



## ***Psylliodes urbanae*: a new species from Central Apennines (Coleoptera: Chrysomelidae: Galerucinae: Alticini)**

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**Abstract.** *Psylliodes urbanae* sp. nov. from Central Apennines (Italy) is described. Male genitalia and some biometric characters allow to distinguish between the new species and the very similar *P. napi* (Fabricius, 1792). Due to its limited distribution in mountain areas, its monophagy, and its subapterism, we propose possible processes leading to its differentiation.

**Riassunto.** *Psylliodes urbanae*: una nuova specie dell'Appennino Centrale (Coleoptera: Chrysomelidae: Galerucinae: Alticini). Nel presente lavoro viene descritta una nuova specie di Chrysomelidae dell'Appennino Centrale, *Psylliodes urbanae* sp. nov. I principali caratteri diagnostici, che permettono di distinguere la nuova specie dall'affine *P. napi* (Fabricius, 1792), riguardano la forma del lobo mediano dell'edeago ed il diverso rapporto tra la lunghezza della tibia posteriore (LHT) e la lunghezza della parte distale dell'incavo che ospita il metatarso (LDTS). Considerando, infine, il ristretto areale di distribuzione nell'area centro-appenninica della nuova specie, la sua supposta monofagia su *Lunaria annua* L. (Brassicaceae) e la sua condizione di sub-atterismo, viene ipotizzato un possibile processo che ha portato al suo differenziamento.

**Key words.** Chrysomelidae, *Psylliodes*, new species, Apennines, endemism, wing reduction.

### **Introduction**

*Psylliodes* Latreille, 1829 is a cosmopolitan genus of the subfamily Galerucinae, tribe Alticini, including over 200 species (KONSTANTINOV & VANDENBERG, 1996; BIONDI & D'ALESSANDRO, 2012), about 170 of which have been reported for the Palaearctic fauna (DÖBERL, 2010; LEONARDI, 2013). They feed on plants of some 30 families but they have been more frequently found on Brassicaceae, Solanaceae, Asteraceae, Poaceae, Amaranthaceae, and Cannabaceae (JOLIVET & HAWKESWOOD, 1995). The systematic of the genus is still not clear, despite some attempts of classification based on morphological characters. For example, LEONARDI (1970) based its classification on the spermathecal characters and the different patterns of the frontal grooves, while NADEIN (2006) tried to use the tegmen in combination with other internal and external morphological structures.

In this paper, we describe *Psylliodes urbanae* sp. nov. from Central Apennines (Italy) that can be attributed to the *Psylliodes napi* species-group *sensu* LEONARDI (1970) and NADEIN (2006). The new species appears very similar to *P. napi* (Fabricius, 1792) in external morphology, and, as often happens within Alticini, only the shape of the median lobe of aedeagus has allowed to realize that it could be dealt as a different species. A discriminant analysis on some biometric characters to look for not immediately visible features has allowed to reliably distinguish between *Psylliodes urbanae* sp. nov. and *P. napi*. Considering the association of the new species to *Lunaria annua* L., we put particular attention to two other taxa both reported as monophagous (*sensu* BIONDI, 1996) on the genus *Lunaria* L.: *Psylliodes napi* var. *flavicornis* Weise, 1883 and *Psylliodes napi* var. *lunariae* Jacquet, 1887.

Beyond the discussion about taxonomy, we speculated about the possible processes that have led to the differentiation of *P. urbaniae* sp. nov. in Central Apennines, based on its presence at medium and high altitude, its monophagy, and its subapterism (*sensu* BIONDI, 1993).

### Material and methods

Material consisted of dried pinned specimens preserved in the institutions listed below. Specimens were examined and dissected using LEICA M205C binocular microscope. Photomicrographs were taken using a Leica DFC500 camera and the Auto-Montage Pro 2006 software (license number: 15224\*syn2459\*153a2112\_maurizio\_266836). Scanning electron micrographs were taken using a Hitachi TM-1000. Geographical coordinates of the localities were reported in the format used in the respective labels. The terminology used follows DÖBERL (1986), FURTH & SUZUKI (1994), and SUZUKI (1988) for the spermatheca; APGIV system (THE ANGIOSPERM PHYLOGENY GROUP, 2016) for the botanical family names. Morphometric measures were taken using the image analysis software Image-Pro Insight 8.0 (license number: 03080000-5385). Statistical analyses and graphics were performed using the package NCSS version 11 for Windows (license number: N9J7-B9F5-L6B3-G8R3-X6R4). Discriminant function analysis (TABACHNICK & FIDELL, 1989) was used to establish appropriate functions separating the species using morphometric characters as predictors.

### Abbreviations

Morphology. LA, numerical sequence proportional to length of each antennomere; LAED, length of aedeagus; LAN, length of antennae; LB, total length of body; LDTS, length of distal socket of hind tibia; LE, length of elytra; LHT, length of hind tibia; LP, length of pronotum; LSP, length of spermatheca; WE, width of elytra; WP, width of pronotum.

Collections and depositories. BAQ: collection of M. Biondi, Dipartimento di Medicina clinica, Sanità pubblica, Scienze della Vita e dell'Ambiente, Università dell'Aquila, Italy; MNHN: Museum National d'Histoire Naturelle, Paris, France; MSNM: Museo Civico di Storia Naturale, Milano, Italy; MZUF: Museo di Storia Naturale dell'Università degli Studi di Firenze, sezione di Zoologia "La Specola", Italy; ZMHU: Museum für Naturkunde der Humboldt-Universität, Berlin, Germany.

### Results

#### *Psylliodes urbaniae* sp. nov.

Type material. Holotype, ♂, Italy, Monti della Duchessa (RI) [Rieti], Val di Fua, 1300 m, on *Lunaria annua*, micropterous, 6.VI.1997, M. Biondi leg. (BAQ).

Paratypes: same data of the holotype, on *Lunaria annua*, 3 ♀♀ (BAQ, MZUF); ditto, 15.VII.1997, on *Lunaria annua*, 3 ♂♂ (BAQ); ditto, 1100-1300 m, on *Lunaria annua*, 9.IX.1992, 6 ♂♂ and 5 ♀♀ (BAQ, MZUF); ditto, 1500 m, on *Lunaria annua*, 16.V.2007, 2 ♀♀, brachypterous/micropterous (BAQ); ditto, 1350-1550 m, on *Lunaria annua*, 27.V.2007, 1 ♂ and 4 ♀♀ (BAQ; MSNM); Italy, Lazio (RI) [Rieti], Monti della Duchessa, Val di Fua, Fosso dell'Omo, 1400 m, 42°10.493' N 13°19.294' E, on *Lunaria annua*, 18.IX.2016, M. Biondi & P. D'Alessandro leg., 1 ♀ (BAQ); ditto, 5.VI.2015, 2 ♂♂ (BAQ; MSNM); Abruzzo (AQ) [Lazio, Rieti], Duchessa-Velino, Val di Fua, 19.VI.1997, P. Audisio leg., 1 ♂ and 1 ♀ (BAQ); Lazio (Roma), Monti Cornicolani, 700 m, Civitella di Licenza, on *Lunaria annua*, 29.VI.1996, P. Audisio leg., 1 ♂, brachypterous (BAQ); Abruzzo (AQ), Pietrasecca, on *Lunaria annua*, 12.IV.1997, P. Audisio leg., 1 ♂ and 2 ♀♀, micropterous (BAQ); ditto, near Pietrasecca, 850 m, on *Lunaria annua*, M. Biondi & G. De Nardis leg., 1 ♂ (BAQ); Lazio (VT), Castro, Fosso Olpeta, on *Lunaria annua*, 3.IV.1983, P. Audisio leg., 1 ♀, subbrachypterous (BAQ); Lazio (FR), Sgurgola, on *Lunaria annua*, 27.III.1997, P. Audisio leg., 1 ♂ and 1 ♀, micropterous (BAQ).

Diagnosis. *Psylliodes urbaniae* sp. nov. is nearly indistinguishable from *P. napi* in external features, unless considering the LHT/LDTS ratio (Figs 3-4) (see the following 'Discriminant analysis'). However, the median lobe of aedeagus allows to distinguish easily between the males of the two species (Figs 5-6); the spermatheca is instead very similar (Figs 7-8).

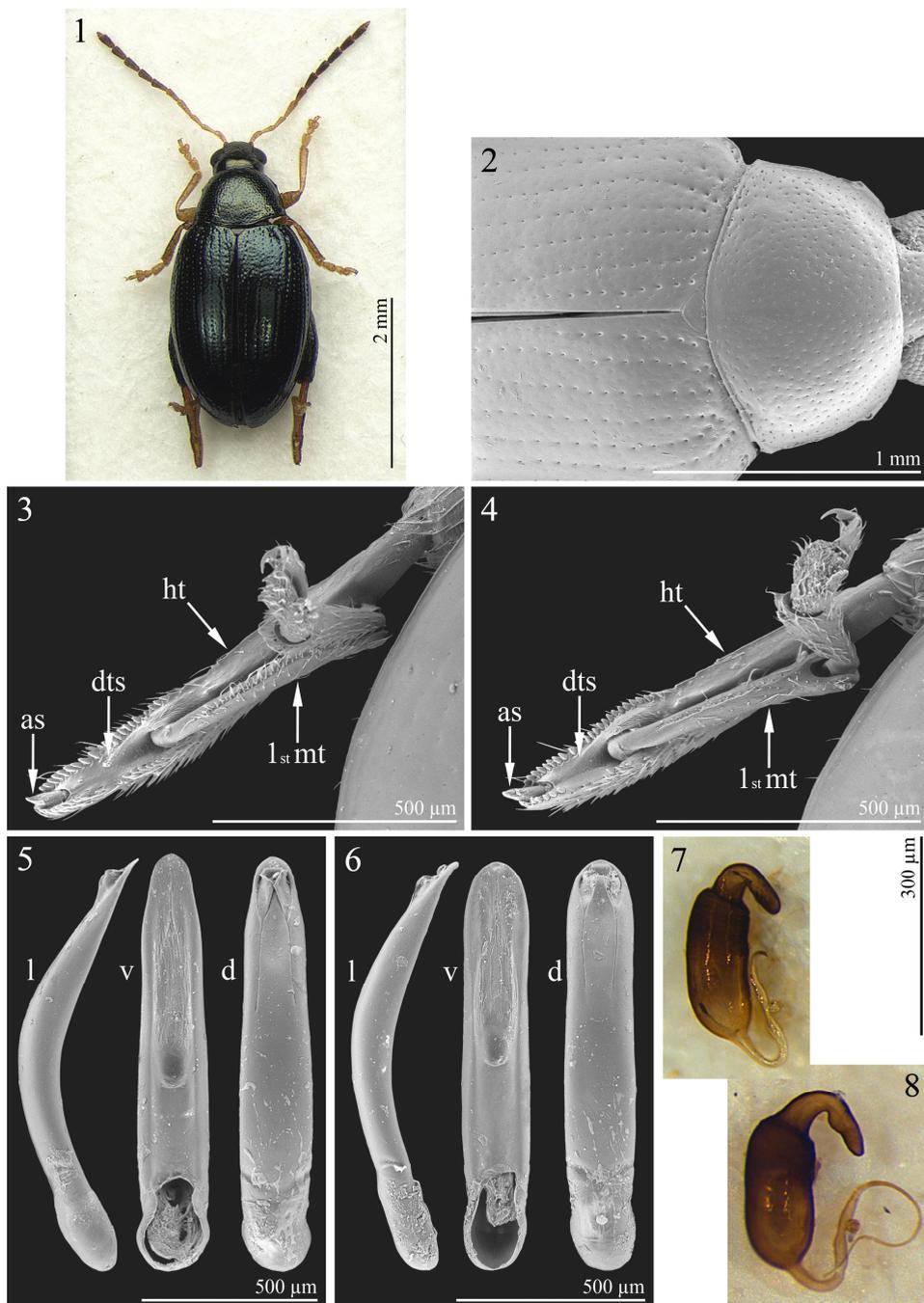
**Description of the holotype.** Body elliptical, elongate, rather convex (Fig. 1); LB = 3.13 mm; maximum pronotal width at base (WP = 1.06 mm); maximum elytral width at basal third (WE = 1.65 mm). Dorsum blueish with green dark metallic reflections. Frons and vertex slightly wrinkled and clearly punctate; frontal grooves absent, but ocular margin strongly marked; frontal tubercles weakly distinguishable; genae and frontal carina moderately elongate; frontal carina about as wide as antennal socket, with rounded margin; antennae slightly longer than half body length (LAN = 1.95 mm; LAN/LB = 0.62; LAN/(LE+LP) = 0.64), yellowish, gradually darkened towards apex; LA: 100 : 88 : 91 : 113 : 91 : 94 : 94 : 94 : 91 : 131. Pronotum (Fig. 2) distinctly trapezoidal and transverse (LP = 0.61 mm; WP/LP = 1.73), laterally straight; anterior angles moderately prominent, obliquely beveled; pronotal punctation small, dense, distinctly impressed, evenly distributed on microreticulate surface. Elytra moderately elongate (LE = 2.43 mm; WE/LE = 0.68), laterally distinctly arcuate; punctuation clearly larger than on pronotum (Fig. 2), arranged in regular striae; interstriae wide, smooth, finely micropunctate. Metatoracic wings strongly reduced: micropterous. Legs yellowish, with dark femora; first pro- and mesotarsomeres moderately dilated; adhesive setae on ventral side of first pro- and mesotarsomeres. Ventral parts blackish; last abdominal ventrite without special preapical impressions. Median lobe of aedeagus (LAED = 1.18 mm; LE/LAED = 2.06) slender (Fig. 5), in ventral view slightly tapered towards apex, slightly narrower in apical part; apex sub-rounded, without a median tooth; ventral surface with a wide ventral sulcus, deeper at base, about as long as half aedeagus; in lateral view, aedeagus clearly and evenly curved; dorsal ligula slightly longer than 1/3 of aedeagus, moderately wide, gradually narrower towards apex.

**Variability.** Paratypes very similar in shape, size, and colour to the holotype. Wings sometimes brachypterous or subbrachypterous (*sensu* BIONDI, 1993). Female distinguishable by the first pro- and metatarsomeres not dilated and without adhesive setae on ventral side. Spermatheca (Fig. 7) with sub-cylindrical basal part; distal part clearly distinct from basal part, thinner, with short collum; appendix of apical part absent; ductus moderately elongate, sub-apically inserted, curved, uncoiled.

Male (n = 18; mean and standard deviation; range): LE = 2.45 ± 0.12 mm (2.23 ≤ LE ≤ 2.63 mm); WE = 1.64 ± 0.09 mm (1.43 ≤ WE ≤ 1.80 mm); LP = 0.67 ± 0.05 mm (0.59 ≤ LP ≤ 0.75 mm); WP = 1.10 ± 0.04 mm (1.03 ≤ WP ≤ 1.08 mm); LAN = 1.92 ± 0.13 mm (1.50 ≤ LAN ≤ 2.08 mm); LAED = 1.20 ± 0.05 mm (1.13 ≤ LAED ≤ 1.33 mm); LB = 3.18 ± 0.16 mm (2.83 ≤ LB ≤ 3.53 mm); LHT = 1.00 ± 0.05 mm (0.91 ≤ LHT ≤ 1.07 mm); LDTS = 0.29 ± 0.02 mm (0.26 ≤ LDTS ≤ 0.31 mm); LE/LP = 3.65 ± 0.26 (3.17 ≤ LE/LP ≤ 4.04); WE/WP = 1.50 ± 0.06 (1.39 ≤ WE/WP ≤ 1.58); WP/LP = 1.64 ± 0.12 (1.43 ≤ WP/LP ≤ 1.84); WE/LE = 0.67 ± 0.02 (0.60 ≤ WE/LE ≤ 0.70); LAN/LB = 0.60 ± 0.04 (0.53 ≤ LAN/LB ≤ 0.66); LAN/(LE+LP) = 0.61 ± 0.04 (0.53 ≤ LAN/(LE+LP) ≤ 0.71); LE/LAED = 2.04 ± 0.10 (1.84 ≤ LE/LAED ≤ 2.22); LHT/LDTS = 3.50 ± 0.10 (3.31 ≤ LDTS ≤ 3.77). Female (n = 20; mean and standard deviation; range): LE = 2.57 ± 0.17 mm (2.28 ≤ LE ≤ 2.80 mm); WE = 1.75 ± 0.12 mm (1.58 ≤ WE ≤ 1.93 mm); LP = 0.68 ± 0.04 mm (0.60 ≤ LP ≤ 0.75 mm); WP = 1.14 ± 0.07 mm (0.99 ≤ WP ≤ 1.24 mm); LAN = 1.89 ± 0.10 mm (1.70 ≤ LAN ≤ 2.08 mm); LSP = 0.36 ± 0.03 mm (0.30 ≤ LSP ≤ 0.41 mm); LB = 3.33 ± 0.23 mm (2.88 ≤ LB ≤ 3.70 mm); LHT = 1.02 ± 0.08 mm (0.83 ≤ LHT ≤ 1.17 mm); LDTS = 0.29 ± 0.03 mm (0.22 ≤ LDTS ≤ 0.33 mm); LE/LP = 3.78 ± 0.25 (3.23 ≤ LE/LP ≤ 4.16); WE/WP = 1.54 ± 0.03 (1.49 ≤ WE/WP ≤ 1.62); WP/LP = 1.67 ± 0.11 (1.47 ≤ WP/LP ≤ 1.86); WE/LE = 0.68 ± 0.02 (0.65 ≤ WE/LE ≤ 0.73); LAN/LB = 0.57 ± 0.03 (0.51 ≤ LAN/LB ≤ 0.61); LAN/(LE+LP) = 0.58 ± 0.02 (0.52 ≤ LAN/(LE+LP) ≤ 0.61); LE/LSP = 7.25 ± 0.46 (6.34 ≤ LE/LSP ≤ 8.12); LHT/LDTS = 3.50 ± 0.13 (3.13 ≤ LDTS ≤ 3.77).

**Ecology.** *Psylliodes urbaniae* sp. nov. lives from medium to high altitude (700-1550 m a.s.l.), and it seems to be monophagous on *Lunaria annua* L. (Brassicaceae), preferably in forest environment. In this regard, it is interesting to observe that two forms attributed to *P. napi*, namely *P. n.* var. *flavicornis* Weise (1883: 219) and *P. n.* var. *lunariae* Jacquet (1887: 2), are associated with *Lunaria rediviva* L. However, after the examination of the type materials preserved at ZMHU (*P. n.* var. *flavicornis*) and MNHN (*P. n.* var. *lunariae*) respectively, we can confirm that these two forms, recognizable for having legs partially blackened, are coinciding and belong actually to the species *P. napi*.

**Distribution.** Italy: Central Apennines (Fig. 9).



Figs 1-8. 1. Habitus of *Psylliodes urbanae* sp. nov. male, Val di Fua. 2. Ditto, pronotum and basal part of elytra. 3-4. Hind tibia and tarsus of *P. urbanae* sp. nov. (3) and *P. napi* (Fabricius, 1792) (4); 1<sup>st</sup> mt: first metatarsomere; as: apical spur; dts: distal tibial socket; ht: hind tibia. 5-6. Aedeagus in lateral (l), ventral (v) and dorsal (d) view of *P. urbanae* sp. nov., Val di Fua (5) and *P. napi* (Fabricius, 1792), Italy, Majella, Ponte di Pietra (6). 7-8. Spermatheca of *P. urbanae* sp. nov., Val di Fua (7) and *P. napi* (Fabricius, 1792), Italy, Majella, Ponte di Pietra (8).

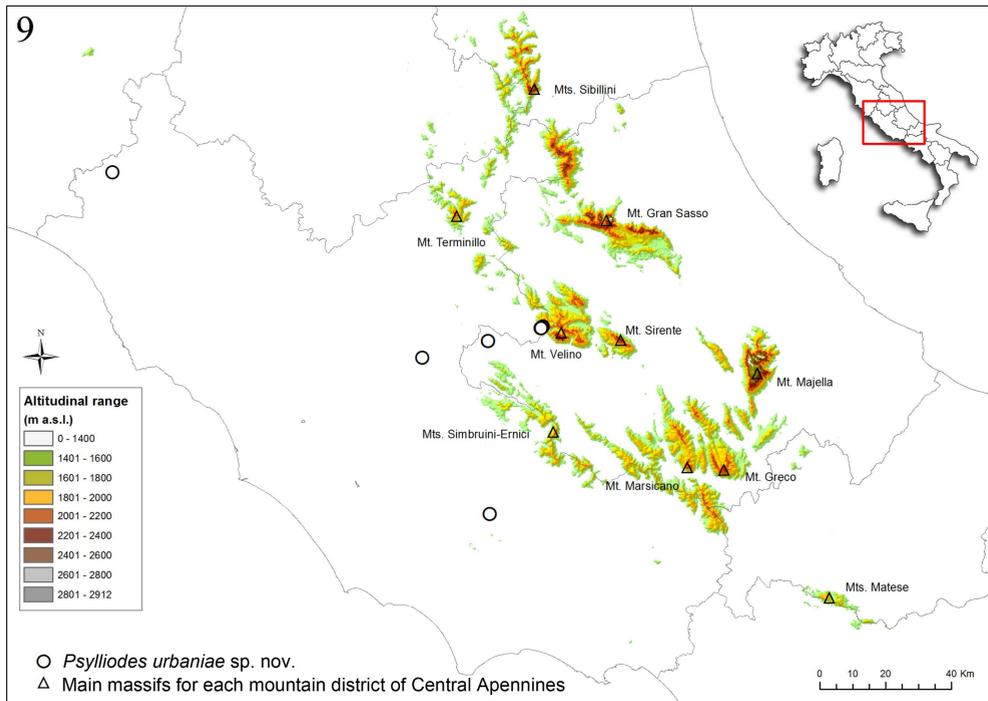


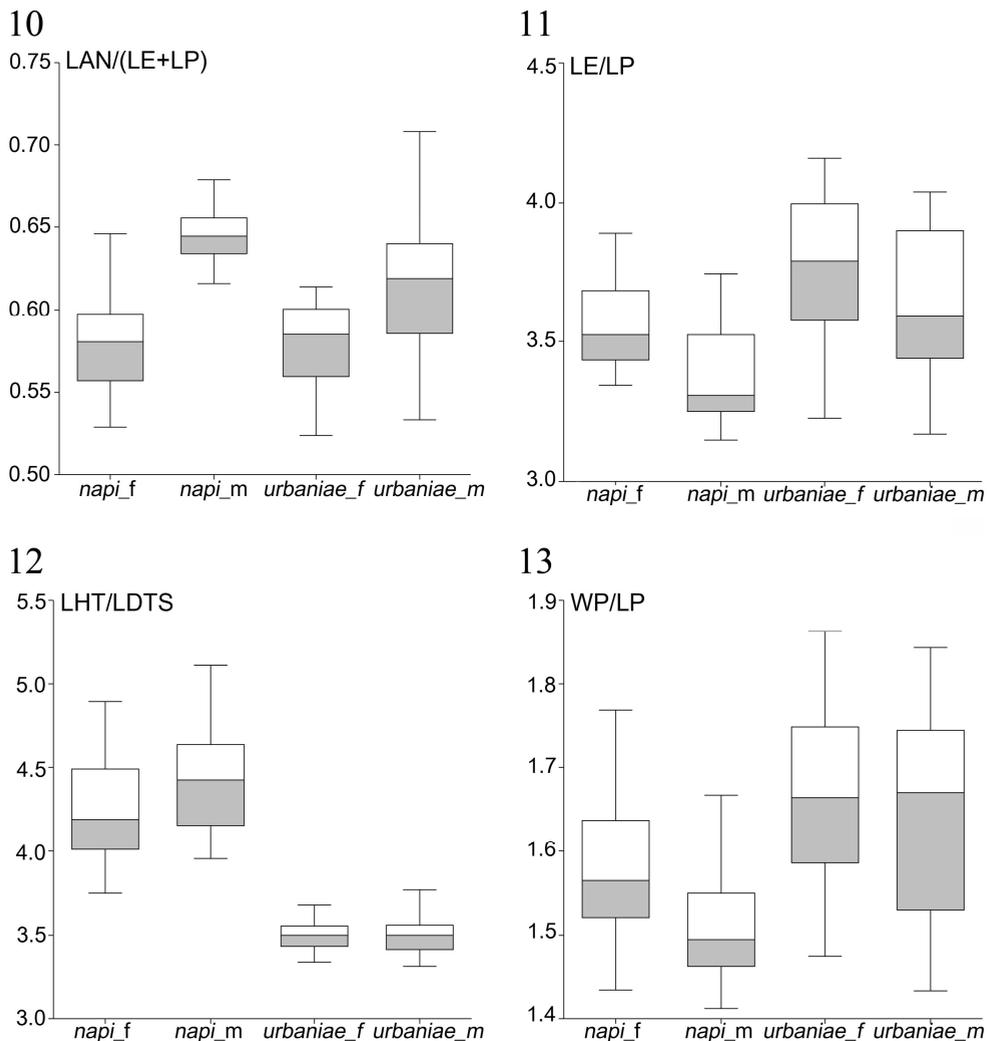
Fig. 9. Geographical distribution of *Psylliodes urbanae* sp. nov.

**Etymology.** We dedicated this species to our friend Fabrizia Urbani (Italy, L'Aquila), as appreciation of her collaboration and help during these last years.

### Discriminant analysis

A forward stepwise discriminant function analysis, considering separately males and females, was performed using eight morphometric variables as predictors (Fig. 14). The main aim of this analysis was to determine morphometric characters to aid in the identification of *P. napi* (20 ♂♂ and 20 ♀♀) and *P. urbanae* sp. nov. (18 ♂♂ and 20 ♀♀). Predictor variables used in the analysis were: length of elytrae (LE), width of elytrae (WE), length of pronotum (LP), width of pronotum (WP), length of antennae (LAN), length of the hind tibiae (LHT), length of the distal part of the hind tibial socket (LDTS), length of aedeagus (LAED) and length of spermatheca (LSP). No data standardization or normalization were performed for these measures.

The analysis carried out on the males shows that the variables LDTS, LAED, and LHT are highly discriminating (Tab. I), while LE, WE, LP, WP, and LAN are not significant. The classification matrix relative to males (Tab. II) shows a percentage of 100% of corrected attributions for the two species analyzed. The discriminant analysis carried on females has also supplied significant results (Tab. I). Also in this case, the variables with higher discriminating power are LDTS and LHT, while LE, WE, LP, WP, LAN, and LSP are not significant. In the classification matrix of females (Tab. II), the percentage of corrected attributions is equal to 97.5% (100% for *P. napi* and 95% for *P. urbanae* sp. nov.), with a failed attribution only for a specimen of *P. urbanae* sp. nov. The group centroids for both males and females are reported in Tab. III. Box and whisker plots showing median, inter-quartile range and range of the ratios LAN/(LE+LP), LE/LP, LHT/LDTS, and WP/LP for males and females are shown in Figs 10-13.



Figs 10-13. Box and whiskers plots for males and females of *Psylliodes urbaniae* sp. nov. and *P. napi* (Fabricius, 1792) showing median, inter-quartile range, and range of the ratios LAN/(LE+LP) (10); LE/LP (11); LHT/LDTS (12); WP/LP (13). Abbreviations: napi\_m and napi\_f = males and females of *P. napi* (Fabricius, 1792); urbaniae\_m and urbaniae\_f = males and females of *P. urbaniae* sp. nov.

### Discussion and conclusion

*Psylliodes urbaniae* sp. nov. can be attributed to the *Psylliodes napi* species-group *sensu* LEONARDI (1970) due to the absence of frontal grooves, the weakly distinguishable frontal tubercles, and the spermatheca with distal part not invaginated in basal part and with simple, uncoiled ductus (Fig. 7). By following the classification by NADEIN (2006), who split the *P. napi* species-group proposed by LEONARDI (1970) into *cuprea* species-group and *napi* species-group, the new species still belongs to the *napi* group, because of the Y-shaped tegmen, the convex body, and the not shagreened dorsal surface. More specifically, it appears closely related to *P. napi* due both to their general strong similarity in the external habitus, and the morphology of the genitalia (Figs 5-8). Based on qualitative

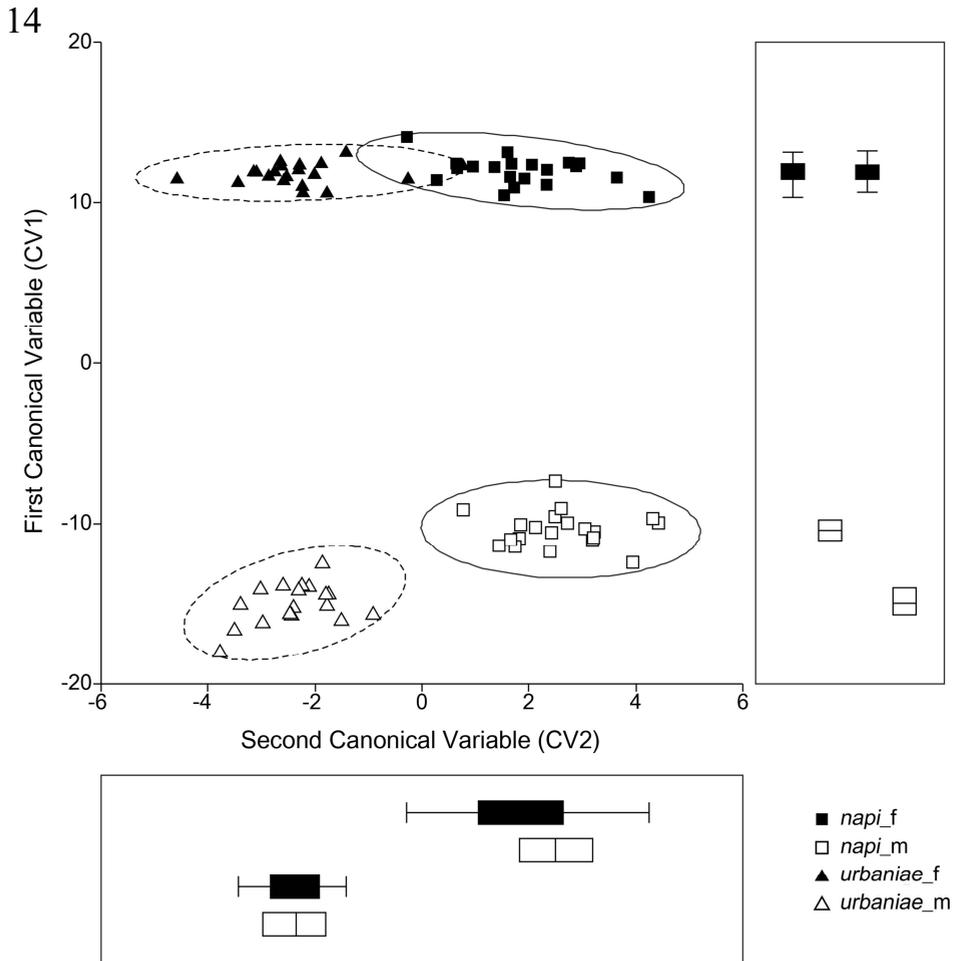


Fig. 14. Discriminant analysis for males and females of *Psylliodes urbaniae* sp. nov. and *P. napi* (Fabricius, 1792), based on morphometric variables as predictors (see text).

characters, *P. napi* and *P. urbaniae* sp. nov. can be reliably distinguished only by the small but constant and distinguishable differences in the shape of the aedeagus (Figs 5-6). However, the quantitative analysis here performed proves that the ratio LHT/LDTS is reliable to separate the two species, both in males and females (Figs 3-4, Fig. 12).

Central Apennines is the region of the Italian territory with the greatest richness of Italian endemic and subendemic leaf beetle species: 29 species, of which 7 are locally exclusively endemic (BIONDI *et al.*, 2013). Moreover, a “parsimony analysis of endemism”, performed by using Chrysomelidae, detected in Central Apennines one of the main areas of endemism in Italy (BIONDI *et al.*, 2013).

After all, the phenomenon of endemization associated with higher altitudes in Central Apennines is well-known, and it is generally associated to the alternation of catathermic and hypsothermic phases during the Pleistocene that favored isolation and differentiation events of montane fauna (STOCH, 2006; SCHMITT, 2007; STEWART *et al.*, 2010). *Psylliodes urbaniae* sp. nov. increases to 8 the number of the exclusive endemic leaf beetle taxa in Central Apennines.

♂♂	Step	F to enter	p-level	Lambda
LDTS	1	286.83	0.000000	0.111513
LAED	2	5.03	0.031308	0.097495
LHT	3	6.37	0.016453	0.082113

♀♀	Step	F to enter	p-level	Lambda
LDTS	1	100.85	0.000000	0.273671
LHT	2	11.96	0.001385	0.206830

Tab. I. Discriminant Stepwise Analysis for males and females of *Psylliodes urbaniae* sp. nov.: “variables in the model”, “F to enter”, p-level and Wilk’s Lambda values.

	Percent	<i>P. napi</i> ♂♂	<i>P. urbaniae</i> ♂♂	<i>P. napi</i> ♀♀	<i>P. urbaniae</i> ♀♀	Total
<i>P. napi</i> ♂♂	100.00	20	0	0	0	20
<i>P. urbaniae</i> ♂♂	100.00	0	18	0	0	18
<i>P. napi</i> ♀♀	100.00	0	0	20	0	20
<i>P. urbaniae</i> ♀♀	95.00	0	0	1	19	20
<b>Total</b>	98.75	20	18	21	19	78

Tab. II. Discriminant Stepwise Analysis: classification matrix for males and females of *Psylliodes napi* (Fabricius, 1792) and *P. urbaniae* sp. nov. Rows: observed classifications; columns: predicted classifications.

♂♂	CV1	CV2	♀♀	CV1	CV2
<i>P. napi</i>	-10.347	2.593	<i>P. napi</i>	11.946	1.844
<i>P. urbaniae</i>	-14.944	-2.380	<i>P. urbaniae</i>	11.851	-2.296

Tab. III. Discriminant Stepwise Analysis: group centroids for males and females of *Psylliodes napi* (Fabricius, 1792) and *P. urbaniae* sp. nov.

Since all the examined specimens show a wing reduction the new species is unable to fly. *Psylliodes napi* itself shows wing-polymorphism (LEONARDI, 1971, 1975). The latter species is widely distributed in Europe, present in Caucasus and Northern Africa too (Sibero-European Chorotype ‘with some extensions’, *sensu* VIGNA TAGLIANTI *et al.*, 1999), and it is oligophagous on several genera of Brassicaceae (LEONARDI, 1975; DÖBERL, 2010). Intra-specific wing-length polymorphism is a widespread phenomenon among leaf beetles caused by a variety of factors (cf. FURTH, 1980). The geographic contexts where flightlessness seems predominant in insects are the isolated mountain tops and the islands. For both cases, the recurrent explanations are that: flight is a disadvantage when individuals may easily blow away from their favorable habitat; in habitats with scarce competitors and/or predators it is not convenient to invest in energy-expensive activities as the wing formation and the flight. Among phytophagous species, differences in host choice by flying and non-flying morphs have been reported: flying morphs tend to feed on more botanical genera or species than non-flying morphs (cf. FURTH, 1980). Considering the close phylogenetic relationships among the species of the *napi* group (LEONARDI, 1970, 1971; NADEIN, 2006), we can speculate on how a process of differentiation/speciation may occur within a wing-length polymorphic phytophagous species:

starting from an ancestral flying, polyphagous or oligophagous species with genetic tendency to the flightless, non-flying individuals may appear and easily proliferate in contexts where both flight is disadvantageous and a host plant is concentrate and easily available. This may have been the process leading to the differentiation of *P. urbaniae* sp. nov.: *P. napi*, widely distributed, oligophagous on several genera of Brassicaceae, and wing-length polymorphic may be the ancestor (or the derived species more similar to the ancestor); *P. urbaniae* sp. nov. may be derived from it (or from the common ancestor), particularly from non-flying individuals of Central Apennines feeding on *Lunaria annua* in forest environment.

*Psylliodes napi* var. *flavicornis*, considered as subspecies by LEONARDI (1975), also lives on the genus *Lunaria*, and shows a reduction of the metathoracic wings (LEONARDI, 1975). It has been reported from the mountain areas of Central Europe (Alps, Sudetes, Carpathians, and Transylvanian Alps) (LEONARDI, 1975). This taxon may have been affected by a similar process involving *P. urbaniae* sp. nov., but with a less degree of differentiation possibly due to a more recent isolation from the ancestral species, or to the maintenance of some degree of genetic flow with the typonominal populations of *P. napi*. A possible alternative hypothesis could be that *P. urbaniae* sp. nov. and *P. napi* var. *flavicornis* are sister taxa derived from a common ancestor associated with the plant genus *Lunaria*. In this case *P. napi* var. *flavicornis* should be considered as a good species and the differentiation of two taxa could be due to the disjunct montane distribution occurred in the last postglacial. Anyway, future phylogenetic and phylogeographical analyses will possibly confirm or not these assumptions.

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