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An application of the synthetic sex attractants from the series “EFETOV-2” for studying Procridinae in Italy (Lepidoptera: Zygaenidae)

K. A. Efetov, E. E. Kucherenko & G. M. Tarmann

Abstract

The enantiomers of 2-butyl 2-dodecenoate synthesised in the Crimean Federal University are known as the sex attractants for Procridinae species (Lepidoptera: Zygaenidae). These compounds, alone and in combination, were applied for studying the Zygaenidae fauna in Italy for four years from 2015 to 2018. We evaluated the effectiveness of the three following variants: EFETOV-2 (the racemic mixture of (2R)-butyl 2-dodecenoate and (2S)-butyl 2-dodecenoate), EFETOV-S-2 (the R-enantiomer alone) and EFETOV-S-S-2 (the S-enantiomer alone). The males of three genera of Procridinae were attracted to the lures from the series “EFETOV-2”: *Rhagades* Wallengren, 1863 (one species), *Adscita* Retzius, 1783 (six species), and *Jordanita* Verity, 1946 (four species). The sex attractants for species *Adscita* (*Adscita*) *italica* (Alberti, 1937), *A. (A.) alpina* (Alberti, 1937), *A. (A.) dujardini* Efetov & Tarmann, 2014, and one endemic species *Jordanita* (*Jordanita*) *tenuicornis* (Zeller, 1847) were found for the first time. Moreover, a hybridisation zone between *A. (A.) italica* and *A. (A.) alpina* was discovered by the application of the sex attractants. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775) was recorded for the first time for more than 100 years in Piemonte and for the first time ever in the Ligurian Alps.

KEY WORDS: Lepidoptera, Zygaenidae, Procridinae, sex attractants, monitoring, distribution, hybridisation, EFETOV-2, EFETOV-S-2, EFETOV-S-S-2, 2-butyl 2-dodecenoate, Alps, Liguria, Italy.

Una aplicación del atrayente sintético sexual de la serie “EFETOV-2” para estudiar los Procridinae en Italia (Lepidoptera: Zygaenidae)

Resumen

Los enantiómeros de 2-butyl 2-dodecenoato sintetizados en la Universidad Federal de Crimea, son conocidos como atrayentes sexual para las especies de Procridinae (Lepidoptera: Zygaenidae). Estos compuestos solos y en combinación, fueron aplicados para el estudio de la fauna de Zygaenidae en Italia durante cuatro años desde el 2015 al 2018. Evaluamos la efectividad de las tres siguientes variantes: EFETOV-2 (la mezcla racémica de (2R)-butyl 2-dodecenoato y (2S)-butyl 2-dodecenoato), EFETOV-S-2 (solo el R-enantiómero) y EFETOV-S-S-2 (solo el S-enantiómero). Los machos de tres géneros de Procridinae fueron atraídos a los señuelos de la serie “EFETOV-2”: *Rhagades* Wallengren, 1863 (una especie), *Adscita* Retzius, 1783 (seis especies) y *Jordanita* Verity, 1946 (cuatro especies). Fue hallado, por primera vez, el atrayente sexual para las especies *Adscita* (*Adscita*) *italica* (Alberti, 1937), *A. (A.) alpina* (Alberti, 1937), *A. (A.) dujardini* Efetov & Tarmann, 2014 y una especie endémica *Jordanita* (*Jordanita*) *tenuicornis* (Zeller, 1847). Sin embargo, con la aplicación de los atrayentes sexuales, fue descubierta una zona de hibridación entre *A. (A.) italica* y *A. (A.) alpina*. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775) fue registrado por primera vez, en más de 100 años, en el Piemonte y por primera vez en los Alpes de Liguria. PALABRAS CLAVE: Lepidoptera, Zygaenidae, Procridinae, atrayente sexual, monitoreo, distribución, hibridación, EFETOV-2, EFETOV-S-2, EFETOV-S-S-2, 2-butyl 2-dodecenoato, Alpes, Liguria, Italia.

Introduction

In recent years, there has been an increasing interest in studies of sex pheromones and sex attractants of Insecta. A considerable amount of research has been published on the application of these molecules for pest management and monitoring of rare and endangered species (WITZGALL *et al.*, 2010; EFETOV *et al.*, 2014b; OLEANDER *et al.*, 2015; LARSSON, 2016; CIZEJ & TREMATERRA, 2017; WILSON *et al.*, 2017; GREGG *et al.*, 2018). Sex pheromones and sex attractants are also used for faunal and evolutionary studies of Lepidoptera (ROELOFS & ROONEY, 2003; SUBCHEV *et al.*, 2016; RAZOV *et al.*, 2017; CAN *et al.*, 2019). Field experiments with sex attractants and/or sex pheromones, such as for Zygaenidae (Lepidoptera), have allowed researchers to discover new species (KEIL, 2016; EFETOV *et al.*, 2018a), detect low-density populations of harmful or rare moths (EFETOV *et al.*, 2019b; TARMANN *et al.*, 2019; VRENOZI *et al.*, 2019) and discover new data about the distribution of Zygaenids (CAN CENGİZ *et al.*, 2018).

To date, the family Zygaenidae is represented by five subfamilies: Inouelinae Efetov & Tarmann, 2017; Procridinae Boisduval, 1828; Chalcosiinae Walker, 1865; Callizygaeninae Alberti, 1954; and Zygaeninae Latreille, 1809 (EFETOV, 2001b; EFETOV *et al.*, 2004, 2006, 2014a, 2015a; EFETOV & HAYASHI, 2008; EFETOV & SAVCHUK, 2009, 2013; EFETOV & TARMANN, 2017a; EFETOV & KNYAZEY, 2014; KNYAZEY *et al.*, 2015a, 2015b; HOFMANN & TREMEWAN, 2017). During the last 30 years, extensive revisional work has been carried out on the Palaearctic Zygaenidae with many new species described on the base of morphological and molecular analysis (EFETOV, 1992, 1996a, 1996b, 1997a, 1997b, 1998, 1999, 2001a, 2006, 2010; EFETOV & TARMANN, 1999, 2013a, 2013b, 2014a, 2016a, 2016b, 2017b; MOLLET & TARMANN, 2007, 2018; TARMANN & DROUET, 2015; EFETOV *et al.*, 2019a, 2019c). Forty five species of Zygaenidae are currently known from Italy, of which three are endemic, viz. *Jordanita (Jordanita) tenuicornis* (Zeller, 1847) (Procridinae), *Zygaena (Mesembrynus) rubicundus* (Hübner, 1817), and *Z. (Zygaena) oxytropis* Boisduval, 1828 (Zygaeninae) (EFETOV, 1994, 2004; BERTACCINI & FIUMI, 1999; NAUMANN *et al.*, 1999; EFETOV *et al.*, 2011b; EFETOV & TARMANN, 2014b; HOFMANN & TREMEWAN, 2017). However, the occurrence of *J. (Gregorita) algerica* (Rothschild, 1917) needs confirmation because the examined historical specimens from Sicily are the only reference material that is available, and no other records are known. Two species, *Z. (Z.) nevadensis* Rambur, 1858, and *Z. (Z.) angelicae* Ochseneheimer, 1808, have been found only recently on Italian territory. The earlier published data on *Z. (Z.) nevadensis* have been proved to be erroneous and this species was newly discovered on La Sila in Calabria (EFETOV *et al.*, 2011b). Recently, based on the differences in habitus, genitalia morphology and DNA barcoding data, one new species, viz. *Adscita (Adscita) dujardini* Efetov & Tarmann, 2014, has been described from Italy, France, Switzerland and Slovenia (EFETOV & TARMANN, 2014b). Here this species replaces *A. (A.) albanica* (Naufock, 1926) which is restricted to the Balkans, Ukraine, and Russia. Despite the fact that the rich Italian Zygaenidae fauna is well investigated, a lot of questions about the origin, distribution, biology, and ecology of Zygaenidae of the Apennine Peninsula remain still open and need to be discussed.

EFETOV *et al.* (2015b) investigated the fauna of Zygaenidae in Italy using the *R*- and *S*-enantiomers of 2-butyl (7*Z*)-dodecenoate which have been identified as female sex pheromone components of *Illiberis (Primilliberis) rotundata* Jordan, 1907, a species known from East Asia (SUBCHEV *et al.*, 2009). The attractiveness of these molecules has been shown for many Procridinae species (EFETOV *et al.*, 2010, 2011a; SUBCHEV *et al.*, 2010, 2012, 2013). In this study we used the *R*- and *S*-enantiomers of another ester, viz. 2-butyl 2-dodecenoate, synthesised in the Crimean Federal University (EFETOV *et al.*, 2014c). These molecules also attracted the males of Procridinae (EFETOV *et al.*, 2016, 2018b, 2019b). There were three main aims of our work: 1) to check the attractiveness of (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate and their mixture for the Italian Procridinae species; 2) to discover new data about the occurrence and distribution of Zygaenidae in Italy; 3) to look for presumed extinct species (e. g. *Rhagades (Rhagades) pruni* ([Denis & Schiffermüller], 1775) in Piemonte).

Materials and methods

Field observations with synthetic sex attractants from the series “EFETOV-2” were undertaken in

Italy for four years from 2015 to 2018. We tested the sex attractants in six provinces (Bolzano, Pordenone, Udine, Vicenza, Macerata, L'Aquila) in 2015; in eleven provinces (Bolzano, Aosta, Sondrio, Lecco, Bergamo, Brescia, Torino, Cuneo, Parma, Imperia, Savona) in 2016; in four provinces (Bolzano, Imperia, Savona, Genova) in 2017; and in four provinces (Bolzano, L'Aquila, Potenza, Cosenza) in 2018. A detailed table with locality information where Procridinae species were attracted is given in Table 1.

2-butyl 2-dodecenoate were synthesised in the Crimean Federal University as described in EFETOV *et al.* (2014c). We tested the attractiveness of (2R)-butyl 2-dodecenoate alone (EFETOV-S-2), (2S)-butyl 2-dodecenoate alone (EFETOV-S-S-2) and their racemic mixture (EFETOV-2). For preparing baits, the different sex attractants were applied onto grey rubber vial caps fixed on cardboard rectangles and labelled. The lures were attached to twigs on bushes or on the clothes of the researcher (when he slowly crossed the biotope). The attracted specimens were collected by netting them near the lure. In some cases, transparent Delta traps were also used. The removable sticky layers were covered with Tanglefoot® insect glue. Control traps (without attractants) were necessarily placed in these localities. All captured and attracted moths were recorded and determined by the genitalia examination.

All maps are compiled from the BioOffice database of the Tiroler Landesmuseen, Innsbruck, Austria.

Two-letter code of studied provinces: BZ - Bolzano, PN - Pordenone, UD - Udine, TO - Torino, CN - Cuneo, PR - Parma, IM - Imperia, SV - Savona, MC - Macerata, AQ - L'Aquila, CS - Cosenza.

Results and discussion

It was shown that (2R)-butyl 2-dodecenoate and (2S)-butyl 2-dodecenoate, alone and in combination, were attractive for the males of eleven Procridinae species and two hybrid populations (Table 2). The total numbers of the attracted specimens to the sex attractants from the series “EFETOV-2” are listed in Table 3.

Table 3.– The total numbers of the males of Procridinae species attracted to the sex attractants from the series “EFETOV-2” in different provinces of Italy. Specimens collected by baited traps are marked with an asterisk. The two-letter code is an abbreviation of the province name.

Attracted species	EFETOV-2	EFETOV-S-2	EFETOV-S-S-2
<i>Rhagades pruni</i>	–	–	18 ♂♂ (TO, CN, IM)
<i>Adscita statices</i>	–	4 ♂♂ (IM)	–
<i>Adscita statices</i> × <i>alpina</i>	–	5 ♂♂ (IM)	–
<i>Adscita alpina</i>	9 ♂♂ (BZ)	40 ♂♂ (CN, SV, BZ)	–
<i>Adscita alpina</i> × <i>italica</i>	7 ♂♂ (SV)	–	–
<i>Adscita italica</i>	17* ♂♂ (AQ)	19 ♂♂ (AQ, CS)	17 ♂♂ (AQ)
<i>Adscita geryon</i>	–	4 ♂♂ (BZ, AQ)	–
<i>Adscita dujardini</i>	1* ♂ (MC)	1* ♂ (MC)	–
<i>Adscita mannii</i>	1* ♂ + 18 ♂♂ (AQ, CS)	19 ♂♂ (BZ, PN, UD, AQ)	8 ♂♂ (IM, AQ, CS)
<i>Jordanita notata</i>	–	1* ♂ + 7 ♂♂ (PN, MC)	3 ♂♂ (IM, AQ)
<i>Jordanita tenuicornis</i>	–	1 ♂ (AQ)	3 ♂♂ (AQ)
<i>Jordanita globulariae</i>	–	4 ♂♂ (PN)	–
<i>Jordanita subsolana</i>	–	419 ♂♂ (TO, IM, PR, BZ, AQ)	–

Some Procridinae species demonstrated a strong preference for only one type of the attractant. For example, the males of *Rhagades* (*Rhagades*) *pruni* were attracted only to the baits with EFETOV-S-S-2. It should be noted that *Rh. (Rh.) pruni* was found for the first time since its last record of more than 100 years ago in Piemonte near Torino in 1906 and for the first time ever in the Ligurian Alps. Three new populations (Fig. 1) of this presumed extinct species were found only with the help of the sex attractant. It has recently been shown that EFETOV-S-S-2 contributed to the rediscovery of *Rh. (Rh.) pruni* in Spain on the southern side of the Pyrenees where this species is also a very rare (EFETOV *et al.*, 2019b). Together, these studies suggest that the sex attractant EFETOV-S-S-2 is a sensitive and efficient tool for monitoring populations of

Rh. (Rh.) pruni. It is interesting that this species is mentioned as a pest on apple (*Malus toringoides* Hughes) and *Prunus* spp. in Japan (TARMANN, 2003).

In contrast to *Rh. (Rh.) pruni* the species *Jordanita (Solaniterna) subsolana* (Staudinger, 1862) (with more than 400 attracted specimens) reacted only to EFETOV-S-2. For instance, during 30 minutes enormous masses of this species (more than 100 specimens) were attracted to the lure with EFETOV-S-2 attached to the hat of the researcher (Fig. 2). The males showed active sexual behaviour: they trembled by wings, moved antennae, opened valves, touched the rubber cap by the end of abdomen and tried to copulate with it. This means that our type of the sex attractant was very effective for detection of *J. (S.) subsolana* in biotopes. New localities could be discovered in three provinces, viz. Torino, Imperia and Parma (Fig. 3).

One more species, viz. *Jordanita (Jordanita) globulariae* (Hübner, 1793), came to the lures with EFETOV-S-2. Thus, *R*-enantiomer attracts *J. (S.) subsolana* and *J. (J.) globulariae*, while *S*-enantiomer has high attractiveness for the males of *Rh. (Rh.) pruni*.

All three variants of the sex attractants from the series “EFETOV-2” were active for *Adscita (Tarmannita) mannii* (Lederer, 1853). They are new synthetic sex attractants for this species. Besides this, the attractiveness of other esters of 2-butanol, viz. (2*S*)-butyl (9*Z*)-tetradecenoate, (2*S*)-butyl (7*Z*)-dodecenoate and (2*R*)-butyl (7*Z*)-dodecenoate, were proved for the males of *A. (T.) mannii* some years ago (SUBCHEV *et al.*, 2010; EFETOV *et al.*, 2015b; RAZOV *et al.*, 2017). It is interesting to note that for this species the *S*-enantiomers of these esters are more active than the corresponding *R*-enantiomers. EFETOV *et al.* (2015b) demonstrated that when three variants of the attractants ((2*S*)-butyl (7*Z*)-dodecenoate, (2*R*)-butyl (7*Z*)-dodecenoate and their mixture) were present in the habitat, the males of *A. (T.) mannii* came mainly to the *S*-enantiomer alone. When only (2*R*)-butyl (7*Z*)-dodecenoate and the mixture were present, some specimens could be found in traps baited with the mixture. The males of *A. (T.) mannii* were captured by the traps baited with (2*R*)-butyl (7*Z*)-dodecenoate only if other types of the lures were absent in biotopes. Our results coincide with those observed in earlier studies. The males of *A. (T.) mannii* were attracted to the lures EFETOV-S-2 (*R*-enantiomer) if we used only this one attractant during observations. In case when all three types of the lures were applied, *A. (T.) mannii* came to EFETOV-S-S-2 (*S*-enantiomer) or EFETOV-2 (the mixture). It should be noted that the differences between our sex attractants and other attractive esters for *A. (T.) mannii* are in the position of the double bond (at the 2nd carbon atom instead of the 7th or 9th) and for (2*S*)-butyl (9*Z*)-tetradecenoate in the carbon chain length of fatty acids (12 C instead of 14 C). There are two important implications from these findings. Firstly, the presence of the *R*-enantiomers of biologically active esters does not influence the attractiveness of the corresponding *S*-enantiomers for *A. (T.) mannii*. Secondly, stereochemistry of molecules is important for the identification of the sex attractants by olfactory receptors of moths.

Three closely related species, viz. *Adscita (Adscita) statice* (Linnaeus, 1758), *A. (A.) alpina* (Alberti, 1937), and *A. (A.) italica* (Alberti, 1937), reacted actively to the investigated sex attractants. The males of *A. (A.) statice* were attracted only to the lures baited with EFETOV-S-2. The males of *A. (A.) alpina* were mainly attracted to EFETOV-S-2 and to EFETOV-2. The males of *A. (A.) italica* were caught by the sticky trap with EFETOV-2 (Fig. 4); in addition, they also came to the lures with EFETOV-S-S-2 and EFETOV-S-2. The sex attractants for *A. (A.) alpina* and *A. (A.) italica* have been found for the first time while the attractiveness of EFETOV-2 and EFETOV-S-2 for *A. (A.) statice* was demonstrated in our previous studies (EFETOV & GORBUNOV, 2016; CAN CENGİZ *et al.*, 2018). In the Ligurian Alps these three species of the subgenus *Adscita* have their distributional borders. In recent years it has been observed that there is a hybrid belt between *A. (A.) statice* and *A. (A.) alpina* in the western Ligurian Alps (GUENIN & TARMANN, 2016). Hybrid zones between these two species have been known throughout the Alps for a long time (white squares on Fig. 5) (TARMANN, 1979; GUENIN, 2013, 2016). However, there was still a distributional gap of about 70 km (as the crow flies) between *A. (A.) alpina* in the West and *A. (A.) italica* in the East in the Ligurian Alps and the Ligurian Apennine. *A. (A.) italica* is an endemic subspecies of the Apennine Peninsula and had its western known distribution around Genova. In summer 2017 the field work to study this so-called distributional gap in the Ligurian Alps and the Ligurian Apennines was carried out with the help of the sex attractants from the series “EFETOV-2”. As soon as the males were detected, the females had to be found because *A. (A.) alpina* and *A. (A.) italica* can

only be distinguished clearly by different structures in the female genitalia (Figs 6, 7). The distributional limits of *A. (A.) alpina* and *A. (A.) italica* were determined and hybrids between *A. (A.) alpina* and *A. (A.) italica* were discovered for the first time. The contact area between these two species is farther to the East than was expected and *A. (A.) alpina* is recorded for the first time from the Apennines (province Savona: Montenotte inferiore) (Fig. 5). Hybrids *Adscita alpina* × *italica* with intermediate female genitalic structures (Fig. 8) were found at five localities, four in the Ligurian Apennines (province Genova: Valle Gorgasso, Madonna della Guardia; province Savona: Piampaludo, Case del Oca) and one in the eastern Ligurian Alps (province Savona: Bardinetto) (black squares on Fig. 5).

Adscita (Adscita) dujardini Efetov & Tarmann, 2014, was found in large numbers in 2015 at its type-locality (more than 50 specimens on flowers of *Geranium sanguineum* L.). In spite of this fact, there were only two males (one in EFETOV-2 trap and one in EFETOV-S-2 trap) glued from 16-VI-2015 to 18-VI-2015. It means that the attractants have no strong attraction for the males of this species. However, the empty control trap (without attractants) indicates that it was not accidentally that the specimens were attracted into traps with the attractants EFETOV-2 and EFETOV-S-2. The males of the endemic Italian species *Jordanita (Jordanita) tenuicornis* (Zeller, 1847) also reacted to two attractants: EFETOV-S-2 and EFETOV-S-S-2. The sex attractants for these two species were found for the first time.

It is worth mentioning about the study of diurnal rhythms of pheromone communication of some species of Procridinae in Italy. It is known that the time of the day when the males react to the sex pheromone is usually synchronized with the time when the conspecific females release pheromones. Therefore observations on calling females or the attraction of males to a pheromone (or synthetic attractant) source could be used for these investigations. On 08-09-VI-2015 a twenty four hour experiment with the attractant EFETOV-S-2 was carried out by G. M. Tarmann in the province Pordenone, Magredi di Cellina, Vivaro S. The researcher slowly crossing the biotope testing the attractant attached to his hat for 30 minutes, with a break for one hour thirty minutes. In total, 12 exposures of the sex attractant were carried out. The males of three species, viz. *A. (T.) mannii*, *Jordanita (Tremewanina) notata* (Zeller, 1847), and *J. (J.) globulariae*, were found in the biotope and attracted to EFETOV-S-2 (Table 4). The males of *J. (T.) notata* and *J. (J.) globulariae* reacted to the lures from 2 to 5 a.m. while the males *A. (T.) mannii* were attracted from 6 to 7 p.m. In our further field observations, most of *A. (T.) mannii* males (27 from 33) were attracted between 3 and 7 p.m. This fact does not contradict the results received in the described experiment. Thus, the sexual activity of *A. (T.) mannii* is high late in the afternoon. The peak of the searching behaviour of *J. (T.) notata* and *J. (J.) globulariae* occurs at night before sunrise.

Table 4.– The diurnal rhythms of sexual activity of the three Procridinae species studied with the help of the attractant EFETOV-S-2 in province Pordenone on 08-09-VI-2015.

Time of observations	<i>Jordanita notata</i>	<i>Jordanita globulariae</i>	<i>Adscita mannii</i>
08:30-09:00	2 ♂♂ and 1 ♀ flying in the meadow	–	3 ♂♂ resting on the grass
10:30-11:00	2 ♂♂ flying in the meadow	–	6 ♂♂ sitting and flying in the meadow
12:30-13:00	–	–	1 ♂ flying in the meadow
14:30-15:00	–	–	–
16:30-17:00	–	–	–
18:30-19:00	1 ♀ sitting on the grass	–	12 ♂♂ attracted
20:30-21:00	–	–	–
22:30-23:00	–	–	–
00:30-01:00	–	–	–
02:30-03:00	6 ♂♂ attracted	1 ♂ attracted	–
04:30-05:00	1 ♂ attracted	3 ♂♂ attracted	–
06:30-07:00	–	–	Ca 20 (♂♂ and ♀♀) sitting on the grass

Conclusions

The males of three genera of Procrinae were attracted to the lures from the series “EFETOV-2”, viz. *Rhagades* Wallengren, 1863 (one species), *Adscita* Retzius, 1783 (six species), and *Jordanita* Verity, 1946 (four species).

The synthetic sex attractants for four Zygaenidae species, viz. *A. (A.) italica*, *A. (A.) alpina*, *A. (A.) dujardini*, and *J. (J.) tenuicornis* (the latter is endemic to Italy), were found for the first time. These attractants are (2*R*)-butyl 2-dodecenoate and (2*S*)-butyl 2-dodecenoate alone or in a mixture.

New localities of the Italian Procrinae species were found, including the hybridisation zone between *A. (A.) italica* and *A. (A.) alpina*.

The presumed extinct species *Rh. (Rh.) pruni* was rediscovered after more than 100 years in Piemonte.

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Table 1.– Localities monitored by the sex attractants from the series "EFETOV-2" in 2015-2018 in Italy. Lures placed in traps are marked with an asterisk.

Region / Province / Locality	Code	GPS coordinates	Altitude	Habitat	Dates	Type of lures
Piemonte / Torino / Alpi Cozie, Valle di Susa, Bardonecchia SW, Pian del Colle	TO1	E 06°40'16" N 45°03'02"	1450 m	Historical dry flowery mountain meadow on terraces on the edge of the forest	09-VII-2016	EFETOV-S-2
Piemonte / Torino / Alpi Cozie, Valle di Susa, Eclause	TO2	E 06°53'36" N 45°05'10"	1385 m	Dry flowery meadows on the edge of bushes, rocky places and stone walls	09-VII-2016	EFETOV-S-2 EFETOV-S-S-2
Piemonte / Torino / Alpi Cozie, Valle di Susa, Femils (NE Salbertrand)	TO3	E 06°54'18" N 45°05'03"	1125 m	Deciduous forest with clearings	08, 09-VII-2016	EFETOV-S-2 EFETOV-S-S-2
Piemonte / Cuneo / Valle Tanaro, Bagnasco W, road to Battifoglio	CN	E 08°01'31" N 44°19'00"	766 m	Dry flowery meadows on the edge of the forest	31-V-2016 04, 06-VII-2016	EFETOV-S-2 EFETOV-S-S-2
Liguria / Imperia / Passo della Colletta	IM1	E 07°43'33" N 44°06'44"	1625 m	Mountain meadows East of the pass with rich vegetation	06-VII-2016	EFETOV-S-2
Liguria / Imperia / Colle Langan	IM2	E 07°43'36" N 43°58'06"	1080-1090 m	Flowery mountain meadows (large complex) in terraces	05-VII-2016	EFETOV-S-S-2
Liguria / Imperia / Monte Ceppo	IM3	E 07°45'38" N 43°56'03"	1470-1480 m	Flowery mountain meadow, rocky vegetation, and bushes	05-VII-2016 22-VI-2017	EFETOV-S-2 EFETOV-S-S-2
Liguria / Imperia / Drego S	IM4	E 07°48'31" N 43°59'37"	1060-1080 m	Dry mountain steppe, meadows, rocky vegetation, and bushes	30-V-2016 04-VII-2016 24-VI-2017	EFETOV-2 EFETOV-S-2 EFETOV-S-S-2
Liguria / Savona / Bormida valley, Callizzano, S of Camping Laghetti	SV1	E 08°07'13" N 44°14'11"	660 m	Meadow north of road	02-VI-2017	EFETOV-S-2
Liguria / Savona / Piampeludo S	SV2	E 08°34'55" N 44°27'44"	868 m	Flowery meadow with dry and moist parts	01-VI-2017	EFETOV-2 EFETOV-S-2 EFETOV-S-S-2
Emilia-Romagna / Parma / Viazzano E	PR	E 10°04'28" N 44°41'36"	170-180 m	Grassy slope with dry meadows on the edge of the forest	01-VI-2016	EFETOV-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Sessvenna, Taufers, Avignatal, Trockenhang	BZ1	E 10°26'37" N 46°38'41"	1450 m	Dry undisturbed steppe slope	16-VII-2016	EFETOV-S-2 EFETOV-S-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Sessvenna, Plantapatsch (Bergstation Lift)	BZ2	E 10°29'37" N 46°42'37"	2120 m	Open grassy area at the border of the forest	22-VII-2015	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Sessvenna, Prämajur W, Ochsenberg	BZ3	E 10°30'07" N 46°42'18"	1900 m	Roadside in the forest	22-VII-2015	EFETOV-S-2

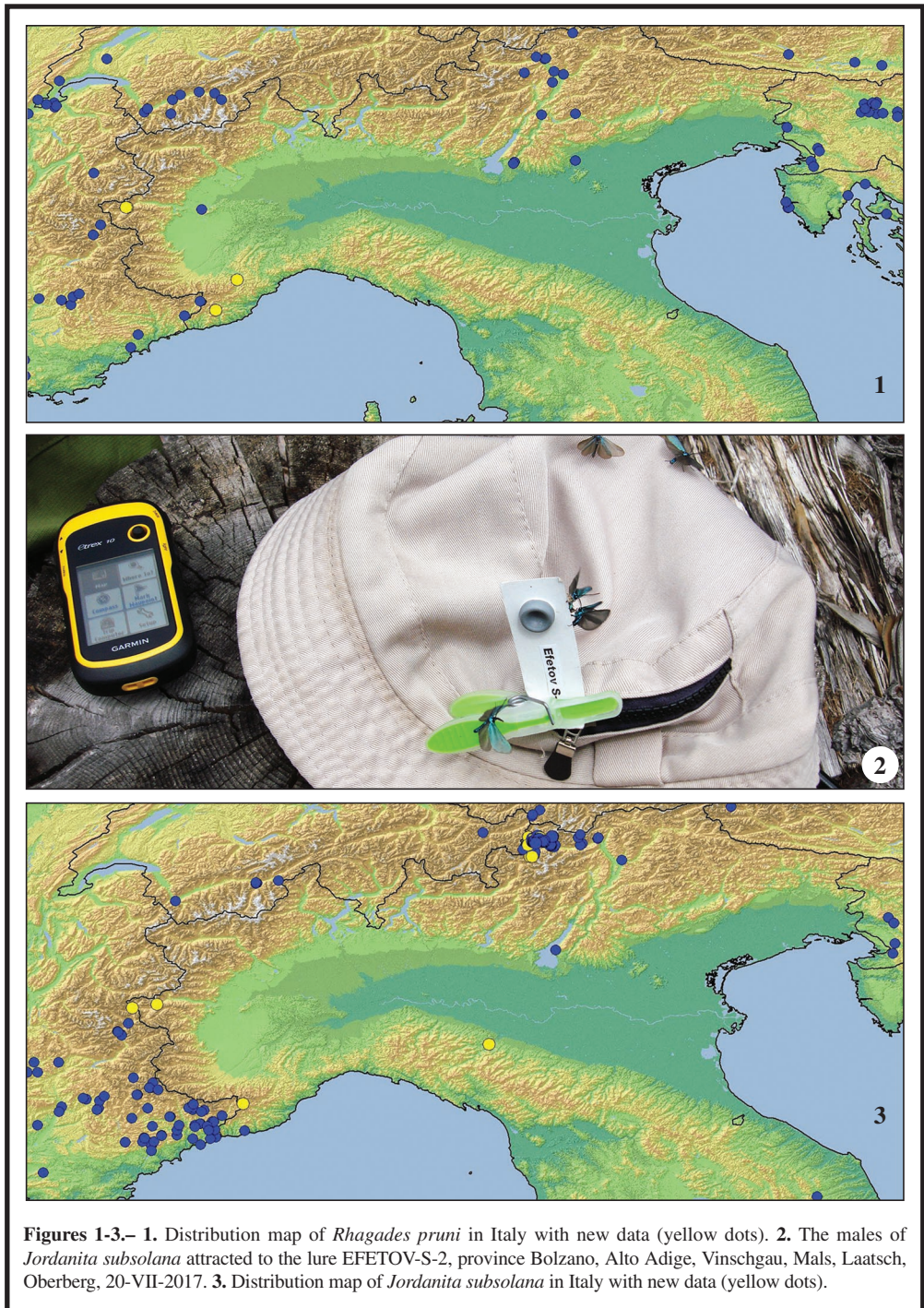
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Sesvenna, Schleiserleiten W, Verschleis	BZ4	E 10°30'54" N 46°41'48"	1320 m	Sleep SW exposed steppe slope with rich vegetation on the edge of the forest	01-VII-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch W, Calvaweide NEU	BZ5	E 10°30'13" N 46°40'02"	1010-1050 m	Newly created pasture due to road building activities with secondary vegetation	06-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch SW, Arzweg, Pasargwald SW	BZ6	E 10°31'20" N 46°39'52"	1080 m	Clearing in the forest	01-VIII-2018	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Eiselweg, Trockenhang	BZ7	E 10°31'21" N 46°40'41"	1340-1415 m	Steep slope with rich steppe vegetation on the edge of the forest	04-VIII-2016 13, 21-VII-2017 22-VI-2018	EFETOV-S-2 EFETOV-S-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Eiselweg, Schafhütte	BZ8	E 10°30'36" N 46°40'29"	1420-1440 m	Rocky steep steppe with rich vegetation on the edge of the forest	13-VII-2017 22-VI-2018	EFETOV-S-2 EFETOV-S-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Oberberg	BZ9	E 10°30'31" N 46°40'38"	1635 m	Forestry trail in the forest with meadows with diverse vegetation	20-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Terzaboden	BZ10	E 10°30'27" N 46°40'48"	1790 m	Pasture with fairly poor diversity	20-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Plovadeng	BZ11	E 10°27'48" N 46°40'48"	2015 m	Meadow with fairly diverse vegetation	20-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Laatsch, Terza S	BZ12	E 10°29'26" N 46°40'44"	2145 m	Meadow with fairly diverse vegetation	20-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Malser Heide N, Burgeiser Marein N	BZ13	E 10°32'27" N 46°44'10"	1495 m	Flowerly meadows	02-VIII-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, "Hoache" (Hochacker)	BZ14	E 10°33'18" N 46°41'26"	1156-1175 m	Flowerly meadows above town of Mals	09, 10-VI-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Örtzaler Alpen, Sankt Martin	BZ15	E 10°32'50" N 46°42'12"	1450 m	Flowerly meadows above the chapel in the forest	27-VII-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Örtzaler Alpen, Griggles	BZ16	E 10°33'13" N 46°42'24"	1500 m	Flowerly moist meadows in the forest	27-VII-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Örtzaler Alpen, Malettes W, Mitterhütt	BZ17	E 10°33'19" N 46°41'57"	1540 m	Flowerly meadows	27-VII-2016	EFETOV-S-2 EFETOV-S-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Örtzaler Alpen, Malettes, Mitterhütt SE	BZ18	E 10°33'26" N 46°41'52"	1545 m	Heavily overgrazed steep "meadow" between rocks and places with rocky soil	13-VII-2015	EFETOV-2 EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Örtzaler Alpen, Malettes NE, Hegefläche	BZ19	E 10°33'19" N 46°42'12"	1600 m	Flowerly meadows	27-VII-2016	EFETOV-S-2

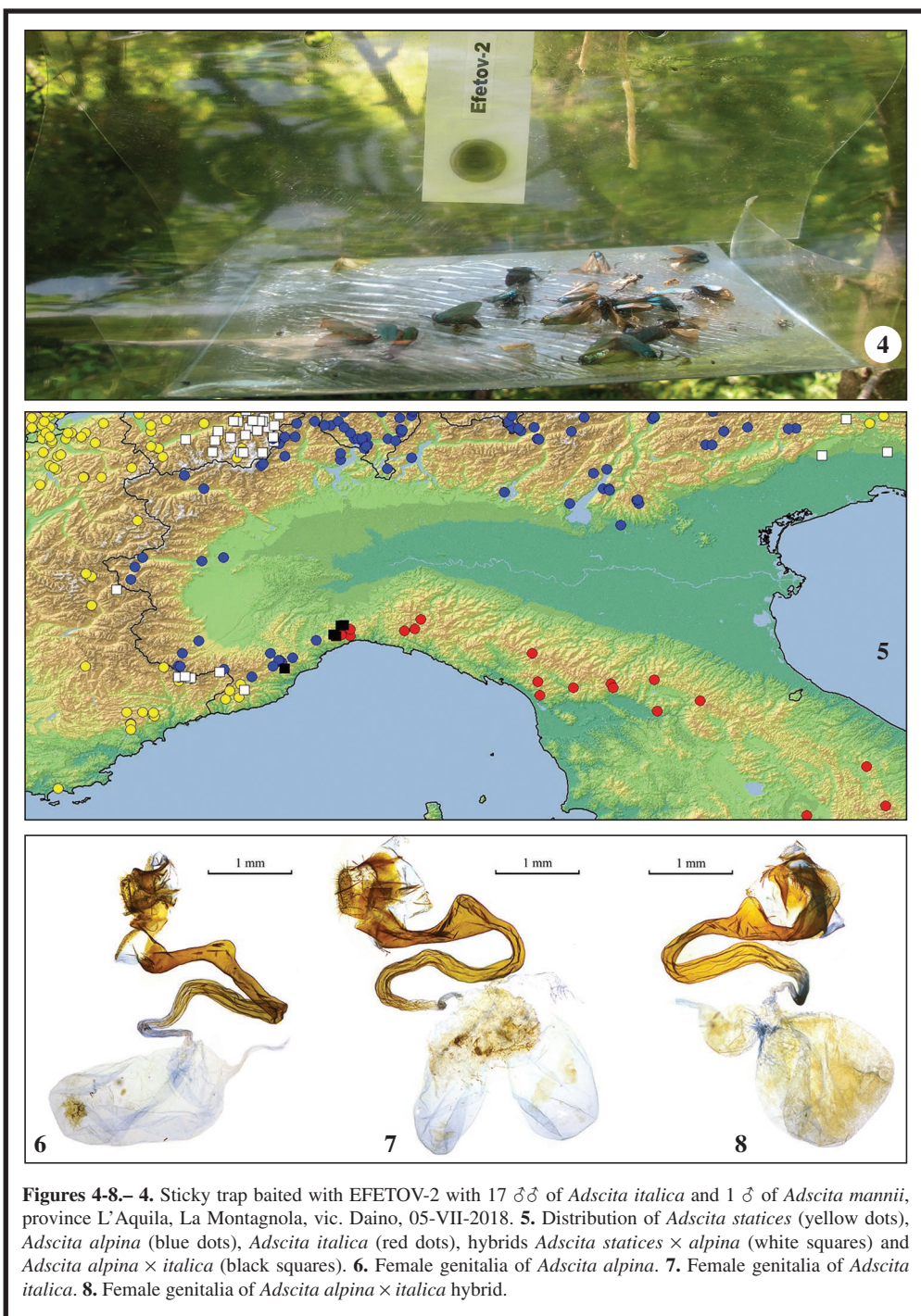
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Malettes E, Raweingraben	BZ20	E 10°34'09" N 46°41'34"	1490 m	Open meadows with rich vegetation in the forest	13-VII-2015	EFETOV-2 EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Tartsch NE, Rauscheck-Graben	BZ21	E 10°34'10" N 46°41'13"	1222 m	Valley with flowery meadows with dry and moist parts	10-VI-2016	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Tartsch, Rawein Trockenhang	BZ22	E 10°35'57" N 46°40'48"	1485 m	Dry steppe slope on the edge of the forest with bushes	12-VII-2017	EFETOV-S-2 EFETOV-S-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Tartsch, Spätweisen	BZ23	E 10°35'23" N 46°40'54"	1565 m	Pasture on the edge of the forest, dry meadows	12-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Mals, Tartscher Wald NE	BZ24	E 10°36'04" N 46°40'58"	1610-1612m	Forest with dry meadows in clearings	12-VII-2017	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Matschertal, Matscher Walweg, Valferz ESE	BZ25	E 10°38'33" N 46°42'41"	1715 m	Flowery band along the old irrigation channels on the edge of dry mountain meadows with rocks	13-VII-2015	EFETOV-2 EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Matscher Tal, Rasifiz ENE	BZ26	E 10°39'43" N 46°43'08"	1706 m	Flowery meadows beside the river	01-VII-2016 21-VI-2018	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Matscher Tal, Außer Gieshof, Brücke	BZ27	E 10°40'42" N 46°43'21"	1788 m	Flowery meadow beside the river	21-VI-2018	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Matscher Tal, Gies N, Tawarill	BZ28	E 10°40'57" N 46°43'38"	1825 m	Open meadows in the forest	14-VII-2015	EFETOV-2 EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Matscher Tal, Gies N, Tawarill	BZ29	E 10°41'06" N 46°43'50"	1900-2020 m	Steep slope with dry forest	14-VII-2015	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Ortlergruppe, Stilsferjochstraße, Franzenshöhe NW, between U-turn 16 and 17	BZ30	E 10°28'27" N 46°32'01"	2330 m	Flowery meadows with rich vegetation above the road along a small creek	23-VII-2015	EFETOV-2 EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Vinschgau, Ortlergruppe, Stilsferjochstraße, between U-turn 30	BZ31	E 10°29'16" N 46°31'55"	2000 m	Bend of the road with a parking place near the forest with flowery meadows	23-VII-2015	EFETOV-S-2
Trentino-Alto Adige / Bolzano / Alto Adige, Burg Sprechstein SE	BZ32	E 11°54'30" N 45°53'23"	1320-1350 m	Flowery meadow	28-VI-2015	EFETOV-S-2
Friuli-Venezia Giulia / Portenone / Magredi di Cellina, Vivaro S	PN	E 12°46'12" N 46°03'23"	120 m	Large plains with steppe vegetation mixed with dry and wet meadows	08, 09-VI-2015	EFETOV-S-2
Friuli-Venezia Giulia / Udine / Val Resia, Resia San Giorgio W	UD	E 13°16'43" N 46°22'37"	390 m	Flowery meadow at the roadside near the forest	24-VI-2015	EFETOV-S-2

Marche / Macerata / Monte San Vicino W, ca. 3 km SW Pian dell'Elmo	MC	E 13°03'21" N 43°19'36"	1180-1190 m	Flowerly mountain meadows	16-18-VI-2015	*EFETOV-2 *EFETOV-S-2 EFETOV-S-2
Abruzzo / L'Aquila / Sperone, 2 km S	AQ1	E 13°43'39" N 41°55'47"	1180-1190 m	Flowerly meadows with bushes and rich vegetation	16, 17-VI-2015	*EFETOV-2 *EFETOV-S-2 EFETOV-S-2
Abruzzo / L'Aquila / La Montagnola, vic. Daino	AQ2	E 13°56'11" N 41°48'09"	1362-1365 m	Bushes on rocky calcareous ground and dry grassy-flowerly open vegetation	28-VI-5-VII-2018	*EFETOV-2 *EFETOV-S-S-2 EFETOV-S-S-2
Calabria / Cosenza / La Sila Graeca, La Fossiatà E	CS1	E 16°36'55" N 39°23'36"	1316-1334 m	Bushes and wet and dry grassy- flowerly open vegetation beside small river	01-VII-2018	*EFETOV-2 *EFETOV-S-S-2 EFETOV-S-2
Calabria / Cosenza / La Sila Grande, Monte Botte Donato E	CS2	E 16°26'18" N 39°17'52"	1670-1695 m	Bushes and dry grassy-flowerly open vegetation	02, 03-VII-2018	EFETOV-2 EFETOV-S-2 EFETOV-S-S-2

Table 2.– The males of Proctridinae species attracted to the sex attractants from the series "EFETOV-2" in Italy in 2015-2018. Specimens collected by baited traps are marked with an asterisk.

Attracted species	EFETOV-2	EFETOV-S-2	EFETOV-S-S-2
<i>Rhagades pruni</i>	–	–	2016: 5 ♂♂ TO2, 3 ♂♂ TO3, 9 ♂♂ CN 2017: 1 ♂ IM4
<i>Adscita statices</i>	–	2016: 3 ♂♂ IM3 2017: 1 ♂ IM3	–
<i>Adscita statices</i> × <i>alpina</i>	–	2016: 4 ♂♂ IM4 (30-V), 1 ♂ IM4 (04-VII)	–
<i>Adscita alpina</i>	2015: 2 ♂♂ BZ25, 7 ♂♂ BZ30	2015: 2 ♂♂ BZ25, 9 ♂♂ BZ30 2016: 1 ♂ CN (31-V), 7 ♂♂ BZ14, 11 ♂♂ BZ21, 1 ♂ BZ26 2017: 2 ♂♂ SV1, 1 ♂ BZ5, 1 ♂ BZ8 2018: 2 ♂♂ BZ26, 3 ♂♂ BZ27	–
<i>Adscita alpina</i> × <i>italica</i>	2017: 7 ♂♂ SV2	–	–
<i>Adscita italica</i>	2018: 17* ♂♂ AQ2	2015: 1 ♂ AQ1 2018: 9 ♂♂ CS1, 11 ♂♂ CS2	2018: 17 ♂♂ AQ2
<i>Adscita geryon</i>	–	2015: 2 ♂♂ BZ25, 1 ♂ BZ29, 1 ♂ AQ1	–
<i>Adscita dujardini</i>	2015: 1* ♂ MC	2015: 1* ♂ MC	–
<i>Adscita mannii</i>	2018: 1* ♂ + 1 ♂ AQ2, 17 ♂♂ CS2	2015: 5 ♂♂ BZ32, 12 ♂♂ PN, 1 ♂ UD, 1 ♂ AQ1	2016: 1 ♂ IM2, 2 ♂♂ IM4 (04-VII) 2018: 2 ♂♂ CS2, 3 ♂♂ AQ2
<i>Jordanita notata</i>	–	2015: 1* ♂ MC, 7 ♂♂ PN	2016: 2 ♂♂ IM2 2018: 1 ♂ AQ2
<i>Jordanita tenuicornis</i>	–	2015: 1 ♂ AQ1	2018: 3 ♂♂ AQ2
<i>Jordanita globulariae</i>	–	2015: 4 ♂♂ PN	–
<i>Jordanita subsolana</i>	–	2015: 2 ♂♂ BZ2, 1 ♂ BZ3, 8 ♂♂ BZ18, 6 ♂♂ BZ20, 19 ♂♂ BZ28, 63 ♂♂ BZ29, 7 ♂♂ BZ31, 1 ♂ AQ1 2016: 1 ♂ TO1, 10 ♂♂ TO3, 2 ♂♂ IM1, 1 ♂ PR, 1 ♂ BZ1, 1 ♂ BZ4, 11 ♂♂ BZ7, 2 ♂♂ BZ13, 5 ♂♂ BZ15, 18 ♂♂ BZ16, 40 ♂♂ BZ17, 2 ♂♂ BZ19 2017: 20 ♂♂ BZ7 (13-VII), 7 ♂♂ BZ7 (21-VII), 69 ♂♂ BZ8, 100 ♂♂ BZ9, 2 ♂♂ BZ10, 6 ♂♂ BZ11, 1 ♂ BZ12, 1 ♂ BZ22, 1 ♂ BZ23, 4 ♂♂ BZ24 2018: 1 ♂ BZ7, 1 ♂ BZ8, 2 ♂♂ BZ6	–





Figures 4-8.— 4. Sticky trap baited with EFETOV-2 with 17 ♂♂ of *Adscita italica* and 1 ♂ of *Adscita mannii*, province L'Aquila, La Montagnola, vic. Daino, 05-VII-2018. 5. Distribution of *Adscita statice* (yellow dots), *Adscita alpina* (blue dots), *Adscita italica* (red dots), hybrids *Adscita statice* × *alpina* (white squares) and *Adscita alpina* × *italica* (black squares). 6. Female genitalia of *Adscita alpina*. 7. Female genitalia of *Adscita italica*. 8. Female genitalia of *Adscita alpina* × *italica* hybrid.