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Application of two molecular approaches (use of sex attractants and DNA barcoding) allowed to rediscover *Zygaenoprocris eberti* (Alberti, 1968) (Lepidoptera, Zygaenidae, Procridinae), hitherto known only from the female holotype

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Abstract. The hitherto unknown males of *Zygaenoprocris* (*Zygaenoprocris*) *eberti* (Alberti, 1968) were attracted by sex pheromones in Afghanistan. Conspecificity with the female holotype was confirmed by using DNA barcoding. A description of the male and data on the habitat of this species are provided. A key to the subgenera of the genus *Zygaenoprocris* Hampson, 1900, and the species of the subgenus *Zygaenoprocris* Hampson, 1900, is given.

Introduction

The subfamily Procridinae (Lepidoptera, Zygaenidae) includes some genera with externally very similar species, e.g. *Fuscartona* Efetov & Tarmann, 2012 (Efetov 1997a), *Chrysartona* Swinhoe, 1892 (Efetov 2006), *Illiberis* Walker, 1854 (Efetov 1997b; Efetov et al. 2004), *Hedina* Alberti, 1954 (Efetov 1997b; Efetov 2010; Efetov and Tarmann 2012), *Goe* Hampson, 1893 (Efetov 1998), *Adscita* Retzius, 1753 (Efetov 2001b; Efetov and Tarmann 2012), *Jordanita* Verity, 1946 (Efetov 2001b; Efetov and Tarmann 2012) etc. Species of the mentioned genera can often be identified only by examination of genitalic structures (Efetov and Tarmann 1999), chaetotaxy of the first instar larvae (Efetov et al. 2006; Efetov and Hayashi 2008), karyotypes (Efetov 2004; Efetov et al. 2004) or DNA analysis. Some species are known only from the type specimens and sometimes only one sex is known. The identification of material of the other sex needs to be verified by molecular methods. One such genus is *Zygaenoprocris* Hampson, 1900 (Efetov and Tarmann 1994; Efetov 1996; 2001a), which is currently represented by 13 species (Efetov and Tarmann 2012).

Hitherto *Zygaenoprocris eberti* (Alberti, 1968) was known only from the holotype, a female labelled 'Z.-Afghanistan, Koh-i-Baba, S-Seite Shahtu-Pass, 3000 m, 17.–19.7.1966, G. EBERT leg.' and collected on the south side of the pass known as Kotal-e Shahtu that crosses the Koh-i

Baba main chain between Panjao and Yakolang (Figs 4, 10). The discovery of the males of this species became possible by the use of two molecular methods: attraction by sex pheromones followed by confirmation of conspecificity of the collected males with the female holotype by DNA barcoding.

Two esters of fatty acids, 2-butyl (7*Z*)-dodecenoate and 2-butyl (9*Z*)-tetradecenoate, were found in the female pheromone glands of *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907 and both (*R*)- and (*S*)-enantiomers of each compound were synthesized (Subchev et al. 2010). These compounds and their mixtures were screened in the field and proved to be sex attractants for different species of the genera *Illiberis* Walker, 1854 (Subchev et al. 2012; 2013), *Zygaenoprocris* Hampson, 1900 (Efetov et al. 2011), *Adscita* Retzius, 1783, and *Jordanita* Verity, 1946 (Efetov et al. 2010; Subchev et al. 2010).

Methods

During an expedition to Afghanistan in 2011, Axel Hofmann, using baits containing the above-mentioned female sex attractants and their mixtures, collected a series of males of *Zygaenoprocris eberti* in the vicinity of the lakes of Band-i Amir (*ca.* 40 km north-east of the type-locality) in the central Koh-i Baba in Hazarajat in Afghanistan (Efetov et al. 2012). Male specimens were attracted to (2*R*)-butyl (7*Z*)-dodecenoate and the mixture of (2*R*)-butyl (7*Z*)-dodecenoate and (2*R*)-butyl (9*Z*)-tetradecenoate (Fig. 2). The pin-label data are as follows: "Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, Canyon, 3130–3280 m, 5.VII. 2011, [15/11], leg. A. HOFMANN". At this locality *Z. eberti* was syntopic with *Z. chalcochlora* Hampson, 1900 (Figs 3, 10). Amongst the 38 collected males 37 were *Z. eberti* and only one male was *Z. chalcochlora*. In other localities in Afghanistan which were visited during the same collecting tour by A. Hofmann in 2011, only *Z. chalcochlora* could be found. Males of the latter species were seen resting (Fig. 9) and actively flying but a clear preference to any of the presented sex attractants could not be verified.

DNA barcodes were obtained by sampling legs from dry specimens. Legs were prepared in the Department of Biological Chemistry of the Crimean State Medical University (Simferopol). All specimens were identified by K. A. Efetov & G. M. Tarmann. PCR amplification and DNA sequencing were performed at the Canadian Centre for DNA Barcoding following standard high-throughput protocols (which can be accessed at http://www.dnabarcoding.ca/pa/ge/research/protocols), where all obtained DNA extracts are stored now. All sequences were deposited in GenBank according to the iBOL data release policy. Complete specimen data (images, voucher deposition, geographic coordinates, sequence and trace files) can easily be accessed in the BOLD in public project ZYGMO [http://www.boldsystems.org/index.php/MAS_Management_OpenProject?code=ZYGMO]. Sequence divergences for the barcode region were calculated using the Kimura 2 Parameter model by the analytical tools on BOLD.

Results

The conspecificity of the collected males with the holotype of *Zygaenorpocris eberti* was confirmed by the analysis of the DNA barcode, 658-bp region of the cytochrome *c* oxidase I mitochondrial gene (Fig. 5). The treeless habitat at Band-i Amir, Jarkushan N, Hazarajat, 3130–3280

m (Fig. 3), was dominated by *Acantholimon* (Plumbaginaceae), *Cousinia*, *Artemisia* (Asteraceae) and *Astragalus* (Fabaceae) species. As we know from other *Zygaenoprocris* (*Zygaenoprocris*) species, *Acantholimon* and *Cousinia* may be the larval host-plants for the studied species. We provide below a description of the hitherto unknown male of *Z. eberti*.

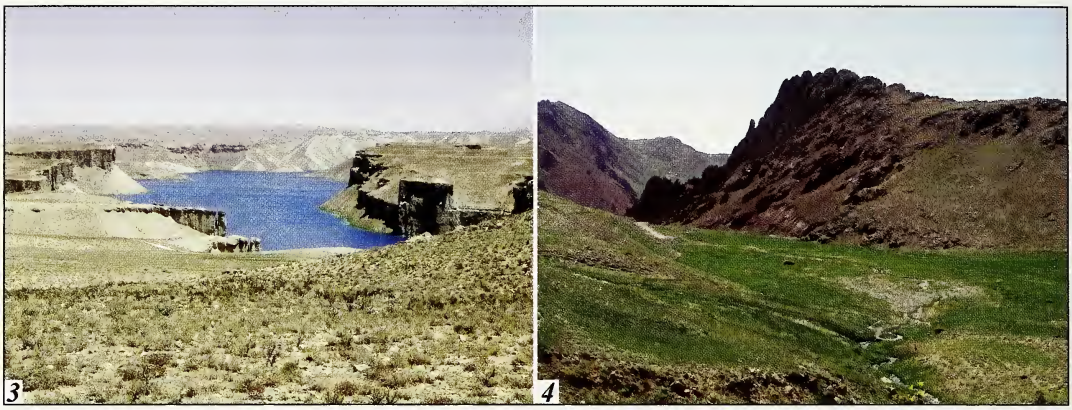
Zygaenoprocris eberti (Alberti, 1968)

Description of male (Fig. 1). Length of body: 5.8–6.5 mm; length of forewing: 8.6–8.9 mm, width: 3.3–3.4 mm; length of antenna: 4.6–5.0 mm. Frons and occiput green with submetallic sheen. Antenna strongly clubbed, thickly covered with shining scales, bipectinate, length of pectination in middle part of antenna 0.7 mm, last segments of antenna with pectination reduced, antennal shaft strongly thickened distally, ratio of width of 4th segment from apex to width of 15th segment is 4. Proboscis well developed, yellow. Tegulae and patagia green with submetallic sheen. Thorax thickly covered with green shiny scales. Forewing upperside bright green with submetallic sheen, thickly covered with shiny scales; underside of forewing grey; fringe grey. Hindwing upper- and underside grey, fringe concolorous. Legs green, thickly covered with shiny scales, foreleg with long tibial epiphysis, hind tibia with one pair of spurs (apical). Abdomen greenish black, thickly covered with shiny scales. The long black hair covers the head, labial palpi, thorax, legs and abdomen.

Male genitalia (Figs 6, 8). Uncus heavily sclerotized, nearly equal in length to tegumen. Valva without any process. Juxta long, 1.5 times longer than uncus. Phallus slightly curved, long, approximately 3 times longer than uncus, with one long straight cornutus, its length 0.5 times length of phallus, distal part of cornutus very slender, with pointed apex.

Differential diagnosis. *Zygaenoprocris eberti* is syntopic and synchronous with *Zygaenoprocris* (*Zygaenoprocris*) *chalcochlora* Hampson, 1900, the type species of the subgenus *Zygaenoprocris* Hampson, 1900. Externally these two species cannot be distinguished but both differ significantly in their genitalia morphology (Figs 6–8; Efetov and Tarmann 1999, figs 108, 166). In the male the phallus is longer in *Z. eberti* and the cornutus is large with a characteristic shape

Figures 1, 2. 1. Male of *Zygaenoprocris eberti* (Alberti, 1968). Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, 3130–3280 m, 5.vii.2011, leg. A. Hofmann. Photo S. Heim. **2.** Mixture of (2*R*)-butyl (7*Z*)-dodecenoate and (2*R*)-butyl (9*Z*)-tetradecenoate ('R12+R14') attracting two males of *Zygaenoprocris eberti* (Alberti, 1968). The two males were so sexually excited that they copulated with each other. Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, 3130–3280 m, 5.vii. 2011. Photo A. Hofmann.



Figures 3, 4. 3. Habitat of *Zygaenoprocris eberti* (Alberti, 1968), and *Zygaenoprocris chalcochlora* Hampson, 1900. Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, 3130–3280 m, 5.vii. 2011. Photo A. Hofmann. **4.** Near the type locality of *Zygaenoprocris eberti* (Alberti, 1968). Afghanistan, Prov. Bamiyan, Koh-i Baba, Shahtu pass, 3000 m. The exact type locality is not known. Photo A. Hofmann.

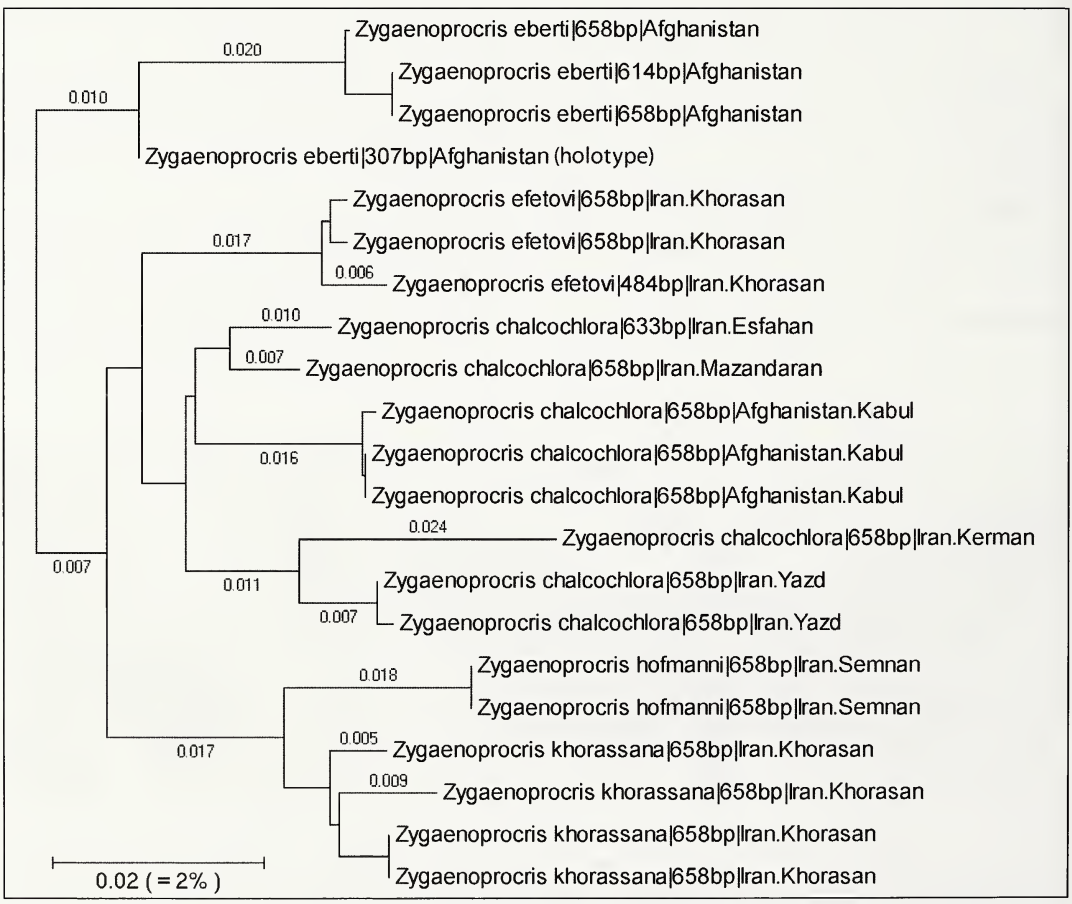


Figure 5. Neighbour-joining tree (K2P) of the DNA barcodes for the different *Zygaenoprocris* (*Zygaenoprocris*) species.

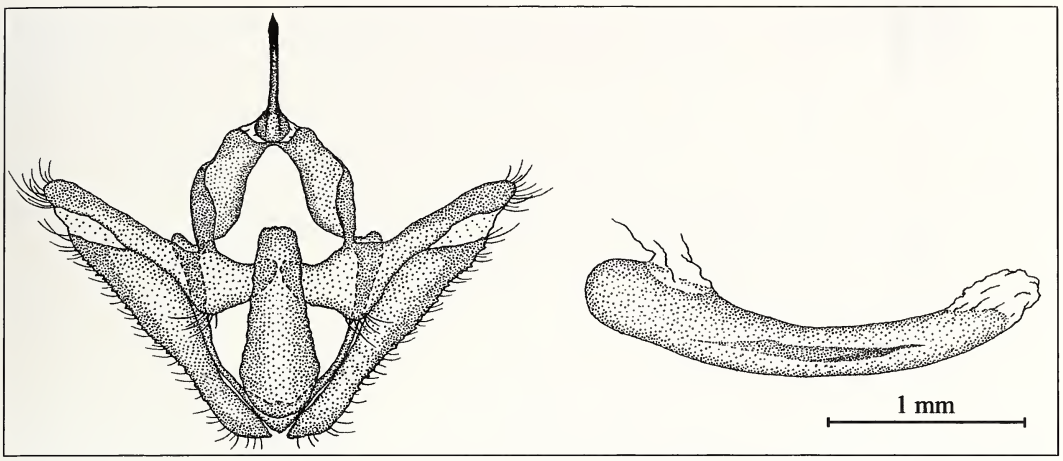


Figure 6. Male genitalia of *Zygaenoprocris eberti*. Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, 3130-3280 m, 5.vii. 2011, leg. A. Hofmann.

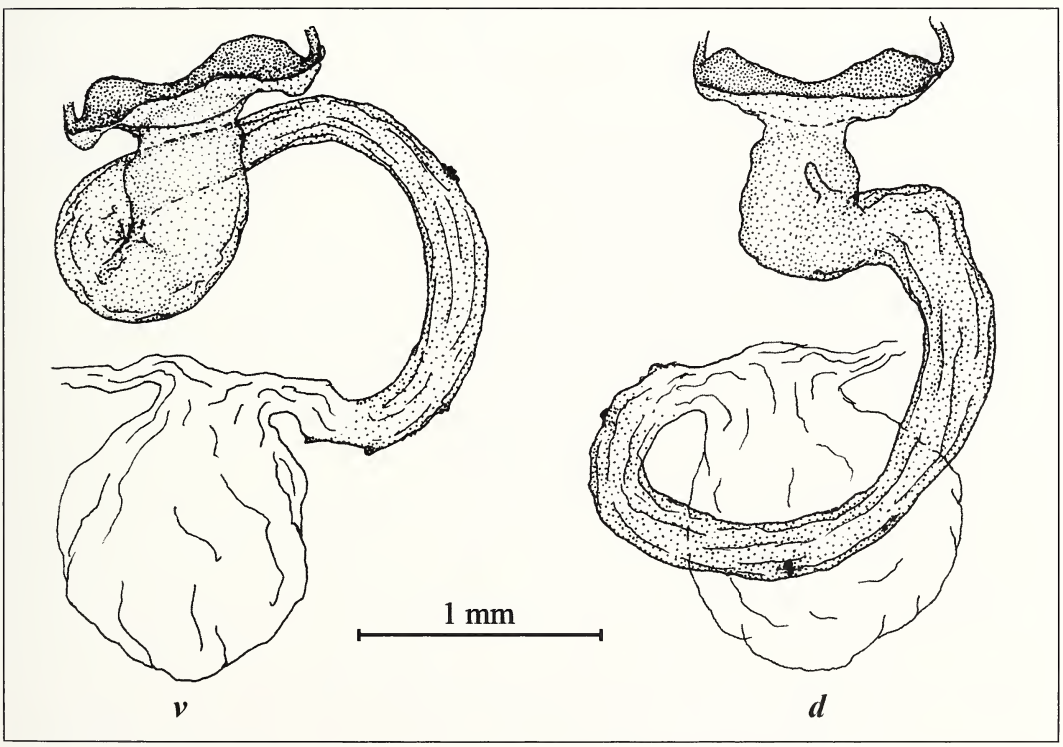
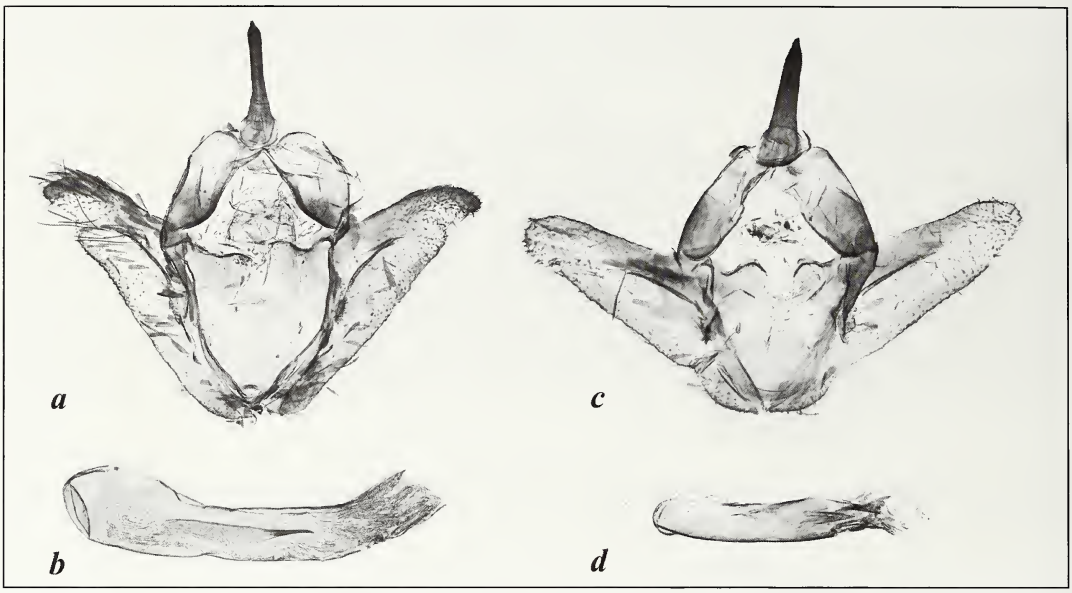


Figure 7. Female genitalia of the holotype of *Procris eberti* Alberti, 1968 (= *Zygaenoprocris eberti*), *v* – ventral view, *d* – dorsal view. 'Z.-Afghanistan, Koh-i-Baba, S-Seite Shahtu-Pass, 3000 m, 17.–19.7.1966, G. EBERT leg.'

(with broad basal part and ending somewhat abruptly in a pointed tip distally). The female of *Z. eberti* has long, broad and strongly curved (twisted) ductus bursae, whereas *Z. chalcochlora* has short, narrow and straight ductus bursae.



Figures 8a–d. a, b. Male genitalia of *Zygaenoprocris eberti*. Afghanistan, Prov. Bamiyan, Band-i Amir, Jarkushan N, 3130–3280 m, 5.vii. 2011, leg. A. Hofmann (Prep. GMT Z 3562). **a**, Uncus, tegumen, vinculum, saccus, valvae; **b**, Phallus (Photo S. Heim). **c**, **d**. Male genitalia of *Zygaenoprocris chalcochlora*. Afghanistan, Prov. Kabul, Kabul N, Darreh Estalif, 1880–2200 m, 21. vi. 2011, leg. A. Hofmann (Prep. GMT Z 3564). **c**, Uncus, tegumen, vinculum, saccus, valvae; **d**, Phallus (Photo S. Heim).

Remarks. As shown earlier, males of *Zygaenoprocris* (*Molletia*) *taftana* (Alberti, 1939), were attracted by (2*R*)-butyl (7*Z*)-dodecenoate (Efetov et al. 2011). Males of *Zygaenoprocris* (*Zygaenoprocris*) *eberti* were attracted by (2*R*)-butyl (7*Z*)-dodecenoate ('R12') as well as by the mixture of (2*R*)-butyl (7*Z*)-dodecenoate and (2*R*)-butyl (9*Z*)-tetradecenoate ('R12+R14') (Fig. 2). Of the 38 males that were collected at both attractants 28 were taken around or on the R12 pheromone baits and 10 around or on the baits with the mixture of R12+R14. Of the 13 dissected specimens taken at R12, 12 were *Z. eberti* and only one *Z. chalcochlora*. All 10 specimens attracted to R12+R14 were *Z. eberti*. The collecting time was between 13.15–14.15 hours.

Key to the subgenera of the genus *Zygaenoprocris* Hampson, 1900

1 Valva extremely narrow (Efetov and Tarmann 1999: figs 113–115; Efetov 2001a: fig. 11) ..... subgenus ***Molletia*** Efetov, 2001a

– Valva broad (Efetov and Tarmann 1999: figs 108–112; Efetov 2001a: figs 10, 12)..... 2

2 Apex of sacculus with triangular, pointed process (Efetov and Tarmann 1999: figs 111, 112; Efetov 2001a: fig. 12), ductus bursae with sclerotized spines (Efetov and Tarmann 1999: figs 169–171; Efetov 2001a: fig. 15)..... subgenus ***Keilia*** Efetov, 2001a

Figure 9. Male of *Zygaenoprocris chalcochlora* Hampson, 1900, resting on *Artemisia*. Afghanistan, Prov. Baghlan, Salang pass, north side, Do Shagh N, Chahar Maghzak vic., 2180–2250 m, 22.vi. 2011. Photo A. Hofmann.

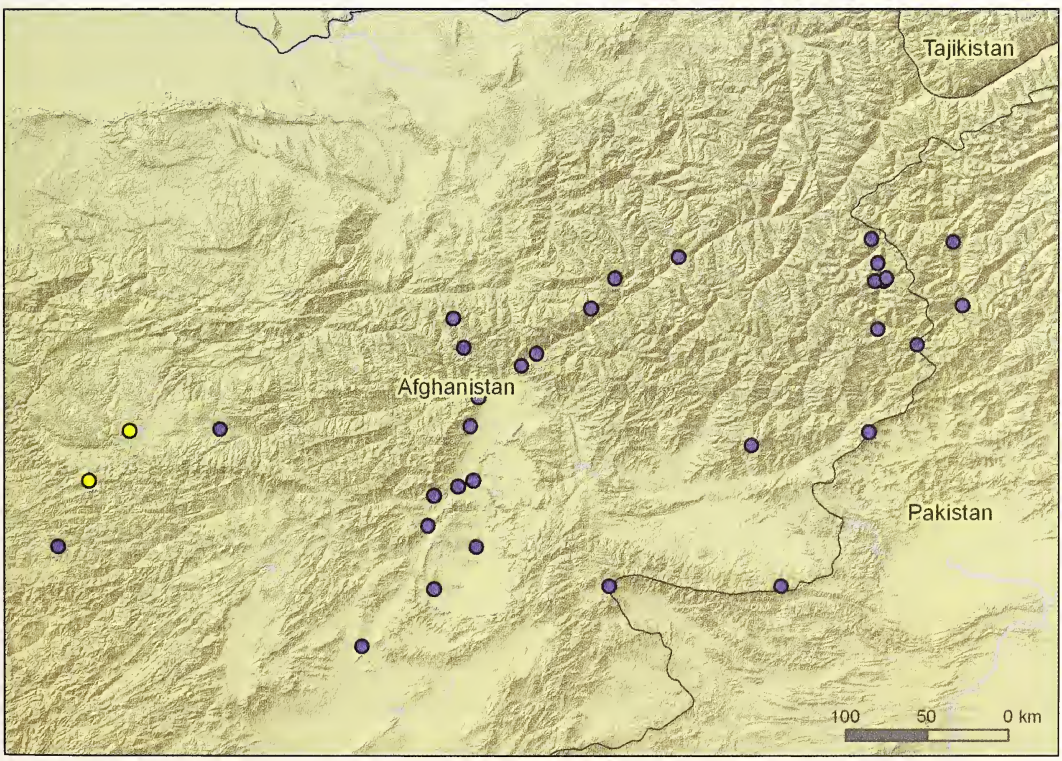


Figure 10. Distribution map of *Zygaenoprocris eberti* and *Z. chalcochlora* in Afghanistan. Blue dots: *Z. chalcochlora*, yellow dots: *Z. eberti* (at the northern locality of *Z. eberti* this species is syntopic with *Z. chalcochlora*).

– Apex of sacculus without triangular, pointed process (Efetov and Tarmann 1999: figs 108–110; Efetov 2001a: fig. 10), ductus bursae without sclerotized spines (Efetov and Tarmann 1999: figs 166–168; Efetov 2001a: fig. 13; Mollet 2003: fig. 2).....3

3 Distal end of phallus with two very characteristic lateral processes with sclerotized dentations apically (Efetov and Tarmann 1999: fig. 110), ductus bursae strongly dilated, forming a praebursa (Mollet 2003: fig. 2)..... subgenus ***Efetovia*** Mollet, 2001

– Distal end of phallus without lateral processes (Efetov and Tarmann 1999: figs 108, 109), ductus bursae not forming a praebursa (Efetov and Tarmann 1999: figs 166–168; Efetov 2001a: fig. 13).....subgenus ***Zygaenoprocris*** Hampson, 1900

Key to the species of the subgenus *Zygaenoprocris* Hampson, 1900

1 Forewing upperside green, bluish green or coppery, with submetallic sheen Figs 1, 9).....2

– Forewing upperside light brown, without submetallic sheen 5

2 Cornutus short, slightly sclerotized (Fig. 8d, Efetov and Tarmann 1994: figs 56–60; 1999: fig. 108; Efetov 2001a: fig. 10), ductus bursae not twisted (Efetov and Tarmann 1999: fig. 166; Efetov 2001a: fig. 13).....3

– Cornutus long, strongly sclerotized (Figs 6, 8b; Efetov and Tarmann 1999: fig. 109), ductus bursae twisted (Fig. 7; Efetov and Tarmann 1999: figs 167, 168).....4

3 Papillae anales narrow, apophyses posteriores long (Mollet and Tarmann 2007: fig. 10) .....***Z. (Z.) chalcochlora*** Hampson, 1900

– Papillae anales broad, apophyses posteriores short .....
.....***Z. (Z.) khorassana*** (Alberti, 1939)

4 Cornutus very long (2.5 times longer than uncus), juxta with sclerotized spines ventrally (Efetov 1996: figs 5, 6; Efetov and Tarmann 1999: figs 109, 109a), ductus bursae narrow, distal part of ductus bursae with smooth walls (Efetov 1996: fig.7; Efetov and Tarmann 1999: fig. 167)..... ***Z. (Z.) rjabovi*** (Alberti, 1938)

– Cornutus shorter (only 1.8 times longer than uncus), juxta without sclerotized spines (Fig. 6), ductus bursae broad, distal part of ductus bursae with folded walls (Fig. 7; Efetov 1996: figs 8, 9; Efetov and Tarmann 1999: fig 168).....***Z. (Z.) eberti*** (Alberti, 1968)

5 Cornutus 6 times shorter than phallus, apex of cornutus obtuse (Mollet and Tarmann 2007: fig. 6).....***Z. (Z.) hofmanni*** Mollet & Tarmann, 2007

– Cornutus only 3 times shorter than phallus, apex of cornutus pointed (Mollet and Tarmann 2007: fig. 5).....***Z. (Z.) efetovi*** Mollet & Tarmann, 2007

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References

Alberti B (1968) Zur Kenntnis des Genus *Procris* F. in Afghanistan (Lepidoptera, Zygaenidae). Reichenbachia 10: 249–253.

Efetov KA (1996) The description of the female of *Adscita* (*Zygaenoprocris*) *rjabovi* (Alberti, 1938) (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 47: 31–35.

Efetov KA (1997a) Two new species of the genus *Artona* Walker, 1854 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 48: 165–177.

Efetov KA (1997b) Three new species of the genus *Illiberis* Walker, 1854, from Taiwan and Vietnam (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 48: 231–244.

Efetov KA (1998) A revision of the genus *Goe* Hampson, [1893] (Lepidoptera: Zygaenidae, Procridinae), with descriptions of two new species. Entomologist's Gazette 49: 49–62.

Efetov KA (2001a) On the systematic position of *Zygaenoprocris* Hampson, 1900 (Lepidoptera: Zygaenidae, Procridinae) and the erection of two new subgenera. Entomologist's Gazette 52: 41–48.

Efetov KA (2001b) An annotated check-list of Forester moths (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 52: 153–162.

Efetov KA (2004) Forester and Burnet Moths (Lepidoptera: Zygaenidae). The genera *Theresimima* Strand, 1917, *Rhagades* Wallengren, 1863, *Zygaenoprocris* Hampson, 1900, *Adscita* Retzius, 1783, *Jordanita* Verity, 1946 (Procridinae), and *Zygaena* Fabricius, 1775 (Zygaeninae). CSMU Press, Simferopol, 272 pp.

Efetov KA (2006) Nine new species of the genus *Chrysartona* Swinhoe, 1892 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 57: 23–50.

Efetov KA (2010) *Illiberis* (*Hedina*) *louisi* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from China. Entomologist's Gazette 61: 235–241.

Efetov KA, Can F, Toshova TB, Subchev M (2010) New sex attractant for *Jordanita anatolica* (Naufock) (Lepidoptera: Zygaenidae: Procridinae). Acta zoologica bulgarica 62: 315–319.

Efetov KA, Hayashi E (2008) On the chaetotaxy of the first instar larva of *Artona martini* Efetov, 1997 (Lepidoptera: Zygaenidae, Procridinae, Artonini). Entomologist's Gazette 59: 101–104.

Efetov KA, Hofmann A, Tarmann GM (2012) The rediscovery of *Zygaenoprocris eberti* (Alberti, 1968) (Lepidoptera: Zygaenidae, Procridinae). XIII International Symposium on Zygaenidae, Innsbruck, 16–23 September 2012: 9–10.

Efetov KA, Parshkova EV, Koshio C (2004) The karyotype of *Illiberis* (*Primilliberis*) *rotundata* Jordan, [1907] (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 55: 167–170.

Efetov KA, Subchev MA, Toshova TB, Kiselev VM (2011) Attraction of *Zygaenoprocris taftana* (Alberti, 1939) and *Jordanita horni* (Alberti, 1937) (Lepidoptera: Zygaenidae, Procridinae) by synthetic sex pheromones in Armenia. Entomologist's Gazette 62: 113–121.

Efetov KA, Tarmann GM (1994) A revision of *Zygaenoprocris* Hampson, 1900, a subgenus of *Adscita* Retzius, 1783 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 45: 81–105.

Efetov KA, Tarmann GM (1999) Forester Moths. The genera *Theresimima* Strand, 1917, *Rhagades* Wallengren, 1863, *Jordanita* Verity, 1946, and *Adscita* Retzius, 1783 (Lepidoptera: Zygaenidae, Procridinae). Apollo Books, Stenstrup, 192 pp.

Efetov KA, Tarmann GM (2012) A checklist of the Palaearctic Procridinae (Lepidoptera: Zygaenidae). CSMU Press, Simferopol – Innsbruck, 108 pp.

Efetov KA, Tarmann GM, Hayashi E, Parshkova EV (2006) New data on the chaetotaxy of the first instar larvae of Procridini and Artonini (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 57: 229–233.

Mollet B (2003) The rediscovery of *Zygaenoprocris* (*Efetovia*) *fredi* (Alberti, 1939) and the description of the female (Lepidoptera: Zygaenidae, Procridinae). In: Efetov KA, Tremewan WG, Tarmann GM (Eds) Proceedings of the 7th International Symposium on Zygaenidae (Lepidoptera) (Innsbruck, Austria, September 2000). CSMU Press, Simferopol, 103–108.

Mollet B, Tarmann GM (2007) Two new species of *Zygaenoprocris* Hampson, 1900 (Lepidoptera: Zygaenidae, Procridinae) from Iran. Entomologist's Gazette 58: 69–84.

Subchev M, Efetov KA, Toshova T, Parshkova EV, Toth M, Francke W (2010) New sex attractants for species of the zygaenid subfamily Procridinae (Lepidoptera: Zygaenidae). Entomologia Generalis (Stuttgart) 32: 243–250.

Subchev MA, Koshio C, Toshova TB, Efetov KA (2012) *Illiberis* (*Primilliberis*) *rotundata* Jordan (Lepidoptera: Zygaenidae: Procridinae) male sex attractant: Optimization and use for seasonal monitoring. Entomological Science 15: 137–139.

Subchev M, Koshio C, Toshova T, Efetov KA, Francke W (2013) (2*R*)-butyl (7*Z*)-dodecenoate, a main sex pheromone component of *Illiberis* (*Primilliberis*) *pruni* Dyar (Lepidoptera: Zygaenidae: Procridinae)? Acta zoologica bulgarica 65: 391–396. doi: 10.1111/j.1479-8298.2011.00485.x