# Systematics of Problepsis wiltshirei (Prout, 1938), comb. nov. (Lepidoptera, Geometridae, Sterrhinae) - an endemic species to the Zagros Mountains in the Middle East 

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#### Abstract

Within Iran, the Zagros Mountains show high biodiversity, with a wealth of endemic species. One of these is the geometrid moth Somatina wiltshirei Prout, 1938, originally described from Iran and Iraq. In the present study, one mitochondrial and up to nine protein-coding nuclear gene regions were used along with a comparative morphological examination to investigate the systematic position of this species. The results support the reclassification of this species as Problepsis wiltshirei comb. nov. Since the original species description is superficial, we provide a re-description supported by rich illustrations of morphological characters and distribution. In addition, Problepsis wiltshirei comb. nov. is reported as a new species for the fauna of Turkey. The importance of the habitat for the conservation of this species is discussed.


## Introduction

In the traditional classification of Scopulini (Geometridae: Sterrhinae), species were classified into genera based on the number of forewing areoles. Genera with one areole included Problepsis Lederer, 1853 and Scopula Schrank, 1802, and species with two areoles were classified in Somatina Guenée, [1858] (Prout 1934-1939). Sihvonen (2005) found evidence for the view that the number of areoles in the forewing is homoplastic and therefore not valuable as the only diagnostic character, as the state of two areoles also occurs in some Problepsis and Scopula species. Thus, even today, quite a few species are erroneously placed in the genus Somatina. Some of those have recently been reclassified (e.g., Sihvonen 2005; Xue et al. 2018; Sihvonen et al. 2020). However, the classification of other species within Somatina (sensu Sihvonen 2005) need to be re-examined.

The type species of Somatina is S. anthophilata Guenée, [1858] described from India. Scoble (1999) listed 50 species within the genus, mainly distributed in Africa, Asia and Australia. Recent phylogenetic studies showed that many Somatina species belong to other genera: In a morphological phylogenetic study, Sihvonen (2005) transferred four Somatina species to Scopula, namely S. indicataria (Walker, 1861), S. mendicaria (Leech, 1897), S. microphylla (Meyrick, 1889), S. nucleata (Warren, 1905) and two species to Problepsis (P. centrophora (Prout, 1915), P.
triocellata Bastelberger, 1908). Scopula microphylla and P. triocellata were classified in Somatina by Hausmann and Scoble (2007), who listed 46 species within this genus. Xue et al. (2018) transferred Somatina transvehens (Prout, 1918) to Problepsis.

In a multi-gene phylogenetic analysis, two more species were transferred from Somatina to Problepsis namely, P. figurata (Warren, 1897) and P. vestalis (Butler, 1875), consequently decreasing the number of Somatina species at present to 41 (Sihvonen et al. 2020).

Somatina species share the following morphological characters (after Sihvonen 2005, none of those are unique synapomorphies): weak discal spots on fore- and hindwing, forewing with two areoles. The male hind tibia is characterized by the presence of a hair pencil. The male genitalia are characterized by socii being not fused, and sacculi and valvuli being asymmetrical. The juxta bears wing-like processes on the anterior margin, with the apex fused to the sacculus of the valva. Sternite 8 in males is variable, x -shaped and with weakly developed or absent mappa, normally without cerata.

The genus Problepsis was described based on the type species Caloptera ocellata Frivaldszky, 1845 and belongs to Scopulini, the largest tribe within the subfamily Sterrhinae (Sihvonen 2005; Müller et al. 2019; Sihvonen et al. 2020). This genus currently comprises 53 species distributed in the Old World and Australia (Hausmann 2004; Sihvonen and Siljander 2005; Stadie and Stadie 2016; Feizpour et al. 2018; Xue et al. 2018).

Problepsis species share the following morphological characters (none of those are unique synapomorphies): ocellate discal spots on fore- and hindwing, forewing mainly with one areole, occasionally with two areoles (Hausmann 2004; Sihvonen 2005). The male hind tibia is laterally flattened, spoon-shaped and characterized by the presence of a hair pencil (Sihvonen 2005; Feizpour et al. 2018). The male genitalia are characterized by fused socii and a dentate or smooth ventral margin of the tegumen (Sihvonen 2005; Xue et al 2018). Sternite 8 in males is elongated, the cerata are absent, rudimentary or fully developed; if present, then often fused to the mappa (Sihvonen 2005).

Recently, Problepsis cinerea (Butler 1886) was reported from the south Iranian province Hormozgan as the only species belonging to the genus Problepsis in Iran (Feizpour et al. 2018). Additionally, Somatina wiltshirei Prout, 1938 is the only species of the genus Somatina described from the Zagros Mountains in Iran and Iraq. Wiltshire (1957) considered S. wiltshirei to be restricted to the Zagros woodland belt.

The Zagros Mountains cover an area of $533,543 \mathrm{~km}^{2}$, extending with a length of 2000 km from Eastern Turkey and Northern parts of Iraq to the whole Western and Southwestern parts of Iran (Mouthereau et al. 2011; Kazemi and Hosseinzadeh 2020). In Iran, these mountains show a high rate of endemism, including reptiles, amphibians and plants (Gholamifard 2011; Safaei-Mahroo et al. 2015; Noroozi et al. 2018; Kazemi and Hosseinzadeh 2020). Additionally, due to its location in low and middle latitudes (between $25-40^{\circ} \mathrm{N}$ ) and milder climate conditions during the Last Glacial Maximum (LGM), this area played an important role as refugia for many biota (van Zeist and Bottema 1977; Rajaei et al. 2013; Ashrafzadeh et al. 2016; Mohammadi et al. 2021).

As a part of the revision of Iranian geometrid moths, the present study aims to clarify the systematic position of $S$. wiltshirei, using an integrative approach; to illustrate species-specific characters, and to give an overview of its distribution in the Zagros Mountains. To achieve this, we used a multi-gene molecular analysis along with the examination of external and internal morphological characters and distribution data. We also discuss the importance of the habitat for the conservation of this species.

## Material and methods

Type specimens, as well as additional specimens used in this study, were borrowed and studied from the following collections (acronyms after Evenhuis 2007):

| IMCA | Insect and Mite Collection Ahvaz University, Iran; |
| :--- | :--- |
| NHMUK | Natural History Museum London, United Kingdom; |
| PCPS | Private Collection of Pasi Sihvonen, Veikkola, Finland; |
| PCWW | Private Collection of Werner Wolf, Bindlach, Germany; |
| SMNK | Staatliches Museum für Naturkunde Karlsruhe, Germany; |
| SMNS | Staatliches Museum für Naturkunde Stuttgart, Germany; |
| ZFMK | Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; |
| ZSM | Zoologische Staatssammlung München, Germany. |

## Criteria for the selection of taxa

To test the combination of Somatina wiltshirei with the genus Problepsis, we studied type material of $S$. wiltshirei, type species of Problepsis (P. ocellata), and type species of Somatina (S. anthophilata) using morphological and molecular data, and additional specimens of both genera were used in the molecular analysis. Moreover, we included P. cinerea in our investigations, as it is the only species of the genus Problepsis in Iran (Feizpour et al. 2018). For the molecular analysis, already available other Scopulini data were used (Murillo-Ramos et al. 2019, Sihvonen et al. 2020). Lissoblemmini was proposed as sister to Scopulini in a previous phylogenetic study and therefore it was chosen as the outgroup in our study (Sihvonen et al. 2020).

## Morphological examinations

Type material and original descriptions were used for the identification of specimens. Documentation and photography of external characters were carried out using a Visionary Digital photography system (LK Imaging System, Dun. Inc., equipped with a Canon EOS 5DSR) and an Olympus E3 digital camera. Preparation of the genitalia was carried out following standard methods (e.g., Robinson 1976). The vesica was everted following the method described by Sihvonen (2001). Photography of the genitalia characters before embedding took place following the methods proposed by Wanke and Rajaei (2018), Wanke et al. (2019) and Wanke et al. (2021) using a Keyence VHX-5000 digital microscope. Genitalia structures were finally embedded in Euparal and photographed using a Keyence VHX-5000 digital microscope.

The morphology of male and female antennae, as well as the male hind leg, were studied using a Zeiss Scanning Electron Microscope (SEM, EVO-LS15). Antennae and hind leg were mounted on holders and sputter-coated with 6 nm gold-palladium using a Leica coating system (EM ACE 200), before imaging with SEM.

For the drawing of the wing venation, wings were placed on a microscope slide and covered with a drop of ethanol ( $70-96 \%$ ). In this setup, all venation is visible and can be photographed. For the photography, we used a Visionary Digital photography system (LK Imaging System, Dun. Inc., equipped with a Canon EOS 5DSR). In Graphic (vers. 3.1 for Mac) these photographs served as templates for the vector drawing of the wing venation by tracing the veins from it.

## Molecular techniques

For the extraction of DNA, the whole abdomen and a leg from a single dry collection specimen were used following the manufacture's protocol of the DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). Amplification of DNA was conducted following Wahlberg and Wheat (2008) and Wahlberg et al. (2016). We attempted to amplify one mitochondrial (cytochrome oxidase subunit I, COI) and up to nine protein-coding nuclear gene regions: Ribosomal Protein S5 (RpS5), wingless (wgl), cytosolic malate dehydrogenase (MDH), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), Elongation factor 1 alpha (EF-1alpha), Arginine Kinase (ArgK), Isocitrate dehydrogenase (IDH), sorting nexin-9-like (Nex9), sarco/endoplasmic reticulum calcium ATPase (Ca-ATPase). Sequences were sent to Macrogen for sequencing. The Genbank accession numbers are provided in Appendix 1.

## Phylogenetic analysis

In addition to the data generated in this study, we retrieved sequences of Scopulini taxa from the dataset of Sihvonen et al. (2020). The final dataset comprises 29 taxa. The concatenated length of the alignment was 6800 bp including gaps.

We ran maximum likelihood analyses with a data set partitioned by codon using RAxML-HPC2 V8.2.12 (Stamatakis 2014) on the web-server CIPRES Science Gateway (Miller et al 2010). We implemented the GTR+CAT option, and support for nodes was evaluated with 1000 rapid bootstrap (RBS) (Stamatakis et al 2008). The final tree was rooted with species of Lissoblemmini (Sihvonen et al. 2020). Trees were visualized and edited in FigTree v1.4.3 software (Rambaut 2012).

## Distribution patterns

Tracing of geographical coordinates was conducted using 'Google Earth Pro' (vers. 7.3.3.7786 for Mac ). Distribution patterns were plotted and prepared in QGIS (vers. 3.16.0 for Mac). The elevation profile in QGIS was prepared using Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010) downloaded from https://earthexplorer.usgs.gov.

## Results

In total, five genes of a single specimen of $S$. wiltshirei, six genes of a single specimen of $P$. cinerea and eight genes of a single specimen of $S$. anthophilata were successfully amplified and sequenced (see Appendix 1). As a result, $S$. wiltshirei clustered within the genus Problepsis, supporting its affiliation to this genus (Fig. 1). Additionally, according to the results of our morphological examination, $S$. wiltshirei shows the generic characters of the genus Problepsis (e.g., ocellate discal spots on the fore- and hindwing, the spoon-shaped hind tibia and the presence of a hair pencil in the male hind legs). The following morphological characters of the male genitalia also support the affiliation with Problepsis: fused socii and a smooth internal margin of the tegumen (compared against morphological data in Sihvonen 2005; Feizpour et al. 2018; Xue et al. 2018; Sihvonen et al. 2020) (see Figs 15-17). A re-description of this species is given in the taxonomy part of the discussion.

## Discussion

## Systematics

In sense of the traditional classifications of the genera Problepsis and Somatina, the latter genus was regarded as polyphyletic (see Sihvonen 2005). Recent studies based on morphology (Sihvonen


Figure 1. Phylogenetic position of Problepsis wiltshirei comb. nov. within the tribe Scopulini, supporting its combination in genus Problepsis. The numbers above the branches are Rapid Bootstrap support (RBS) on the best scoring ML tree (Stamatakis 2008). Values $\geq 85$ (\%) indicate supported clades.

2005; Xue et al. 2018), as well as multi-gene phylogenetic studies, resulted in the assignment of several Somatina species to Problepsis (Sihvonen et al. 2020). These results support the possibility of the monophyly of the genus Somatina, when non-Somatina species are reclassified (Sihvonen 2005; Xue et al. 2018; Sihvonen et al. 2020). In addition, Sihvonen (2005) identified three nonunique synapomorphies for Somatina, which support its monophyly (asymmetrical sacculi of valvae; asymmetrical valvuli of valvae; juxta with wing-like processes on anterior margin, with apex fused to sacculus of valva) (compare also Figs 15-17).

Our present results show $S$. wiltshirei nested within Problepsis $($ RBS $=84)$. Therefore, we transfer S. wiltshirei from Somatina to Problepsis comb. nov. Among the species included in our phylogenetic hypothesis, $P$. cinerea was recovered with low support as the sister species to $P$. wiltshirei $(\mathrm{RBS}=18)$.

Additional Problepsis species and possibly more genetic data are needed to find the most closely related species of $P$. wiltshirei. Based on COI sequences, as available on BOLD database, the genetically closest neighbour of $P$. wiltshirei are $P$. ocellata and $P$. cinerea. Both with a genetic distance of $4.2 \%$, calculated using K2P model: Kimura (1980) with MEGA X (Kumar et al. 2018; Stecher et al. 2020).

## Taxonomy

## Problepsis wiltshirei (Prout, 1938), comb. nov.

Figs 2-9, 14B, 15, 18, 21-25

Somatina wiltshirei Prout, 1938. In: Seitz, A. (Ed.), Die Großschmetterlinge der Erde. Supplement zu Band 4, 220. 2 § Syntypes (Iraq: Kurdistan, Rowanduz [Rawanduz Gorge], Berserini [Berserini Gorge]) examined based on photo; 1 Syntype specimen [sex is not given in the original publication], [Iran]: Fars, Ardekan Talochosroe [Tall Khosrow, today in prov. Kohgiluyeh and Boyer-Ahmad]) (in NHMUK).

Material examined. 2 \&, Iran, Esfahan, Gandoman S, Gerdeish-e, 200 m, 12./13.vi.2002, leg. J.-U. Meineke, A. Hofmann, Kallies et al., g.preps 0759, 0760/2020 D. Wanke; 1 \& , Iran, Khuzestan [now Kohgiluyeh va Boyerahmad], Yassoudj [Yasuj], Sisakht, 2250 m, 13.vi.1972, leg. Ebert, Pazouki; 1 §̃, 2 , same data, 13.-14.vi.1972, leg. Ebert \& Falkner, g.prep. ( ${ }^{\top}$ ) 0762/2020 D. Wanke; $2 \delta^{\lambda}$, same data, Sisakht 50 km NW, 15.-18.vi.1975, leg. Ebert, Falkner, g.prep. 0761/2020 D. Wanke; $1{ }^{〔}$, same data, 15 km SE Yassudj [Yasuj], $2050 \mathrm{~m}, 15 . \mathrm{vi} .1972$, leg. Ebert \& Falkner, g.prep. 0907/2020 D. Wanke; 1 §, S-Iran, Prov. Fars, Tange Surkh, 50 km NW Ardekan, 2250 m NN, 12.-15.vi.1975, leg. Ebert, Falkner; 2 §, S-Iran, Fars, Daschte Ardjan, Kotal-Pirehsan, 2000 m, 18.vi.1972, leg. Ebert \& Falkner; 1 ¢, S-Iran, Miyan-Kotal, östl. Kazerun, $51^{\circ} 40^{\prime} \mathrm{E}, 29^{\circ} 30^{\prime} \mathrm{N}, 1900 \mathrm{~m}, 4 .-7 . v i .1969$, leg. G. Ebert; 1 ㅇ, S-Iran, Fars, Kaserun, Mian-Kotal, $1900 \mathrm{~m}, 11 . v i .1972$, leg. Ebert \& Falkner; all in SMNK.

1 ㅇ, Iran, Kohkiluye va Boyerahmad, Yasuj, Sisakht, Dena, 2799 m, $30^{\circ} 57^{\prime} 23.6^{\prime \prime} \mathrm{N}, 51^{\circ} 23^{\prime} 28.9^{\prime \prime} \mathrm{E}, 30 . v i i .2016$, leg. Sh. Feizpour, g.prep. 0712/2020 D. Wanke; in SMNS.
$1 \delta^{\pi} /$ ب, Iran, Khuzestan, Emamzadeh, Abdollah-low altitude; Saite $4 \mathrm{~b}, 31^{\circ} 22^{\prime} 244^{\prime \prime} \mathrm{N}, 50^{\circ} 7^{\prime} 51^{\prime \prime} \mathrm{E}, 1408 \mathrm{~m}, 23 . i x .2018$, Trap1, leg. Mohammad Ahmadi; $1 \delta^{\top} /$ Q , Iran, Khuzestan, Emamzadeh, Abdollah-high altitude; Saite $4 \mathrm{a}, 31^{\circ} 23^{\prime} 10^{\prime \prime} \mathrm{N}$, $50^{\circ} 9^{\prime} 29^{\prime \prime} \mathrm{E}, 2120 \mathrm{~m}$, 13.vii.2018, Trap 2, leg. Mohammad Ahmadi; 1 §/?, Iran, Prov. Khuzestan, Mal aqa, 1100 m , $31^{\circ} 35^{\prime} 57^{\prime \prime} \mathrm{N}, 50^{\circ} 00^{\prime} 50$ "E, 30.vii. 2011, leg. Mehdi Esfandiari; $1 \delta^{\AA} /$ ? , Iran, Prov. Fars, Bolhayat \& Kotal-e-Pirzan, 2000 m , $29^{\circ} 36^{\prime} 48^{\prime \prime} \mathrm{N}, 51^{\circ} 56^{\prime} 28^{\prime \prime} \mathrm{E}, 2$. \& 9.vi.2011, leg. Mehdi Esfandiari; 1 § $/$ 多, Iran, Prov. Fars, Kohmare Sorkhi, 1900 m , $29^{\circ} 28^{\prime} 111^{\prime \prime} \mathrm{N}, 52^{\circ} 08^{\prime} 44^{\prime \prime} \mathrm{E}, 28 . i v .2011$, leg. Mehdi Esfandiari; all in IMCA.

1 \&, Türkei [Turkey], prov. Hakkari, Çığıl Suyu-Tal [Zap-Tal], 22 km SW Hakkari, 28.vi.1984, LF, leg. Werner Wolf; in PCWW.
$1 \bigcirc^{\lambda}, 1$, Iran, Fars, Straße Ardekan-Talochosroe [Tall Khosrow, today in prov. Kohgiluyeh und Boyer Ahmad], Comé [Komehr], 7.viii.1937, 2600 m , coll. Brandt; in ZFMK.
$1 O^{\lambda}$, Iran, Fars, Straße Ardekan-Talochosroe [Tall Khosrow, today in prov. Kohgiluyeh und Boyer Ahmad], Comèe [Komehr], 2600 m, viii.1937, coll. Brandt, ZSM g.prep. No. 1602; in ZSM.

Re-description. Wings and body (Figs 2-9). Wingspan ठ 24-28 mm, $q 29-32 \mathrm{~mm}$; females slightly larger than males. The proboscis well developed. The length of the labial palpi about equal to the diameter of the eye. Frons, thorax and abdomen concolorous with the wings. Chaetosemata arranged as two patches. Antennae ciliate-fasciculate in males and filiform in females (Figs 2, 3). Male hind tibia with reduced tarsal segments; flattened laterally; longitudinally spoon-shaped; with hair pencil. Hair pencil consisting of two types of differently modified scales as illustrated for $P$. cinerea by Feizpour et al. (2018). One type of scales apically flattened, while the other scales are tubular and hollow (Figs 4, 5). Ground colour of wings beige, intermixed with some slightly darker ochreous or brown scales; basal areas slightly lighter beige. Fore- and hindwing with a small, light yellowish ocellus, bordered with a thin dark outer line. Discal spots sometimes not visible; more pronounced in the forewings (Figs 6-9).

Venation (Fig. 14B). Two areoles present in forewing. Veins R1, the common stalk of R2-4 and R5 arising from the second areole. In hindwing $\mathrm{Sc}+\mathrm{R} 1$ slightly curved in basal area, approximating to the cell in the postbasal area; A1+2 originating separately; A3 absent.

Male genitalia (Fig. 15). Uncus absent. Socii strongly developed fused at apex. Internal margin of tegumen smooth. Valva with two curved arms (dorsal and ventral arm of valva), both slerotized, narrow and long, apically pointed (Fig. 15a, d). Aedeagus strongly sclerotized, slender, tapering towards the apex; its basal part dorso-ventrally flattened; vesica without cornuti (Fig. 15b). Sternum A8: anterior margin with two indentations; lateroanteriorly on both sides pointed. Lateral sides towards posterior part concave; posterior margin curved. Cerata located in posterior half of sternum A8, directed towards centre (Fig. 15c).


Figures 2-13. Morphological characters of Iranian Problepsis species and Somatina anthophilata. 2-5. SEM photos of Problepsis wiltshirei comb. nov.; 2. Part of ciliate-fasciculate antennae of male (Iran, Yasuj, Sisakht, g.prep. 0762/2020 D. Wanke); 3. Detail of filiform antennae of female (Iran, Fars, Mian-Kotal); 4. Male hind tibia with hair pencil (Iran, Yasuj, Sisakht, g.prep. 0761/2020 D. Wanke); 5. Close up on tubular and hollow modified scale of hair pencil (Iran, Yasuj, Sisakht, g.prep. 0761/2020 D. Wanke); 6-9. Wing pattern of Problepsis wiltshirei comb. nov.; 6. Male paratype (Iraq, Kurdistan, Berserini); 7. Male (Iran, Fars, Straße Ardekan-Talochosroe, g. prep. 1602 ZSM); 8. Female (Iran, Yasuj, Sisakht, g.prep. 0712/2020 D. Wanke); 9. Male (Iran, Yasuj, Sisakht, g.prep. 0761/2020 D. Wanke); 10. Female of Problepsis cinerea (Iran, Hormozgan, Geno protected area); 11-13. Wing pattern of S. anthophilata; 11. Paralectotype (India); 12. Male (Thailand, Lampang, Muban Phichai); 13. Female (Thailand, Lampang, Muban Phichai). $a=$ upperside; $b=$ underside.


Figure 14. Wing venation of male specimens of Problepsis species; A. Problepsis ocellata (type species for the genus) and B. Problepsis wiltshirei comb. nov. Note: Problepsis ocellata (A.) with one areole in the forewing and Problepsis wiltshirei comb. nov. (B.) with two areoles in the forewing.

Female genitalia (Fig. 18). Papillae anales short and broad. Apophyses anteriores $2 / 3$ length of apophyses posteriores. Antrum strongly sclerotized. Lamella antevaginalis strongly sclerotized; basal part broad, laterally extended; posteriorly curved, folded, tapered to lateral side. Ductus bursae short, strongly sclerotized. Corpus bursae membranous; signum present as a sclerotized, narrow and dentate ridge.

Diagnosis. P. wiltshirei cannot be confused with any other Problepsis or Somatina species within this region. In Iran only Problepsis cinerea is known from South Iran and it does not occur within the range of $P$. wiltshirei comb. nov. Additionally, it cannot be confused with this species, as it differs strongly by wing pattern (see Figs 6-10) and by genitalia (Figs 16, 19). Somatina pythiaria nigrimacula Hausmann, 2009; a species distributed in Oman has been shown differing by a greyer suffusion on wing pattern, the different structure of the sternum A8 and molecular data (Hausmann 2009).

Phenology. Flying from July to October, possibly in two generations (Wiltshire 1943). This coincides with the investigated specimens, but can be expanded from April to October.

Biology. Larva described by Wiltshire (1943) as grayish, intermixed with a complex pattern of grey dots and marks. Pale grey dorsal and ventral lines, the latter rather whitish on somites 4-8. Pupal period lasting 8 to 15 days. The cocoon is woven between leaves and litter (Wiltshire 1943).


Figures 15-17. Male genitalia of Iranian Problepsis species and Somatina anthophilata; 15. Problepsis wiltshirei comb. nov. (a. Iran, Yasuj, Sisakht, g.prep. 0762/2020 D. Wanke; b, c. Iran, Yasuj, Sisakht, g.prep. 0761/2020 D. Wanke; d. Iran, Yasuj, g.prep. 0907/2020 D. Wanke); 16. Problepsis cinerea (a-c. Pakistan, Kaghan-Tal, 375/2017, S. Feizpour); 17. Somatina anthophilata (a-c. Thailand, Lampang, Chae Hom, g.prep. 1177/2021 D. Wanke). $a=$ genitalia capsule; $b=$ aedeagus; $c=$ sternum A8; $d=$ genitalia capsule lateral view.

Wiltshire (1943) noted Fraxinus sp. (Oleaceae) and Acer sp. (Sapindaceae) as food plants for P. wiltshirei. As Problepsis species have been observed feeding on Oleaceae species (Robinson et al. 2002; Stadie and Stadie 2016) Acer sp. is an exceptional food plant, which needs confirmation.

Habitat. This species occurs in the Middle Heights of the mountains, especially the woodland zone (Wiltshire 1957) and mountain steppe, at elevations from 1100 m up to 2800 m (Figs 21-25). The habitat is covered with different herbaceous plants and shrubs, dominated by Prunus sp. (Rosaceae), Artemisia sp. (Asteraceae), Astragalus sp. (Fabaceae) and Acantholimon sp. (Plumbaginaceae).

Distribution. So far recorded in the Zagros Mountains, from northern Iraq (Kurdistan) into south-western Iran (Kohgiluyeh-va-Boyer-Ahmad and across the border to the provinces, Khuzestan, Esfahan and Fars) (Fig. 25). Additionally, here we record this species for the first time for the fauna of Turkey (see examined material). The large gap between the populations in northern Iraq, Turkey and central Zagros in Iran is possibly caused by insufficient sampling in these areas.

## Zagros Mountains as a refuge for Problepsis wiltshirei

Major issues in conservation biology for protection efforts are the identification of areas with high biodiversity, high rates of endemism and past events, like glacial refugia or environmental changes (Médail and Diadema 2009; Cañadas et al. 2014; Noroozi et al. 2018; Kazemi and Hosseinzadeh 2020).

The Zagros Mountains have been identified as an area with a high species diversity of flora and fauna (Rechinger 1963-2015; Firouz 2005; Noroozi et al. 2008; Sayadi and Mehrabian 2016). Akbarirad et al. (2016) showed that brush-tailed mice of the genus Calomyscus (Calomyscidae: Rodentia) are highly diverse, due to the topography of these mountains which cause their geographic isolation. Similar findings were made for the Iranian herpetofauna, where the mountains and their diverse environmental conditions play an important role in the separation and isolation of species (Gholamifard 2011; Kazemi and Hosseinzadeh 2020). The important role of the Zagros Mountains can also be seen through various groups of arthropods (e.g., Paknia et al. 2008; Marusik and Zamani 2015; Zamani et al. 2018). For Lepidoptera, high species diversity of different families has been observed in the Zagros Mountains (e.g., Nazari 2003; Trusch and Hausmann 2008; Rajaei et al. 2012; Tshikolovets et al. 2014; Keil 2015; Yakovlev 2015; Wanke et al. 2020).

Regarding the endemism rate, the Zagros Mountains show the highest richness compared to other Iranian mountain ranges (Akhani 2004; Noroozi et al. 2016; Kazemi and Hosseinzadeh 2020; Khajoei Nasab and Khosavi 2020). It has been shown that $45 \%$ of Zagros vascular plants are restricted to this region (Noroozi et al. 2019). Additionally, this applies to endemic alpine plant species, where the highest rates of endemism were found in the Zagros (Noroozi et al. 2016). Comparably high endemism has been found in Lepidoptera (e.g., Rajaei 2012; Tshikolovets et al. 2014; Keil 2015).

Moreover, the Zagros Mountains have played a crucial role as a refuge for diverse fauna and flora during glaciation events. Malekoutian et al. (2020) found in a phylogeographic analysis, the occurrence of the Yellow-spotted Mountain Newt (Neurergus derjugini) derives from three different refugia in the Zagros mountains. Similar findings for the survival of Iranian Brown Bears (Ursus arctos) in Zagros refugia during the Last Glacial Maximum (LGM) were proved by Ashrafzadeh et al. (2016). Based on genetic and paleo-bioclimatic data, Rajaei et al. (2013) found this region to be a refuge for two Gnopharmia species and their host plants (Prunus scoparia) during the LGM $23,000-18,000$ years ago). Furthermore, it has been shown that the highest haplotype diversity of these two Gnopharmia species is present in southwestern parts of the Zagros Mountains and served as a population source for the postglacial expansion of these species (Rajaei et al. 2013).


Figures 18-20. Female genitalia of Iranian Problepsis species and Somatina anthophilata; 18. Problepsis wiltshirei comb. nov. (Iran, Esfahan, Gandoman, g.prep. 0759/2020 D. Wanke); 19. Problepsis cinerea (g.prep. 374/2017, S. Feizpour); 20. Somatina anthophilata (Thailand, Lampang, Muban Phichai, g.prep. 1176/2021 D. Wanke).


Figures 21-25. Habitat in the Zagros Mountains and distribution map of Iranian Problepsis species; 21, 22. Iran, Fars, Dasht-e Arjan at 2158 m altitude; 23, 24. Iran, Kohkiluye va Boyerahmad, Tange-Tamoradi at 2254 m altitude; 25. Distribution pattern of Iranian Problepsis species.

The results of our study confirm that $P$. wiltshirei is an endemic species in the Zagros mountains and has so far been restricted to two areas of this mountain range. The first area in northern Iraq and south-eastern of Turkey falls into the Irano-Anatolian biodiversity hotspot, a region of remarkable species endemism, covering high elevations of central and eastern Turkey, Armenia, NE Iraq and Iran (Mittermeier et al. 1999; Noroozi et al. 2018). In its second area of distribution, P. wiltshirei inhabits the southwestern parts of the Zagros Mountains, a habitat outstanding for its rich biodiversity (e.g., Hosseinzadeh et al. 2014; Farashi and Shariati 2017; Noroozi et al. 2018).

Although several areas are protected in the Zagros Mountains (e.g., Arjan, Bakhtegan, Karkheh, Bamu etc.), this unique nature reserve is currently threatened, mainly by human activity. Every year 15,000 ha of Iranian forests burn (in 2020 wildfires burned down over 50,000 ha of oak forests in the Zagros Mountains) and centuries-old trees are destroyed in the process (Kheshti 2020). In ecosystems such as the Zagros Mountains, these high fire intensities threaten its species diversity and richness (Heydari et al. 2016). Further threats to biodiversity are overgrazing by sheep and goats in the marginal arid areas, as well as the land erosion caused by agriculture (Jowkar et al. 2016). Also, poaching and sporadic poisoning of animals occurs from time to time, even within protected areas, causing significant damage to its fauna (Jowkar et al. 2016). P. wiltshirei is distributed in this threatened area of high biodiversity and we still know only very little about its distribution and biology. Our study emphasizes the importance of further investigations of the Lepidoptera fauna of the Zagros, to better understand biodiversity hotspots and areas of endemism in the context of species conservation.

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Appendix 1
Table A1. Sterrhinae taxa used in this study, with identification, process code, and GenBank accession numbers for each gene. Data from Sihvonen et al. $(2020)^{(1)} \&$ Wanke et al. (current paper) ${ }^{(2)}$.

| Taxon identification | Code | ArgK | Ca-ATPase | COI-begin | COI-end | EFla-begin | EFla-end | GAPDH | IDH | MDH | Nex9 | RpS5 | WntGeo |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Lissoblemma hamularia ${ }^{(1)}$ | MM00220 | - | - | JF784708 | - | - | JF785331 | - | - | - | - |  |  |
| Aletis helcita ${ }^{(1)}$ | PS268 | MK738556 | MK738887 | MK739619 | - | MK740001 | MK740001 | MK740647 | - | - | MK741674 | MK742030 | MK742585 |
| Aletis monteironis ${ }^{(1)}$ | PS270 | MK738557 | MK738888 | MK739620 | - | - | - | - | - | - | - | - | MK742586 |
| Aletis variabilis ${ }^{(1)}$ | 0x 17 | - |  | MG767890 | MG767890 |  | MG768276 | MG767671 | - |  |  | MG767479 | MG767344 |
| Aletis forbesi ${ }^{(1)}$ | 0x28 | - | - | MG767889 | MG767889 | - | MG768275 | - | - | - | - | MG767478 | MG767343 |
| Scopulo (Isoplenia) trisinuata ${ }^{(1)}$ | PS229 | - | MK738851 | MK739391 | MK739391 | MK739967 | MK739967 | MK740614 | MK740872 | MK741191 | MK741640 | MK742000 | MK742550 |
| Somatina anthophilata ${ }^{(2)}$ | SMNS_Lep_002232 | MZ798167 | MZ798169 | MZ753906 | MZ753906 | MZ798171 | MZ798171 | - | MZ798173 | MZ798174 | - | MZ798175 | MZ798177 |
| Problepsis wiltshireit ${ }^{(2)}$ | SMNS-DNA-157 | MZ798179 | - | MW803363 | - | MW803364 | MW803364 | - | - | - | - | MW803365 | MW842913 |
| Problepsis cinerea ${ }^{(2)}$ | SMNS_Lep_002234 | MZ798168 | MZ798170 | MZ753905 | - | MZ798172 | MZ798172 | - | - |  | - | MZ798176 | MZ798178 |
| Problepsis vestalis ${ }^{(1)}$ | PS249 | MK738538 | MK738869 | MK739406 | MK739406 | MK739983 | MK739983 | MK740630 | MK740881 | - | MK741657 | MK742014 | MK742568 |
| Problepsis figurata ${ }^{(1)}$ | PS272 | MK738559 | MK738890 | MK739424 | MK739424 | MK740003 | MK740003 | MK740649 | - | - | MK741676 | MK742032 | MK742588 |
| Problepsis flavistigma ${ }^{(1)}$ | PS271 | MK738558 | MK738889 | MK739423 | MK739423 | MK740002 | MK740002 | MK740648 | MK740896 | - | MK741675 | MK742031 | MK742587 |
| Problepsis digammata ${ }^{(1)}$ | PS216 | MK738512 | MK738839 | MK739614 | - | MK739958 | MK739958 | MK740605 | MK740860 | MK741179 | MK741628 | MK741990 | MK742538 |
| Problepsis centrophora ${ }^{(1)}$ | PS255 | MK738544 | MK738875 | MK739411 | MK739411 | MK739989 | MK739989 | MK740636 | MK740886 | - | MK741663 | MK742019 | MK742573 |
| Scopula nemorivagata ${ }^{(1)}$ | PS232 | MK738524 | MK738853 | MK739616 | - | MK739968 | MK739968 | MK740616 | - | MK741193 | MK741642 | MK742001 | MK742552 |
| Scopula johnsoni ${ }^{(1)}$ | R1872 | - | - | MG767915 | MG767915 | - | MG768299 | - | MG768488 | MG768103 | - | MG767500 | MG767348 |
| Scopula tenera ${ }^{(1)}$ | SH1155 | - | - | MG767781 | MG767781 | MG768176 | MG768176 | - | MG768396 | MG768017 | - | MG767408 | - |
| Scopula immorata ${ }^{(1)}$ | MM00586 | - | - | GU828645 | GU828443 | GU828978 | GU829261 | - | GU830032 | GU830351 | - | GU830646 | GU829536 |
| Scopula nr karischi ${ }^{(1)}$ | SH0432 | - | - | MG767773 | MG767773 | MG768170 | MG768170 | MG767575 | MG768391 | MG768013 | - | MG767404 | - |
| Scopula punctilineata ${ }^{(1)}$ | PS251 | MK738540 | MK738871 | MK739407 | MK739407 | MK739985 | MK739985 | MK740632 | MK740883 | - | MK741659 | MK742016 | MK742570 |
| Scopula nr laevipennis ${ }^{(1)}$ | R1050 | - | - | MG767897 | MG767897 | MG768281 | MG768281 | MG767677 | - | MG768090 | - | - | - |
| Scopula nr vitellina ${ }^{(1)}$ | SH0448 | - | - | MG767771 | MG767771 | MG768168 | MG768168 | MG767573 | MG768389 | MG768011 | - | MG767402 | - |
| Scopula sp ${ }^{(1)}$ | PS241 | MK738532 | MK738861 | MK739617 | - | MK739976 | MK739976 | MK740623 | - | MK741201 | MK741650 | MK742008 | MK742560 |
| Scopula nr calcarata ${ }^{(1)}$ | SH0421 | - | - | MG767770 | MG767770 | MG768167 | MG768167 | - | MG768388 | MG768010 | - | MG767401 | MG767318 |
| Scopula nr serena ${ }^{(1)}$ | PS 243 | MK738533 | MK738863 | MK739618 | - | MK739978 | MK739978 | MK740625 | - | MK741203 | MK741652 | MK742010 | MK742562 |
| Scopula amala ${ }^{(1)}$ | USNM664273 | - | - | KY370874 | - | - | - | - | - | - | - | LT674233 | LT674262 |
| Scopula nr nigrinotata ${ }^{(1)}$ | PS262 | MK738550 | MK738881 | MK739417 | MK739417 | MK739995 | MK739995 | MK740642 | MK740892 | - | MK741668 | MK742024 | MK742579 |
| Scopula ternata ${ }^{(1)}$ | MM08463 | - | - | MK739598 | - | MK740231 | - | MK740574 | - | MK741142 | - | MK741945 | - |
| Scopula frigidaria ${ }^{(1)}$ | MM10459 | - | - | - | - | MK740233 | - | - | - | MK741144 | - | MK741947 | MK742493 |

