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Pissodes irroratus Reitter 1899, a species from East Russia new to Europe (Coleoptera: Curculionidae: Molytinae)

by

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with 5 figures.

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Abstract

Pissodes irroratus Reitter, 1899 (Coleoptera, Curculionidae, Molytinae) is a species from east Russia newly discovered in Europe in various localities in the Alps (France, Switzerland, and Italy). We provide here a description of morphological characters and molecular data (mitochondrial standard barcode gene) for this species. We also give diagnostic characters to distinguish this species from its closest relative, *Pissodes harcyniae* (Herbst, 1795), and other west palearctic species of the genus.

Key words. Curculionidae, Molytinae, Alps, molecular phylogeny.

Introduction

Fig. 1 *Pissodes irroratus* Reitter , habitus, ♀ (dor./lat.).

The holarctic genus *Pissodes* (Curculionidae: Molytinae) contains eight species in the west palearctic region: *Pissodes castaneus* (De Geer, 1775); *P. gyllenhali* (C.R. Sahlberg, 1834); *P. harcyniae* (Herbst, 1795); *P. piceae* (Illiger, 1807); *P. pini* (Linnaeus, 1758); *P. piniphilus* (Herbst, 1797); *P. scabricollis* Miller, 1859; *P. validirostris* (C.R. Sahlberg, 1834) [Alonso-Zarazaga 2013]. The larval stages of most species develop under the bark of declining co-

niferous trees, with the exception of *P. validirostris* which completes its larval development in cones of pine trees [Roques 1976].

In Europe, *Pissodes harcyniae* is distributed from Scandinavia (Norway, Sweden, and Finland) to the alpine areas of France, Italy and Croatia. This species is known to develop in the top branches of various coniferous trees, such as *Pinus sylvestris* L.*, Abies alba* Mill. and *Picea abies* L. [Hoffman 1954]. The examination of series of specimens identified as *P. harcyniae* from various collections revealed a divergent morphotype bearing almost-black integument and white vestiture, compared to the typical reddish integument and ocher vestiture in *P. harcyniae*. After examination of the holotype, it was established that these specimens belonged to *Pissodes irroratus* Reitter, 1899, a species described from the Kamchatka Peninsula of Russia and distributed in east Siberia and the Russian Far East [Alonso-Zarazaga 2013]. *Pissodes irroratus* [Fig. 1] is newly reported from Europe where it is distributed in various localities in the Alps. This work provides a description of morphological features of adult females (no males were found in Europe yet) and diagnostic characters of *P. irroratus* (including the barcode fragment of the mitochondrial Cytochrome oxidase I) to distinguish the species from *P. harcyniae* and other congeneric species in the west palearctic region.

Material and Methods

Pissodes irroratus was detected as a result of re-examination of series of individuals previously identified as *P. harcyniae* in various European collections. Additional material was captured using flight interception traps baited with a multilure blend (α-pinene, fuscumol, fuscumol acetate, geranyl acetone, monochamol, isopropanol and ethanol) formulated to trap long-horned beetles associated with coniferous trees. Traps were deployed during the summer of 2015 in various mid-elevation areas of the southeastern French Alps near Briançon. A total of 184 individuals were examined from Europe, among which 21 (all females) belonged to *P. irroratus*. An additional four specimens of *P. irroratus* (3 females, 1 male), including the holotype, from eastern Russia were examined.

The mitochondrial cytochrome c oxidase I (COI) gene has been shown to be useful for discrimination among some *Pissodes* species in North America [Langor and Sperling 1997] and China [Zhang et al. 2007]. We used the barcode region (a 660 base-pair fragment of the mitochondrial Cytochrome oxidase I) to provide a molecular characterization of *P. harcyniae* and *P. irroratus*. Two specimens of *P. irroratus* newly collected in Villard-Saint-Pancrace and two specimens from East Russia (codes: COLNF2851-16, COLNF2852-16, see details in the following section) were genotyped using the standard barcoding protocol [Hebert et al. 2003]. Sequence electropherograms were carefully checked to avoid pseudogenes that may occur in beetle species [Haran et al. 2015]. Genetic distances between *P. irroratus* and *P. harcyniae* were computed with MEGA 4.0.2 [Tamura et al. 2007] using sequences of two *P. harcyniae* available on Genbank (accession numbers KJ963137.1 and KJ963019.1). A preliminary examination of phylogenetic relationships was conducted among *P. irroratus*, *P. harcynae* and four other west palearctic *Pissodes* species using our molecular data and other available sequences (Genbank and CURCI: three *P. pini*, KJ963528.1/ KJ963507.1/ 988-PSP_3133; two *P. piniphilus*, KJ965145.1/ 1048-PSP_3193; three *P. validirostris*, KJ966092.1/ KJ962385.1/ KJ964435.1; three *P. castaneus*, 2550-PSP_23524/ 1531-PSP_3291/ 1283-PSP_4099). Two outgroup species from the subfamily Molytinae were also included in analysis: *Hylobius abietis* (Linnaeus, 1758), KM450385.1 and *Lepyrus palustris* (Scopoli, 1763), KM448550.1. Phylogenetic relationship based on barcode sequences was reconstructed using PhyML [Guindon & Gascuel 2003], using 1000 bootstrap replicates.

We examined the morphology of adult specimens borrowed from the following collections:

CCcoll.	Claude Chauvelier private collection, Orléans, France
CURCI	Curculio-Institute collection, Mönchengladbach, Germany
DRcoll.	Daniel Rougon private collection (incl. A. Méquignon coll.), Orléans, France
HNHM	Hungarian Natural History Museum, Budapest, Hungary
JHcoll.	Julien Haran private collection, Montpellier, France
MNHN	Museum National d'Histoire Naturelle, Paris, France
NHM	Natural History Museum, London, United Kingdom
NMBE	Naturhistorisches Museum, Bern, Switzerland
RAS	Russian Academy of Sciences, St. Petersburg, Russia
URZF	Institut National de la Recherche Agronomique (INRA), unité de recherche en zoologie forestière, Orléans, France
ZMHB	Museum für Naturkunde, Berlin, Germany

Material of *P. irroratus* examined

France: La Ribo (05), 44°46'51.29''N 6°52'05.07''E ,1400m, 21.6.2015, G & J Rousset leg., C. CCcoll (1 ♀) ; Villard-Saint-Pancrace (05), 44°51'40.41''N 6°36'09.97''E, 1230 m, 9.6.2015, A. Bernad leg, JHcoll. (16 ♀); Same data, deposited at MNHN (1 ♀); Same data, deposited at URZF (1 ♀). **Italy**: Alto Adige vipiteno, L. Magnano leg., L. Magnano det (*P. harcyniae*) NHM (1 ♀). **Switzerland**: Valais, Chemin, 5.6.1966, leg. P. Scherler, vid. A. Horion, vid. L. Dieckmann, *P. scabricollis* det. Ch. Germann 2003, NMBE (1 ♀). **Russia**: around Irkoutsk, in «la Toïka», 1902, P. Labbé leg., MNHN general collections (1 ♀); Kamtschatka, 1890, leg. O. Herz, HNHM (1♀**,** Holotype); Magadan reg.,

Kulu vil., 16.vi.1976, Glushkova leg. (1 ♀, RAS, COLNF2851-16); Upper Kolymy, 30 km NE of Sporny vil., 26.vi.1981, sample #72, A. Ryabukhin leg. (1 ♂, RAS, original label in Russian, COLNF2852-16)
DNA voucher. **1**♀, Villard-Saint-Pancrace (05), 44°51'40.41''N 6°36'09.97''E, 1230 m, 9.6.2015, A. Bernad leg, deposited in JHcoll: DNA voucher n° Pis-434.

Morphology of adult females [Figs. 1, 2]

Body integuments dark-reddish, almost black, with contrasted spots and bands of white scales.
Length. 2.6 – 4.1 mm (measured from anterior margin of eyes to the tip of elytra).
Head. Head strongly punctuated; inter-ocular space slightly impressed with a spot of white scales on inner eye margin. Rostrum elongated, regularly curved and expanded at apex; punctation dense and coarse in basal half, scattered with shiny teguments in apical half; base of rostrum with an ocher scale in each puncture (on fresh specimens). Antennae inserted in middle of rostrum; covered with black or dark brown setae. Antennae with scape elongated, clavate, reddish at base; funiculus 7-segmented, 1st segment 1.5 times longer than broad; the 2nd as long as broad; segments 3 to 7 broader than long; club acuminate.
Pronotum. Broader than long, length-width ratio 0.78; widest behind the middle; constricted at apex, anterior margin reddish; base of the sides rounded. Punctation dense and coarse, forming a longitudinal smooth line more or less distinct, raised in its middle; spaces between punctures not shiny; scales brown and white, the white scales scarce and mainly distributed at the edges and at the base of the disc, forming two small and contrasting spots in the central part of the disc, within a small depression of integuments.
Elytra. Length-width ratio 1.75; sides sub-parallel, converging apically; shoulders and apical cali marked. Scutellum densely covered with white scales concealing the integument. Striae coarse and irregular; intervals rough; odd intervals raised and as broad as even intervals, for intervals 1-5; sutural interval moderately but distinctly raised, mainly in basal half. Vestiture consists of brown and white scales forming a more-or-less distinct checkerboard pattern on the disc; white scales form a contrasted transverse band in the apical half of elytra, on intervals 2 – 6 (sometimes on intervals 4 – 6 only), always disrupted in its center; some specimens with two spots on intervals 4 – 6 in the basal half of elytra.
Legs. Normal conformation; fore-femora unarmed; integument of tibiae and femora reddish in middle, lighter than the body; legs covered with well-spaced white and brown narrow scales; tarsal claws unarmed.
Venter. 2nd sternite large, longer than 1st and as long as 3-5 together. Vestiture made of short, well-spaced, white scales, not concealing the integument.
Female genitalia. For spermatheca and spiculum ventrale, see [Fig. 2A].
Male genitalia. With only one male specimen (from Russia) available for study, we considered this to be too little material to compose a comprehensive description. We do provide images of the median lobe of the aedeagus [Fig. 2B].

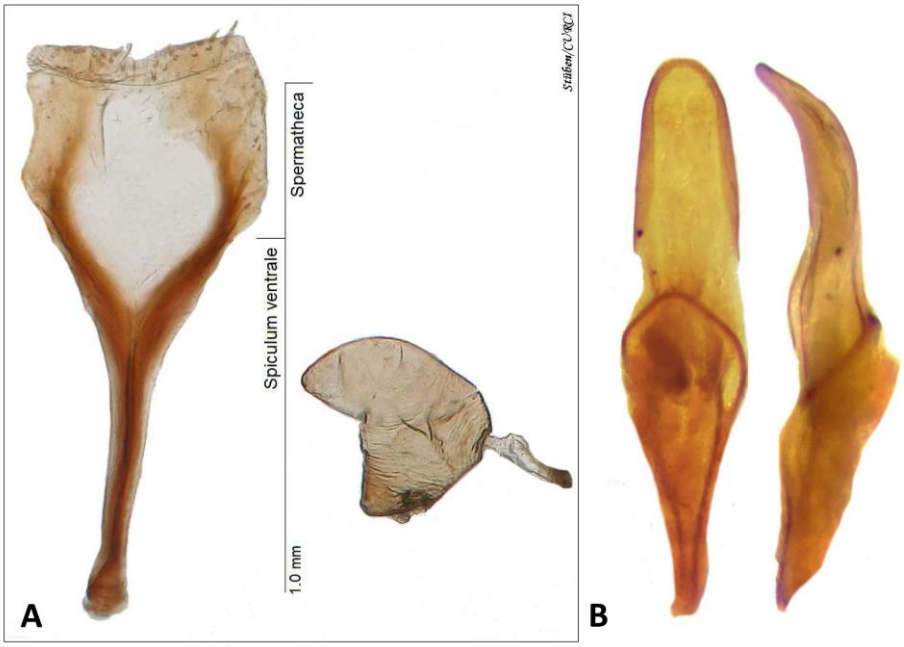

Fig. 2 **A**: *Pissodes irroratus* Reitter, female genitalia: spiculum ventrale (left), spermatheca (right).
B: Male genitalia: median lobe, dorsal and lateral view.

Discussion

Morphological analysis. *Pissodes irroratus* is distinct from all other western palearctic *Pissodes* species by its dark-reddish (almost black) integument. This species is also characterized by the bands of white scales on the elytra, in contrast to other palearctic *Pissodes* that have always brown-ocher bands, at least partly. *Pissodes irroratus* is most similar to the *P. piniphilus*/*P. harcyniae* group (characterized by the basal rounded corners of the pronotum) and is particularly close to *P. harcyniae* with which it was sometimes mixed in collections. *Pissodes irroratus* can also be distinguished from *P. harcyniae* by the width of the elyral intervals (equal in *P. irroratus*; odd intervals wider than even intervals in *P. harcyniae*), the sutural interval (raised in basal half in *P. irroratus*; flat in *P. harcyniae*), and the width of bands of light scales on elytra (reduced in *P. irroratus*: basal band often absent, apical band between intervals 2-6; wide in *P. harcyniae*: basal band always present between intervals 4-6, apical band between intervals 2-7).

Due to the small size of some specimens, *P. irroratus* is sometimes also mixed with *P. scabricollis* (Miller, 1859) in collections. Both species can be easily distinguished by the basal corners of the pronotum (rounded in *P. irroratus*; forming an angle in *P. scabricollis*), the color of the integument (almost black in *P. irroratus*; reddish in *P. scabricollis*) and the color of the bands on the elytra (white in *P. irroratus*; ocher in *P. scabricollis*). In addition, the spaces between the punctures of the pronotum are narrower in *P. irroratus* than in *P. scabricollis*.

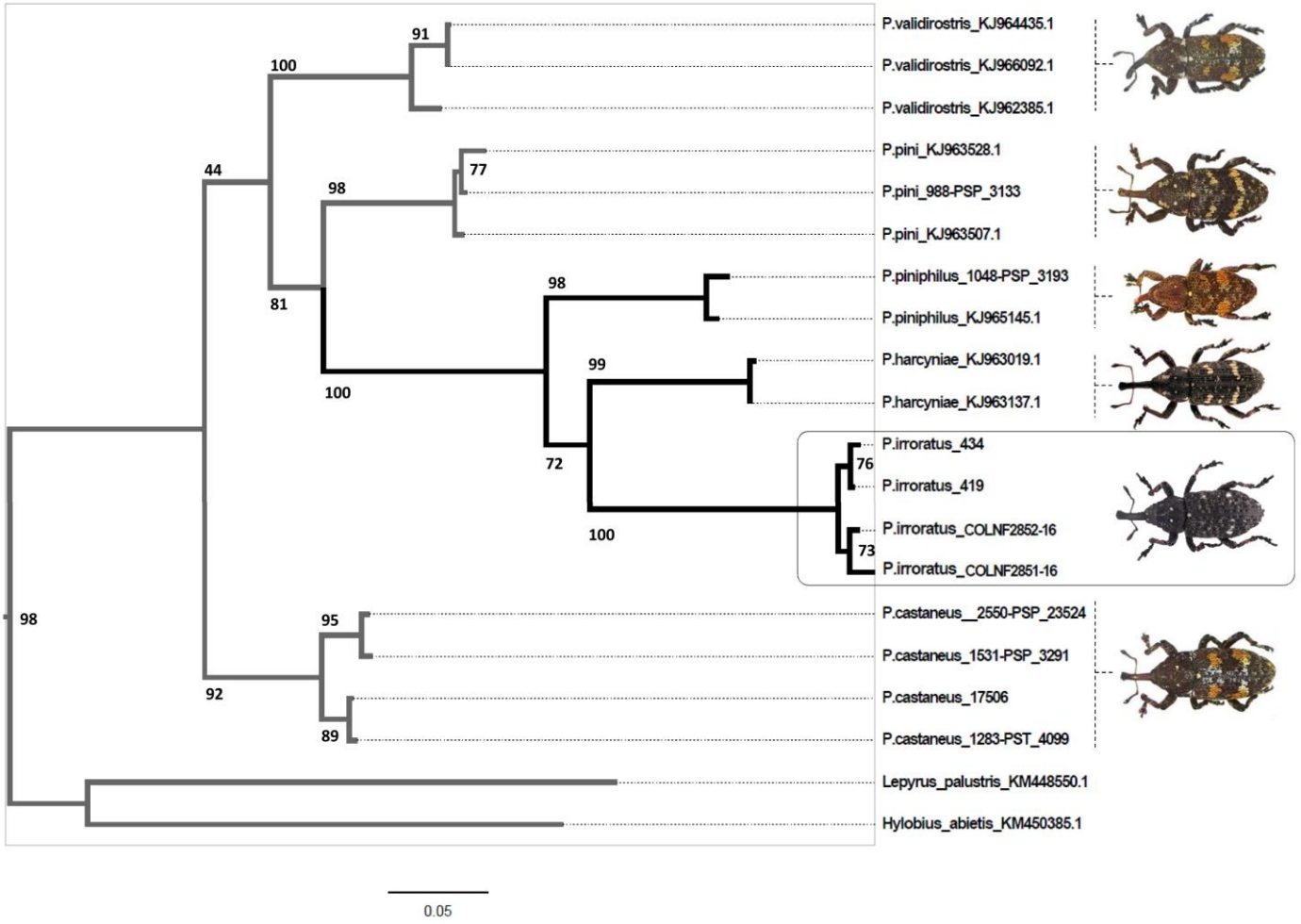

Fig. 3 PhyML phylogenetic tree based on COI barcode sequences of six species of west palaearctic *Pissodes*, including *P. irroratus* Reitter. Node values refer to percent support based on bootstrap analysis performed for 1000 replicates. Photographs: *P. harcyniae*, see: http://www.zin.ru/animalia/coleoptera/rus; *Pissodes species*, see: http://claude.schott.free.fr

Molecular analysis. Barcode sequences showed that *P. irroratus* is closer to *P. harcyniae* than any other examined species, with interspecific genetic distances ranging from 18.3% to 19.1%. Genetic distances among specimens identified as *P. irroratus* ranged from 1.1% to 2.3%, showing that specimens from the west and east palearctic region are indeed conspecific.

Phylogenetic analysis provided the first overview of molecular relationships among six of the nine western Palearctic species of *Pissodes* [Fig. 3]. *Pissodes irroratus* clustered with the other two species with rounded basal corners of the pronotum (*P. piniphilus*, *P. harcyniae*). High bootstrap values indicated that the relationships highlighted within this cluster were reliable. However, relationships with other *Pissodes* species was less supported. The addition of other European species (*P. scabricollis, P. piceae*, and *P. gyllenhali*) and other genes (nuclear) in this analysis is required

to further elucidate phylogenetic relationships within this genus. Work is also under way (Langor, unpublished) to examine the phylogeny of west palearctic species using morphological characters.

Ecology. Within the European range of *P. irroratus*, specimens were found only at middle elevations (1230 – 1400 m) in the western Alps. They were recorded in sites also inhabited by *P. piniphilus* and *P. pini*. The site at Villard-Saint-Pancrace, where most individuals were collected, consisted of an open forest with a mix of coniferous and deciduous trees (*Pinus*, *Larix*, *Sorbus*, *Betula*, *Salix* and *Prunus,* see [Fig. 4]). The exact host taxa could not be identified in the course of this study. However, given the known biology of other species of the genus and given the fact that lures used in traps were designed for long-horned beetles associated with coniferous trees, *Pinus* or *Larix* are the most likely host genera for *P. irroratus*. Adult females were recorded from June to August.

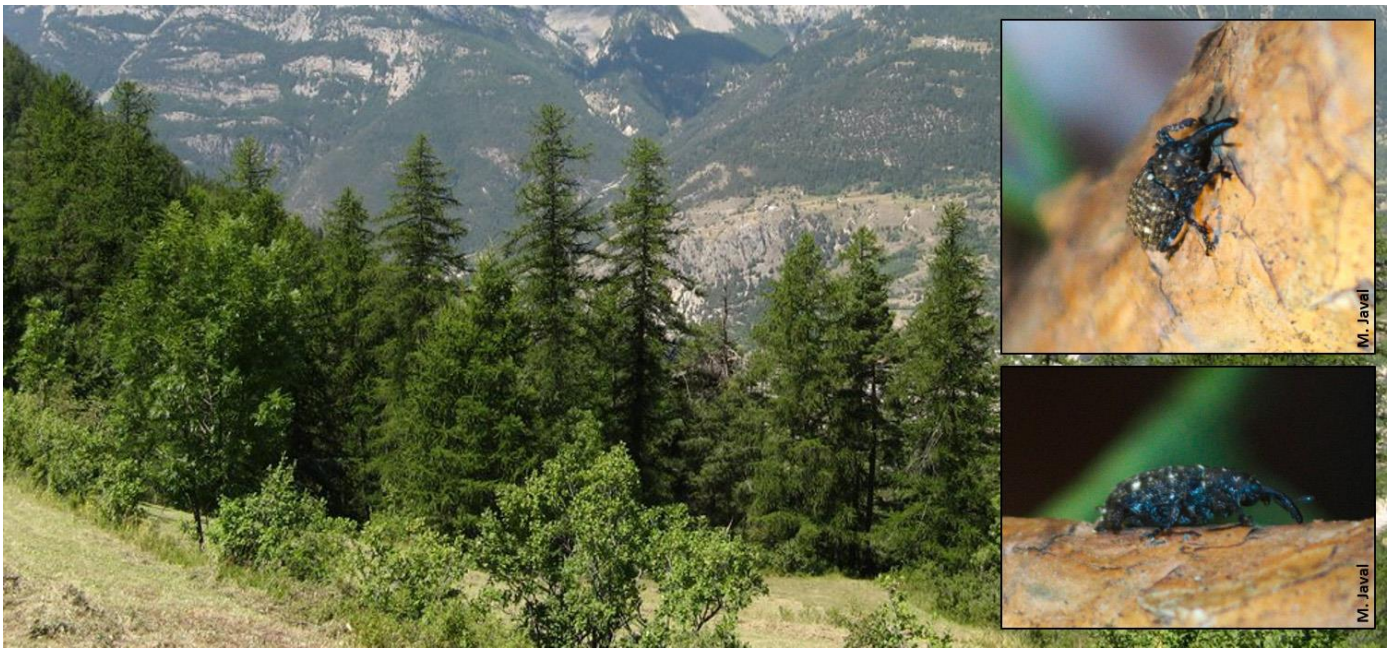

Fig. 4 Habitat where *P. irroratus* Reitter was recorded near Villard-Saint-Pancrace (France, 05).

Distribution. *Pissodes irroratus* was originally described from Russia and is distributed from the Irkutsk area to the Kamchatka Peninsula. Based on morphological comparisons to the holotype and high genetic similarity to specimens from east Russia, *P. irroratus* appears to also occur in Europe, albeit only at high elevations. The easternmost population in Europe is over 6000 km from the nearest known collection locality in Russia. Large Palearctic distributions are known in *Pissodes* species [Alonso-Zarazaga 2013] but such discontinuous range is not known to date in other species. This apparently disjunct distribution pattern may reflect an evolutionary history wherein populations from Europe and Russia were maintained during the last ice age within distinct glacial refugia. Alternatively, it is possible that European populations came from a human-mediated introduction of individuals from Russian populations. Finally, very little material of *P. harcyniae*, with which *P. irroratus* has been frequently confused, has been examined from eastern Europe, Russia, and former Soviet Bloc countries to the south of Russia, so it might be that there is in fact not a real disjunct distribution but rather simply a gap in our knowledge. The species is presently known in its European range from four localities from the southeastern French Alps to the northwestern Italian Alps [Fig. 5]. This distribution coincides well the distribution range of European larch (*Larix decidua*) and the Swiss stone pine (*Pinus cembra*) in the central Alps (*). However, it must be noted that traps baited with the same lures and deployed in sites neighboring Villard-Saint-Pancrace (i.e., Montgenèvre, 1650m, 44°55’31.57’’N 6°41’59.55’’E / Névache, 1450m, 44°59’14.94’’N 6°39’59.30’’E) did not capture any specimens although the environment was quite similar. Examination of more material from collections and additional sampling is need to clarify the distribution of this species in Europe and further east.

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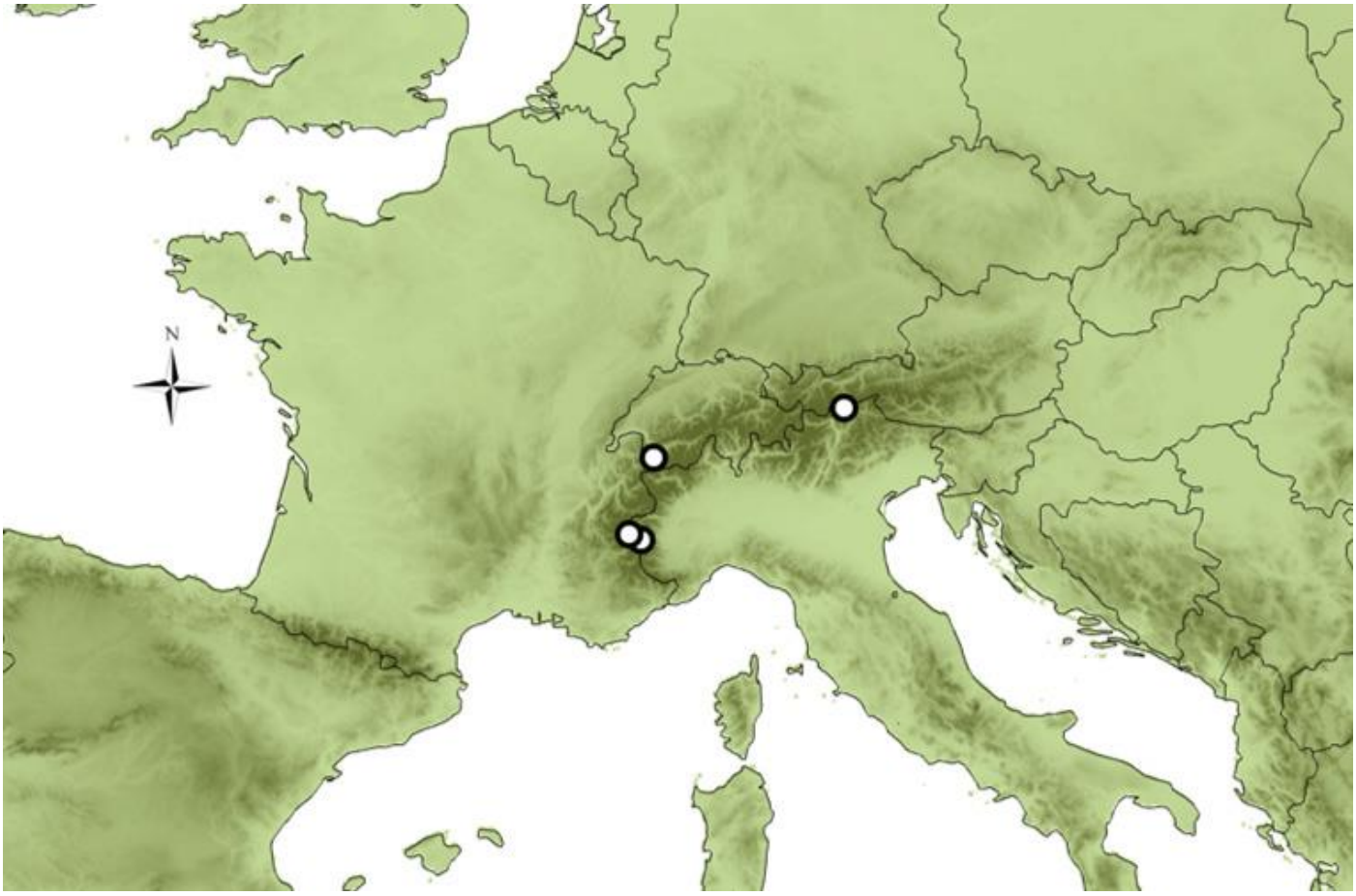

Fig. 5 Distribution of localities where *P. irroratus* Reitter was recorded in Europe..

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