50 µL of Supreme NZYtaq Green PCR Master Mix (NZYTech), 0.5 µM of each primer, 2.5 µL of the template DNA solution, and PCR-grade water up to 25 µL. The thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s; annealing at 50 °C for 30 s; extension at 72 °C for 45 s; and a final extension step at 72 °C for 5 min. A negative control that contained no DNA was included in every PCR round to check for cross-contamination during the experiments. PCR products were run on 1 % agarose gels stained with Real Safe (Durviz), and imaged under UV light. All PCR products yielded one single band of the expected size. PCR products were sequenced using both forward and reverse PCR primers. Electropherogram analysis and overlapping was conducted in Geneious 10.0.3 (Biomatters Ltd). During electropherogram analysis, the primer annealing regions and the low quality regions at both ends of each electropherogram were trimmed (error probability limit of 0.03).

For sequences analysis, all available CO1 sequences from *P. oblonga* (Gory & Percheron, 1833) and *P. morio* (Fabricius, 1781) on GenBank and BOLD (Ratnasingham & Hebert, 2007) were

downloaded. A total of one sequence of *P. oblonga* and four of *P. morio* were obtained (Table S1.1). We also included sequences of the close related species *Cetonia aurata* (Linnaeus, 1761) and *Protaetia fieberi* (Kraatz, 1880). In addition, sequences of *Oryctes nasicornis* (Linnaeus, 1758) and *Trichius zonatus* Germar, 1829 were used as outgroups (Table S1.1). Nucleotide alignment and Neighbor-Joining tree were performed using Geneious 7.1 (Biomatters Ltd), based on Tamura-Nei genetic distance model. Node supports were assessed through 100 bootstrap pseudo-replicates. All eight new sequences obtained from our samples were submitted to GenBank (Table S1.1). Genetic distances between species are reported as minimum uncorrected pairwise distances, while intraspecific variation is reported as maximum uncorrected pairwise distances. DNA extracts are stored at the IVE.

**Table S1.1.** Species, accession code. Length of the sequence, and locality, for the *Protaetia* individuals sampled for barcoding (those from Spain, submitted to GenBank) plus those extracted from GenBank and BOLD. The genus included are: *Cetonia* (Cetoniidae, Cetoniinae), *Protaetia* (Cetoniidae, Cetoniinae), *Oryctes* (Dynastidae, Dynastinae), and *Trichius* (Cetoniidae, Trichiinae).

Species	GenBank	BOLD	Length	Locality
<i>Cetonia aurata</i>	KM449087.1		658	France: Provence-Alpes-Cote d'Azur, Var, Draguignan, Le Plan-de-la-Tour, Ortslage
<i>Oryctes nasicornis</i>	KM441141.1		658	Germany: Rhineland Palatinate, Noerdliche Oberrheinebene, Bad Duerkheim, Freinsheim, Kastanienhain Im Hahnen
<i>Protaetia cuprea</i>	KM451924.1		658	Germany: Baden, Suedliches-Oberrhein-Tiefl, Breisgau-Hochschwarzwald, Vogtsburg-Altvogtsburg, Vogelsang
<i>Protaetia fieberi</i>	KM286168.1	PSFOR251-13	658	France: Poitou-Charentes, Deux Sevres, Foret domaniale de Chize
<i>Protaetia morio</i>	KM285794.1	PSFOR245-13	658	France: Languedoc-Roussillon, Pyrenees Orientales, Sorede
<i>Protaetia morio</i>		GBMIX1807-15	658	France: Provence-Alpes-Côte d'Azur, Sérignan-du-Comtat
<i>Protaetia morio</i>		GBMIX1821-15	658	France: Provence-Alpes-Côte d'Azur, Sérignan-du-Comtat
<i>Protaetia morio</i>		GBMIX2044-15	610	France: Languedoc-Roussillon, Montpellier
<i>Protaetia morio</i>	KY827322		658	Spain: Montán, Castellón. Close to Andilla fire.
<i>Protaetia morio</i>	KY827323		658	Spain: Dos Aguas, Valencia. Cortes fire.
<i>Protaetia morio</i>	KY827324		658	Spain: Montán, Castellón. Close to Andilla fire
<i>Protaetia morio</i>	KY827325		658	Spain: Dos Aguas, Valencia. Cortes fire.

<i>Protaetia oblonga</i>	KM285778.1	PSFOR252-13	658	France: Languedoc-Roussillon, Pyrenees Orientales
<i>Protaetia oblonga</i>	KY827326		658	Spain: Montán, Castellón. Close to Andilla fire.
<i>Protaetia oblonga</i>	KY827327		658	Spain: Dos Aguas, Valencia. Cortes forest fire.
<i>Protaetia oblonga</i>	KY827328		658	Spain: Montán, Castellón. Close to Andilla fire.
<i>Protaetia oblonga</i>	KY827329		658	Spain: Dos Aguas, Valencia. Cortes fire.
<i>Trichius zonatus</i>	KM439893.1		658	Germany: Rhineland Palatinate, Ahrtal, Ahrweiler, Grafschaft-Bengen

## Results

From the COI phylogenetic tree (Fig. 2 main text) we obtained one well supported clade for all species belonged to *Protaetia* genus. Our two morphological species (*P. morio* and *P. oblonga*) are clearly in distinct clades.

For *P. morio*, the two different populations here studied (Cortes and Andilla-Montán areas), even the French specimens included (from Languedoc-Roussillon and Provence-Alpes-Côte d'Azur departments) show a very constant similarity sequences with no important changes or substitutions. It is important to note that the type locality for *P. morio* is “Europa australi, Italia” (Fabricius, 1781) and the two specimens from Provence-Alpes-Côte d'Azur included are close to the Italian populations to suggest that they belong to the type population of the species. This results show little genetic structure in the French and Iberian Mediterranean populations for *Protaetia morio* and there are no evidences of cryptic species, at least, with the specimens analyzed here.

In contrast, the clades that include the *P. oblonga* specimens show some population structure (Fig. 2 main text). Most of the specimens barcoded have exactly the same sequences, including those from the two areas studied and the one from France. Type locality for *P. oblonga* is “France méridionale” (Gory & Percheron, 1833) and the French specimen included (from Languedoc-Roussillon) comes exactly from this area. This scenario indicates that *Protaetia oblonga* sensu stricto includes populations from France (north) and Valencia (south). But one of our specimen shows a genetic divergence of 4.1 %; this corresponds approximately to four to five million years of isolation from the previous taxon based on typical insect mitochondrial substitution rates (Queck et al. 2014). This specimen does not show any morphological differences from the other *P. oblonga*. This data suggests the possibility of cryptic species inside the Iberian *P. oblonga* morphospecies, and claim a detailed phylogenetic and morphologic analysis to elucidate this situation. For our present study, we considered *Protaetia oblonga* specimens as a whole (morphospecies).

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## S2 Appendix: Review of the biology of *Protaetia* (Coleoptera: Cetoniidae)

The beetle family Cetoniidae includes over 2.500 species, mostly in tropical and subtropical Africa (Ritcher 1966). Only 49 species belonged to 11 genera are cited from Europe (Jong et al 2014). Adults of the European Cetoniine species are diurnal and good flying insects; they are attracted mainly by the nectar of flowers but they can also eat stamens and pollen (e.g., genus *Oxythyrea* and *Tropinota*). They also feed on the juices of ripening fruits like grapes, apples, peaches, and figs. In some cases, they can become a pest (Ritcher 1966). Copulation takes place during the day and females lay their eggs directly in the substrate that will feed their larvae. The food of Cetoniidae larvae is varied, including flesh organic matter in the soil, piles of decomposing plant material, decaying wood and other debris accumulated in the hollows of trees, pack-rat and rabbit dung and ant nests (Wheeler 1908, 1910; Paulian 1959; Ritcher 1966; Micó & Galante 2003). The life cycle takes between 1-3 years from egg to adult, depending on species and localities.

The genus *Protaetia* Burmeister, 1842, is the most diverse Cetoniine genus of the Palaearctic region, including 55 species (Krajcik 1998), and ca. 250 species worldwide. As other beetles, the main predators of *Protaetia* species are wasps (on larvae; Svensson et al. 2012, Schwarz et al. 2013), small mammals (larvae and adults; Lanszki 2003), and some birds (on adults; Overskaug et al. 1995, Fuisz et al. 2013, Hámori 2014, . umrada & Hanžel 2012, Keckésová & Noga 2008, Kitowski & Pawlega 2010, Saida Tergou et al. 2014).

The two *Protaetia* species considered here (*P. morio* and *P. oblonga*) are distributed in the western Mediterranean basin, and occurs especially in dry mediterranean habitats. Both taxa belong to the subgenus *Netocia* Costa, 1852, that comprises a total of 18 European species (Jong et al. 2014). Despite the relative large size of these beetles and its popularity among entomologists and amateurs, little is known about their biology (Micó & Galante 2003), compared with their more widespread relative, like *Protaetia cuprea* (Fabricius, 1775) and *Protaetia aeruginosa* (Linnaeus, 1767). Following we describe what is known of the biology of the two studied species.

*Protaetia (Netocia) morio* (Fabricius, 1781)

This species is distributed in North Africa (Morocco, Tunisia and Algeria), Iberian Peninsula, France (reaching the outskirts of Paris and Brittany; Darnaud et al. 1978), southern Switzerland, and Italy (including Sicily and Sardinia) (Baraud 1985).

Adults are active flyers and their activity is always diurnal. They show flower attraction as other Cetoniidae and has been recorded on flowers of many species, like *Sinapis*, *Verbascum* (Gangloff 1991), *Pyracantha* and *Sambucus* (Tauzin 2008). They are also common in overripe fruits (Caillol 1913), like figs and other fruits (Paulian 1959; Degallier 1976; Montegud, pers. observ.), and have been also observed in tree wounds (Fauconnet 1887) and in mushrooms (Bedel 1911). There are many records showing that this species is well captured by traps; it was collected on aerial traps filled with red wine or beer and pitfalls setting for Carabidae species (Du Chatenet 1986; Tauzin 2008).

The immature stage is poorly known. Caillol (1913) already suggested that the larvae of *P. morio* lives in the soil close to old trees, mainly willows, where they were frequently infected by the wasp parasite *Scolia hirta* (Scoliidae, Hymenoptera) (Schrank, 1781). Apparently the larvae do not seem to require any special substrate, as they can develop in a range of conditions with abundant organic matter, including animal faeces (Tauzin 2008); they promote organic matter decay. Devecis (1992) observed females laying eggs even in cricket holes. Larvae can be fed in captivity with a substrate composed of ripe fruits (Micó & Galante 2003); they were also observed feeding on ground grapes in an artificial human habitat (Tauzin 2008).

The life cycle is typically annual, from the egg to adult, but like others Cetoniidae, their metabolism can slow down in cold conditions (Tauzin 2008). Also, the composition and quality of the organic matter in the food of the larvae can accelerate or delay the development. If larvae feed on ripe fruits they can complete the life cycle in 2-3 months (Micó & Galante, 2003). This species overwinters as adult inside the cocoon or as larvae, probably depending on temperature and organic matter quality. Three instar larvae were described by Micó & Galante (2003). Our data suggest that the peak of the adult activity is in May (Fig. S1).

*Protaetia (Netocia) oblonga* (Gory & Percheron, 1833)

This species occurs in North Africa, Iberian Peninsula, southern France and Italy (south to Riviera); it is significantly more common in Spain than in France (Bedel 1911); in the latter country it is

mainly restricted to the south-est, below 500 m of altitude, and in hot and dry conditions (Tauzin 2009).

Adults are active diurnal flyers. They feed on flowers, including many thistle species (Tauzin, 2009) like *Cirsium* (Caillol 1913; Paulian 1959), *Centaurea* (Caillol 1913), and *Onopordum* (Tauzin 2009). They also feed on ripe fruits like in mulberries (Paulian & Baraud 1982), plums, figs and apricots (Tauzin 2009; Montegud, pers. observ.). Some few records are from inside beehives, attracted by honey (Moretto 1978). In France, the activity of adults is typically during June and July (Paulian 1959), although there are some records up to November; similar is observed in our Spanish populations (Fig. S1).

Little is known about the immature stage and life cycle. Old records indicate that larvae were found on the ground (Marquet 1898). They can be observed in large numbers near trees on organic material accumulations like fruits or leaves (Tauzin 2009). Some records show also larvae in other specific places rich in organic matter like in abandoned ant nests and rabbit latrines (Micó & Galante 2003). Recent studies indicate that larvae prefer to live around plant root system (Cistaceae, Lamiaceae) with a rich organic soil (Micó & Galante 2003), where they feed from the organic nutrients provided by plant tissues decomposition, and from faecal from animals (Tauzin 2009). In dry environments, larvae are often observed below stones or rocks where the humidity is maintained. There are no evidence on the larval development of *P. oblonga* in tree cavities or other common habitats observed other European *Cetoniinae* species from subgenus different to *Netocia*. The larvae was detailed described by Micó & Galante (2003) and breded in captivity with a mix of milled rabbit dung and decaying plant matter.

*P. oblonga* life cycle is typically annual (Tauzin 2009), although there are reports of 2 years life cycle (Micó & Galante 2003). In some northern localities or at higher altitude, metabolism of the larvae can slow down and the life cycle extended (Tauzin 2009). Three instars are observed. The overwinter state seems to be always the larvae (Micó & Galante 2003).

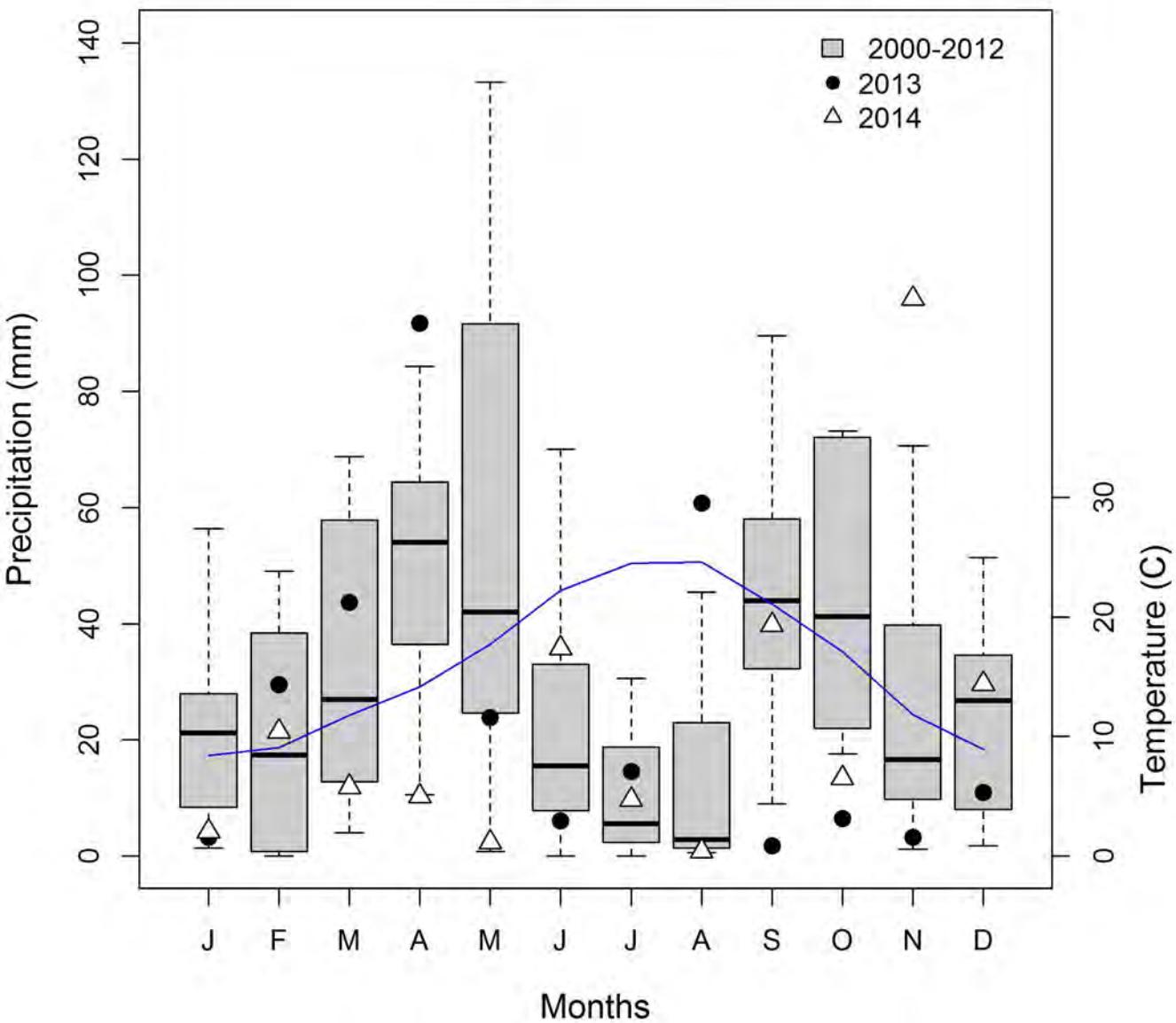
## References (appendix S2)

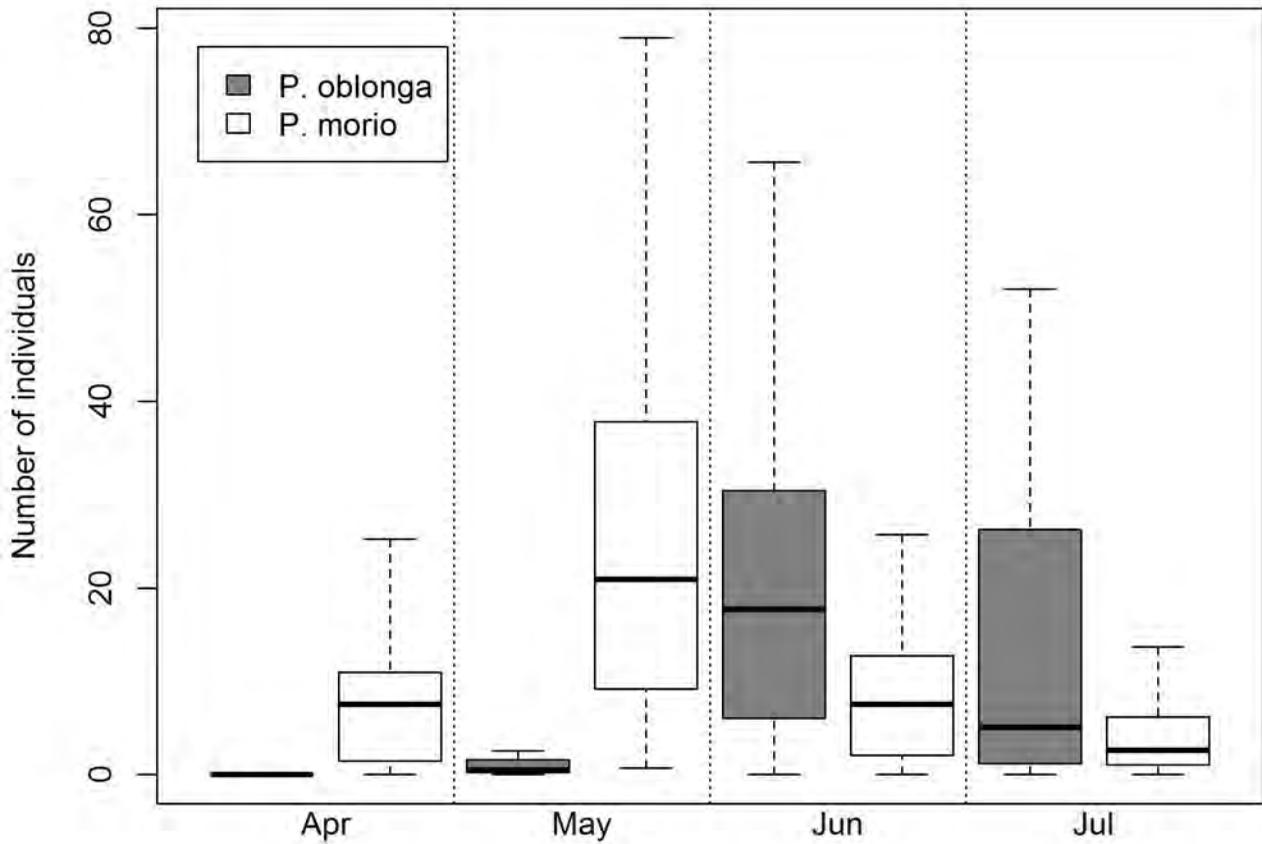
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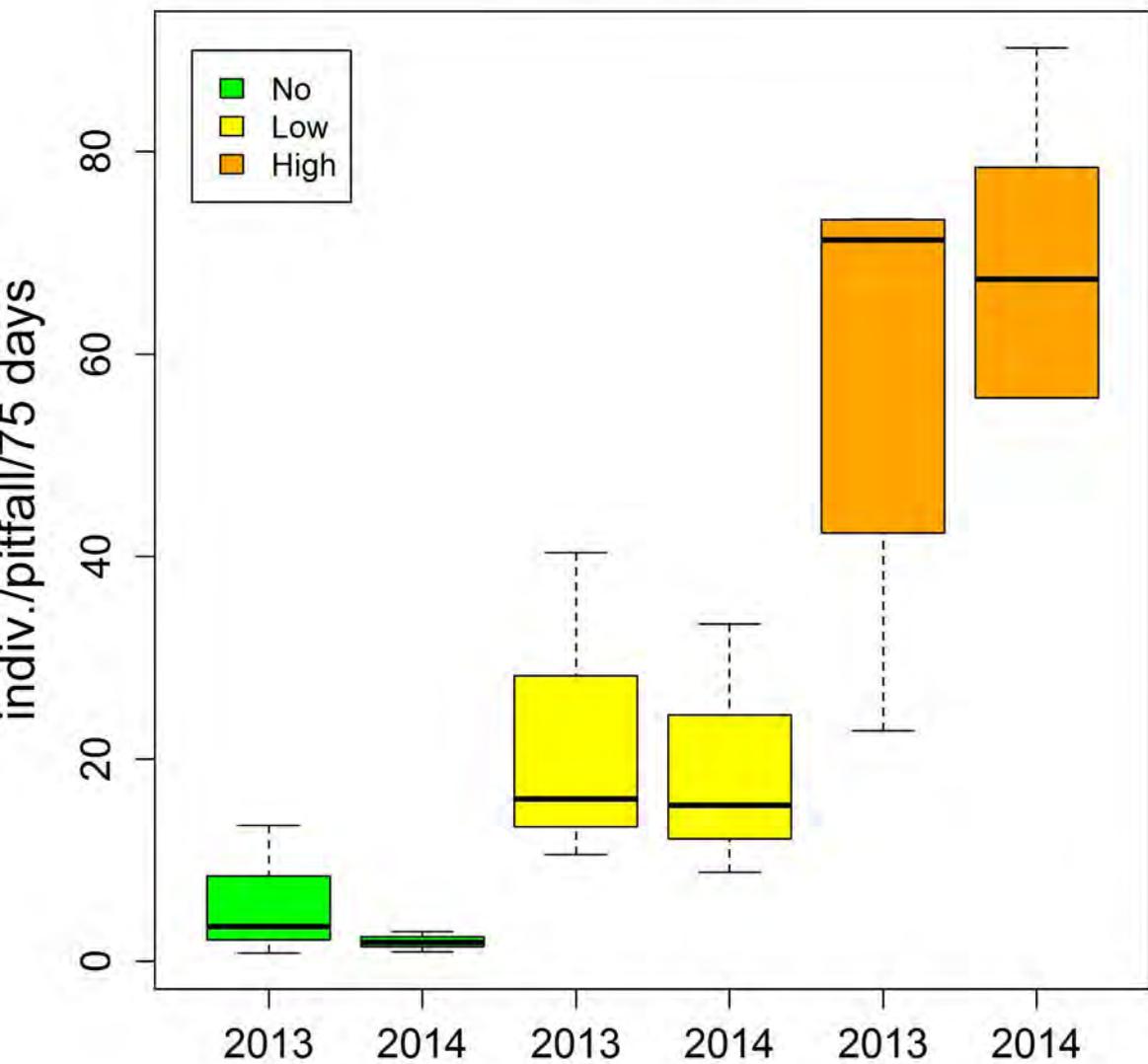
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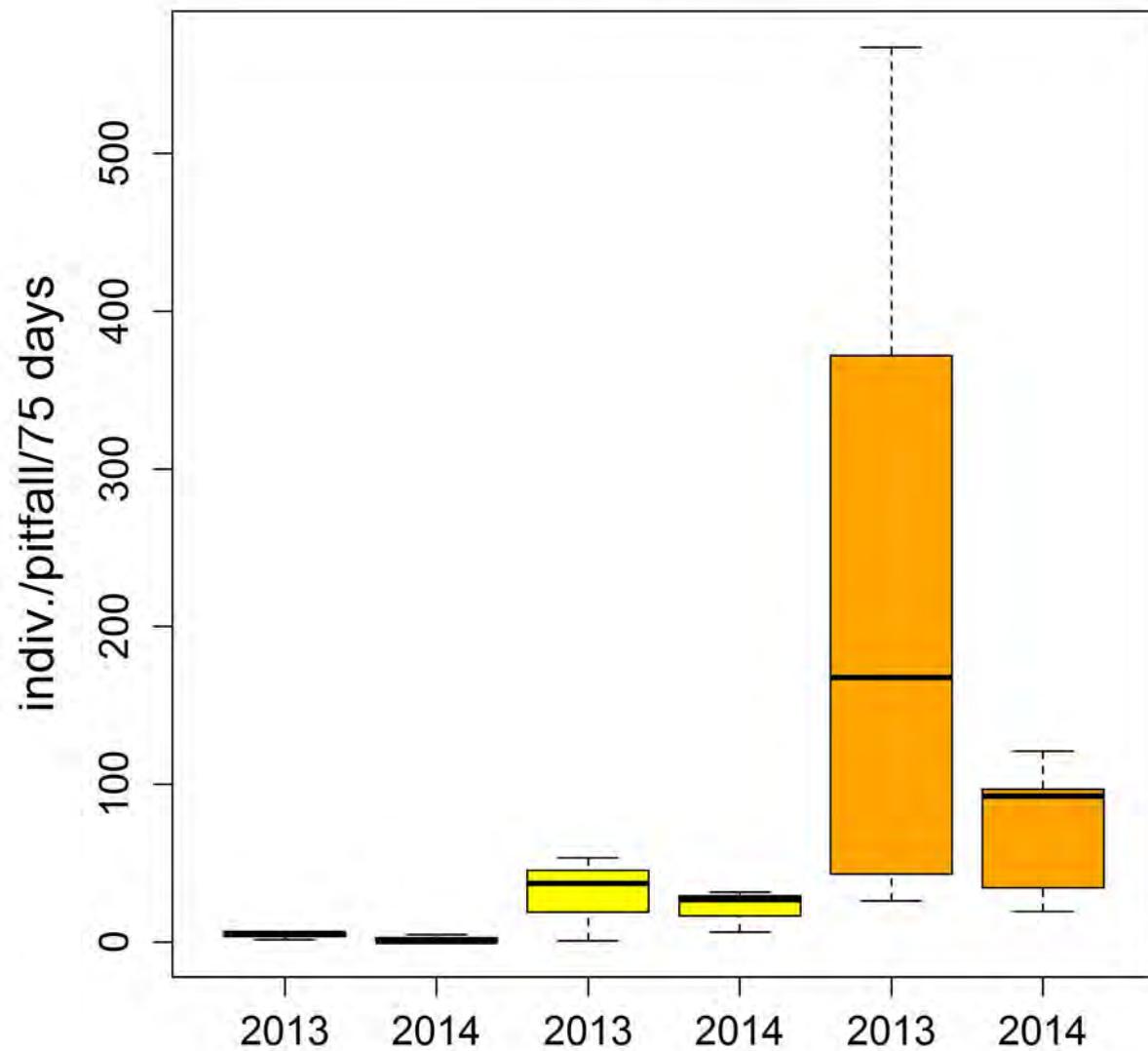


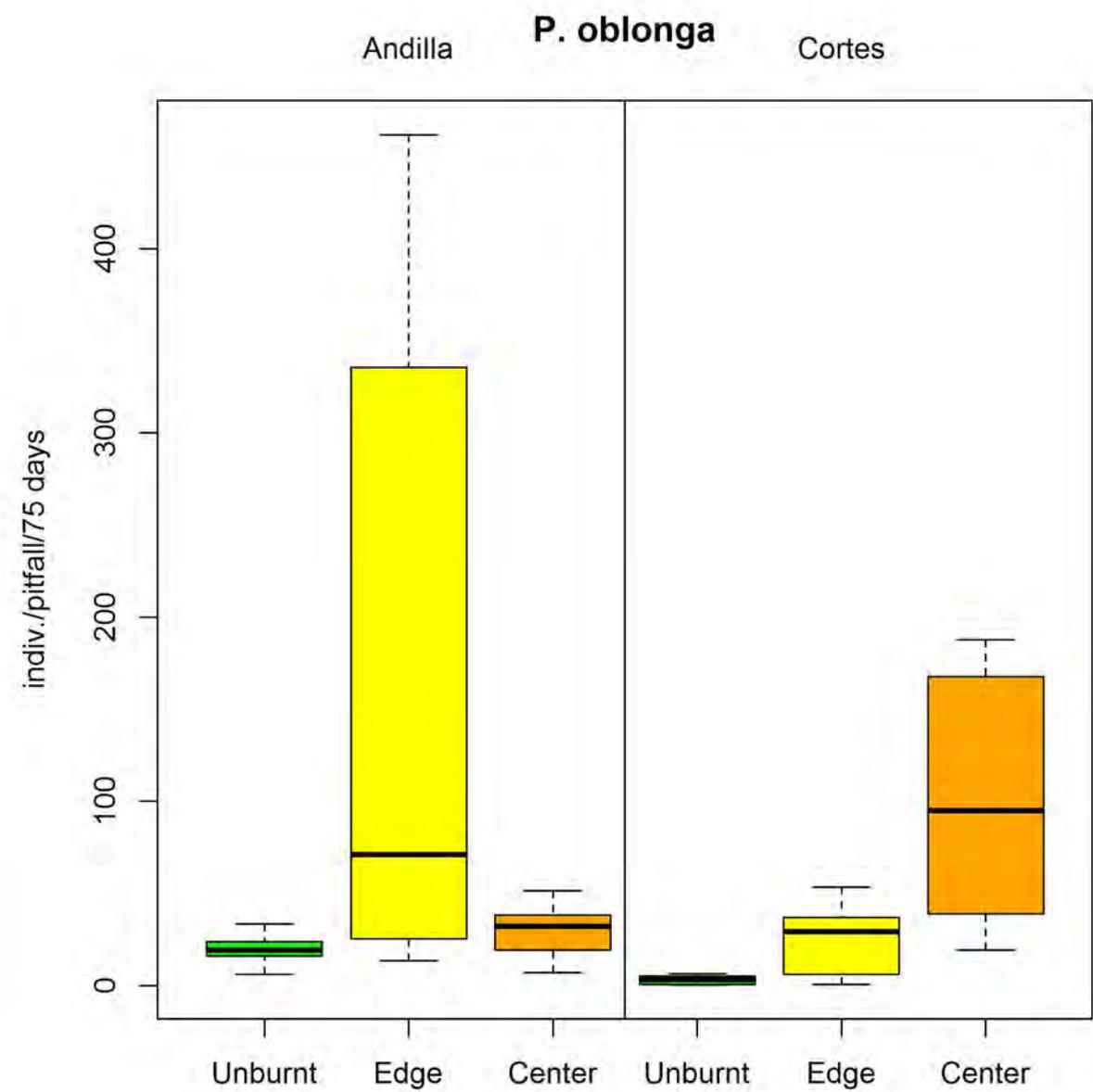
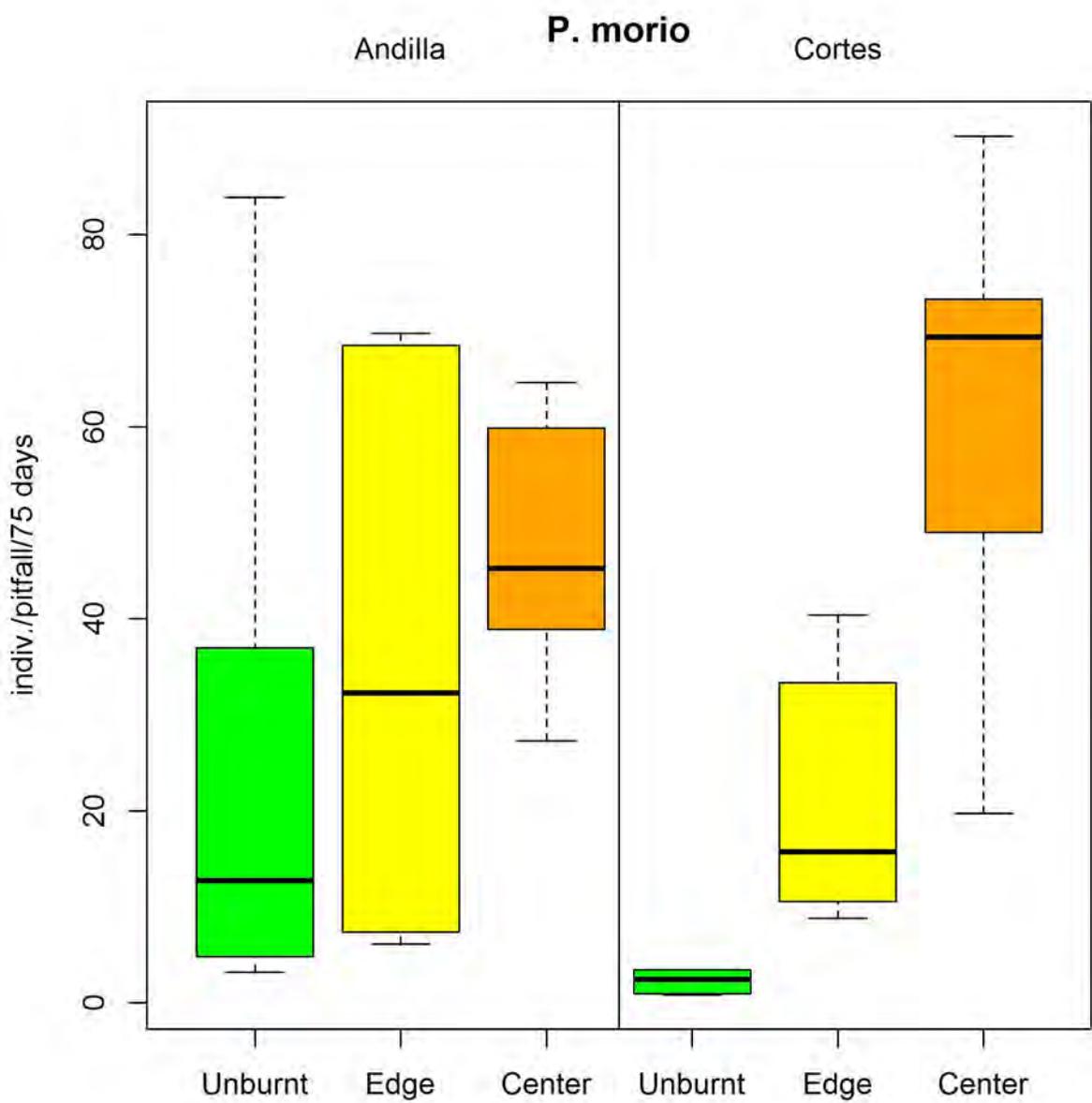


**P. morio**



**P. oblonga**





**S1 Table. Spatial autocorrelation**

Moran's autocorrelation values (I and p-value) for the abundance of the two *Protaetia* species, for the two years and for the two sites, and the autocorrelation of the residuals after fitting the most parsimonious model (lowest AIC, Table 2a). Significance is tested with 1000 permutations.

Site	Year	Species	Abundance		Model residuals	
			I	p	I	p
Cortes	2013	PO	0.443	<b>0.033</b>	-0.008	0.321
Cortes	2013	PM	0.464	<b>0.018</b>	0.130	0.178
Andilla	2013	PO	-0.387	<b>0.017</b>	-0.161	0.365
Andilla	2013	PM	0.138	0.096	-0.038	0.386
Cortes	2014	PO	0.625	<b>0.003</b>	-0.008	0.308
Cortes	2014	PM	0.445	<b>0.030</b>	0.130	0.153
Andilla	2014	PO	-0.145	0.380	0.132	0.100
Andilla	2014	PM	0.019	0.250	-0.153	0.358