Description of the hitherto unknown female of *Agrotis proverai* Zilli, Fibiger, Ronkay & Yela, 2010 (*Lepidoptera, Noctuidae*) with a discussion on its taxonomic status and some notes on its conservation

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Abstract

Half a century after the discovery of the species in Central Italy, the female of *Agrotis proverai* was collected for the first time. The female is unable for flight like those of the other species of the *Agrotis fatidica* group; a great variability in the wing size of the females has been observed.
The *imago* and *genitalia* of the female are illustrated for the first time and the variability of adults of both sexes is outlined. The taxonomic status of the species is discussed and some considerations are made on its conservation, especially in regard to the effects of climate change.
Key words: *Agrotis proverai*, Noctuid moths, climate change, Gran Sasso e Monti della Laga National Park.

Riassunto

Descrizione della femmina finora sconosciuta di* Agrotis proverai *Zilli, Fibiger, Ronkay &Yela, 2010 (Lepidoptera, Noctuidae) con una discussione sul suo status tassonomico e alcune note sulla sua conservazione
Dopo circa mezzo secolo dal ritrovamento della specie in Italia Centrale è stata per la prima volta raccolta la femmina di *Agrotis proverai*. La femmina non è in grado di volare come quelle delle altre specie del gruppo *Agrotis fatidica*; è stata osservata una grande variabilità nello sviluppo alare delle femmine. Vengono illustrati per la prima volta l'imago ed i genitali femminili e viene delineata la variabilità degli adulti di entrambi i sessi. Viene discusso lo stato tassonomico della specie e svolte alcune considerazioni sulla sua situazione di conservazione soprattutto in rapporto agli effetti del cambiamento climatico.

Parole chiave: *Agrotis proverai*, Noctuidae, cambiamento climatico, Parco Nazionale del Gran Sasso e Monti della Laga.

Introduction

Agrotis proverai was collected for the first time in the Gran Sasso massif at Campo Imperatore in 1972 at an altitude of 2100 meters and was initially identified as *Agrotis fatidica* (Hübner, [1824]) (Prola *et al.*, 1978). Subsequently, in 2010 the Apennine population was attributed to a species new to science: *Agrotis proverai* Zilli, Fibiger, Ronkay &Yela, 2010. According to the Authors, "*the species has been collected solely in a restricted montane area of Gran Sasso d'Italia with alpine meadows, at an altitude of 2000-2100 m. Attempts to find the species at higher or lower elevations or in similar habitats from other mountains massifs of the Central Apennines have been unsuccessful*". (Zilli *et al.*, 2010)
The attribution of the Apennine population to the new taxon was made on the basis of the examination only of male specimens as at that date no female of the species had ever been found.
The species is therefore endemic to Central Italy. Campo Imperatore was considered the only flight location of *Agrotis proverai* and furthermore in an extremely narrow altitudinal belt.

A revision of the group was recently performed (Ronkay & Huemer, 2018) and two new species were described: *Agrotis mayrorum* Ronkay & Huemer, 2018 and *Agrotis mazeli* Ronkay & Huemer, 2018. In that review, the specific status of the taxon *proverai* remained unchanged.
So far the female of *A. proverai* had remained still unknown. It was presumed that the females were brachypterous as in the congeners *Agrotis fatidica*, *Agrotis mayrorum* and *Agrotis mazeli*. Therefore it was assumed that the ineptitude of the females to fly, together with the rarity and localization of the species,

was the reason for the failure to find females for over 40 years after the discovery of the species.
Two of the authors (GG) (GF) have been carrying out lepidopterological research a few years ago within the Gran Sasso and Monti della Laga National Park thanks to the sensitivity and willingness of the Park Authorities.
One of the objectives of the research was precisely the deepening of knowledge on *Agrotis proverai*, its distribution and its conservation. The species is a formidable indicator of the state of conservation of the environment, in particular with regard to the impact of climate warming on the Park's wildlife populations. The research was carried out both in Campo Imperatore from an altitude of about 2100 meters, up to the slopes above the "Giardino Alpino" *locus typicus* of the species, and in Campo Pericoli at about 2350 meters altitude.
The research made it possible to outline the current distribution of the species in the area and to discover the female that had remained unknown half a century after the discovery of the species in the Apennines.

Material and methods

Collecting was carried out using light traps provided with 8-watt blacklight tubes or mixed light lamps powered with a battery and a 150-watt lamp.
The search for females was carried out on sight at night with the aid of headlamps.
The searches were made more difficult by the lack of roads to reach the higher prairies of Campo Pericoli (Fig. 1) and by the need to carry the necessary equipment (batteries and lamps) by hand. The other two authors (AF) (GLT) also collaborated in the high-altitude research.
Studied specimens are preserved in the following collections:
Research collection of Guido Govi, Forlì, Italy; GG
Research collection of Gabriele Fiumi, Forlì, Italy; GF
Research collection of Giuseppe Longo Turri, Quinzano (Verona), Italy; GLT
Research collection of Alessandro Floriani, Milano, Italy; AF

Results

Already during the first searches carried out in August 2015, it was possible to find *Agrotis proverai* albeit in a very limited number of specimens. The searches were carried out in the vicinity of the Alpine Garden at an altitude of about 2100 meters and therefore in the vicinity of the place where the species was first found. At this altitude, it was possible to collect only 2 male specimens.
The research was gradually extended to higher altitudes with the use of light traps up to the grasslands of Campo Pericoli at an altitude of over 2300 meters and here the species was found to be much more abundant.
In 2019, a specific expedition was therefore planned to the Campo Pericoli basin aimed at finding females with overnight stays in the area (Fig. 2). The research benefited from particularly favorable weather conditions with no wind and relatively high temperatures. Finally, it was possible to find the female of the species. A total of 9 female specimens were collected, currently preserved in the authors' collections.

Fig. 1. Italy. Abruzzo. Gran Sasso. The Campo Pericoli basin from the slopes of Corno Grande (24 August 2021).

External morphology: males

Concerning the external morphology, we were able to examine a large number of specimens mainly collected in the Campo Pericoli area at an altitude about 250 meters higher than the series of specimens based on which the species was described (Campo Imperatore mainly in the vicinity of the Alpine Garden at an altitude of 2000 - 2100 meters) and illustrated both in the description of the species (Fibiger *et al.*, 2010) and in the revision of the group (Ronkay & Huemer, 2018).

Our observations confirmed that *Agrotis proverai* has an average smaller dimensions than *A. fatidica* (wingspan varying from 34 to 39 mm with an average of 37 mm). Many of the specimens we have collected have a darker color than that of the specimens illustrated in the description of the species. The species, therefore, seems to have darker forms as the altitude increases. The same observation is reported for the Gran Sasso massif for *Mythimna ferrago* (Fabricius, 1787) (Raineri *et al.*, 1991) which in the specimens collected at 2500 meters shows forms similar to those of the northern phenotype, while at lower altitudes, always on the Gran Sasso, has lighter color. We also report an analogous observation for *Euxoa decora* (Denis & Schiffermüller, 1775) which in Campo Pericoli shows medium-dark specimens similar to those flying on the limestone massifs of the Alps, while at lower altitudes of 300-400 meters it has specimens on average with much lighter phenotype (Fig. 3).

Although the specimens collected in Campo Pericoli, at an altitude higher than *locus typicus*, are on average darker, the differential character highlighted in the description of the species is confirmed, i.e. the fill color of the reniform stigma which in *A. proverai* remains darker than in *A. fatidica*, in particular in the distal half, so much so that this stigma appears as a dark spot even in the darkest specimens, while the opposite is true for the typical *A. fatidica*, in which the reniform stigma appears as a light spot on a dark background.

In our opinion, another differential character that appears fairly constant was overlooked by the Authors in the description of the species, that is the length of the claviform spot

Fig. 2. *Agrotis proverai*: specimens photographed in nature (Campo Pericoli, 21 August 2019).

of the upper side of the forewing, which in *A. proverai* is on average shorter than in *A. fatidica*.
In the specimens that we have examined, on the other hand, the fact that the front wings are visibly narrower than those of *A. fatidica*, another differential character of *A. proverai* stressed by the Authors in the description of the species, seems less evident to us.

External morphology of females

Contrary to what the previous Authors hypothesized, the species is only partially brachypterous, as a wide variability of the wing development has been observed, passing from specimens with poorly developed wings and a wingspan of 28 mm, up to specimens with fully developed wings and with a wingspan of 38 mm; the average wingspan in the 9 female specimens collected is 32 mm (Fig. 4).
The pattern on the wings corresponds to that of the male but the color is on average much darker. In particular, the color is gradually darker as the wingspan is reduced, almost as if it undergoes a sort of concentration as the wingspan shrinks. The antennae are filiform as in the other congeneric species.
It is worth mentioning that even the females with the greatest wing development - corresponding to that of the male specimens - are in any case unable for flight and have been observed while walking on the paths frantically flapping their wings or while they were perched on blades of grass; one of the females was observed in copula. Females have been found in greater numbers in the vicinity of the 150 watt light source and therefore it seems possible to hypothesize that, despite having a limited capacity to move, they are attracted to light and therefore, like the males, they have a positive phototropism. As a confirmation of the females' non-aptitude to fly, it should be noted that females have never been found in the trap lamps.
According to Fibiger (FIBIGER, 1990), the females of *Agrotis fatidica* have a wingspan of 24-35 mm although those coming from Norway have a much greater wingspan than those coming from the Alps so that they could possibly be suitable for flight, unlike those from the Alps. Therefore the females of *Agrotis proverai*, together with those of *Agrotis fatidica* coming from Norway, seem to be those with the most developed wings in the group; in this regard, the photo of the copulating specimens photographed in nature is particularly eloquent, as the one at the top of the photo, larger in size, is the female.

Morphology of female genitalia

We examined the *gynopygium* of a specimen of *Agrotis proverai* (Fig. 5) and compared it with those of *A. fatidica* illustrated in the third volume of Noctuidae Europaeae (FIBIGER, 1997) and with those illustrated in the review of the group (RONKAY & HUEMER, 2018). The gynopygium of *A. proverai*, in the single observed specimen, has longer anal papillae than in *A. fatidica*, longer apophyses than in *A. fatidica* (as in *A. mayrorum*) and shorter *ductus bursae* than *A. fatidica*.

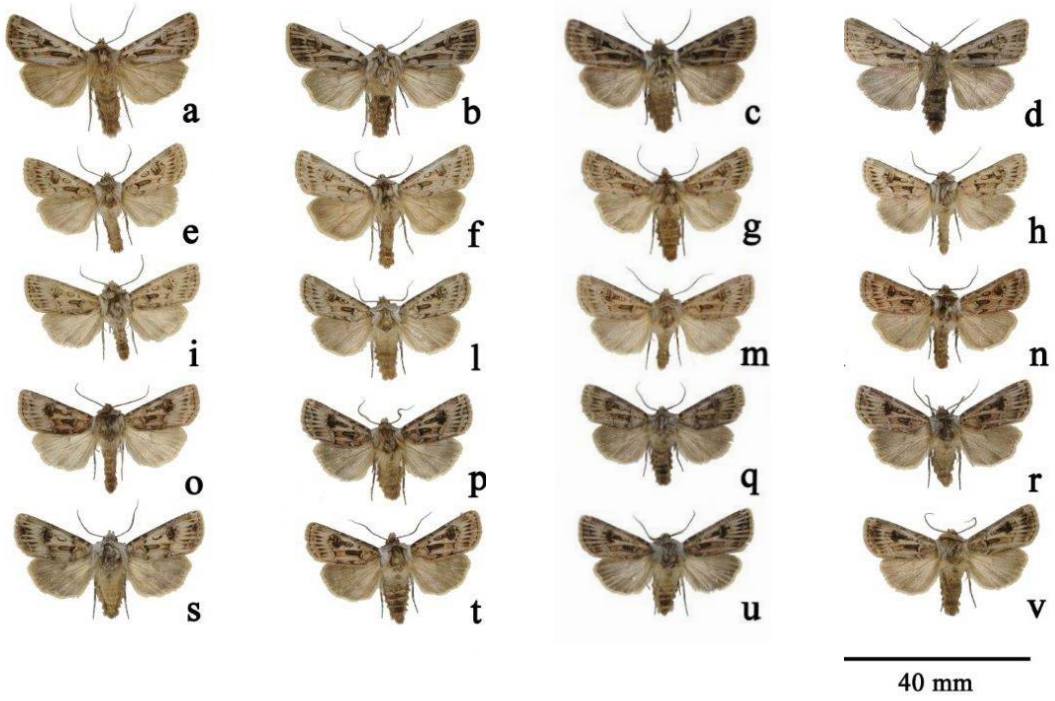

Fig. 3. Variability of male of *Agrotis fatidica* and *Agrotis proverai*. 3a. *A. fatidica* Italy, Trentino, Passo Pordoi, m 2250, 29.VIII.2015; 3b - 3c. *A. fatidica* Italy, Lombardia, Passo dello Stelvio, m 2600, 9.VIII.2020; 3d. *A. fatidica* Italy, Lombardia Passo dello Stelvio, m 2600, 20.VIII.2021; 3e. *Agrotis proverai* Italy, Gran Sasso, Campo Imperatore, m 2100, 19.VIII.2014; Italy, Gran Sasso, Campo Pericoli, m 2300-2350, 19/VIII.2015 / 21.VIII.2019 / 24.VIII.2021. All specimens in coll. GG.

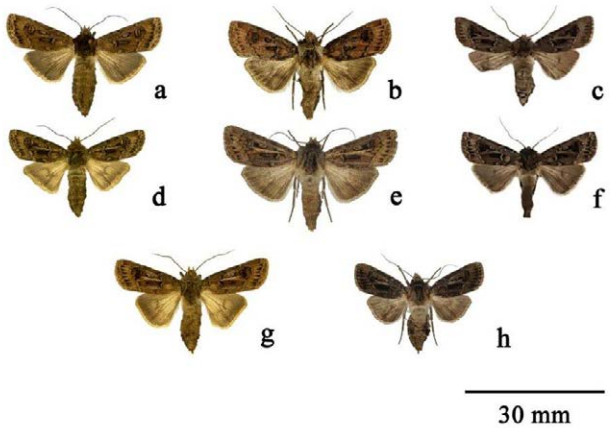

Fig. 4. Variability of female of *Agrotis proverai*. All specimens from 4a to 4h, *A. proverai* Italy, Abruzzo, Gran Sasso d'Italia, Campo Pericoli, m 2350, 21.VIII.2019. All specimens in coll. GG, GF, GLT, AF.

Ecological conclusions

Agrotis proverai is present and also very widespread in Campo Pericoli at an altitude of 2300-2400 meters above sea level. In the observations conducted in the years 2019 and 2021, it even appeared as the prevalent species. In the opinion of the authors, in this environment climate change is the main threat to the species' survival. In fact, in the last years of observation, the species seems to have become extremely rare in the prairies at an altitude of about 2,100 meters where it was originally widespread and where the specimens that led to the discovery and description of the species were found.

We were able to collect only two specimens in Campo Imperatore in 2015 at an altitude of about 2100 meters. In the following years, even in evenings with very favorable climatic conditions, at the afore mentioned altitude of 2100 meters, it was no longer possible to find other specimens of *Agrotis proverai*, while the species, in the same evenings, was found abundant only at a higher altitude of at least 200 meters, in the basin of Campo Pericoli.

The observations hitherto conducted, therefore, lead to fears of the local extinction of the species at lower altitudes and the restriction of its distribution range, limited to higher altitudes. Even within the limits of observations still limited to a small number of years and therefore potentially conditioned by other factors of population fluctuation, the first results appear alarming and seem to outline an elevation of about 200 meters in the distribution of the species over a period of time of about 40 years.

Similar observations (Sistri *et al.*, 2022) have recently been conducted also on *Erebia pandrose sevoensis* Willien & Racheli, 1975, an endemic subspecies of the Monti della Laga for which historical data have provided evidence of uphill movements of several meters per year, thus indicating that this species suffers from the increase in temperature. According to the same authors, the distribution models of the species suggest that these mountain populations will suffer a generalized loss of climatic suitability, which, according to projections, could lead to the extinction of the Apennine population in a few decades.

Like *Erebia pandrose sevoensis* also *Agrotis proverai*, for its iconicity and relative ease of observation and its distribution restricted to a single mountain group, different from that of *Erebia pandrose sevoensis,*could represent an excellent indicator of the state of conservation and evolution of the populations of the most orophile invertebrates of the Gran Sasso and Monti della Laga National Park.

Taxonomical conclusions

Barcoding analysis was performed on three of the specimens collected with the usual methods. The results obtained do not differ from those of Ronkay & Huemer (2018) and therefore confirm the limited genetic distance between *Agrotis proverai* and *Agrotis fatidica*, the genetically closest species of the group, which is approximately 0.5%.

As regards the copulating apparatus of the male according to Fibiger (Fibiger, 1990), the male genitalia of the species of the *A. fatidica* group seem to be extremely variable and it is not possible to find specific differences.

According to the authors (Zilli *et al.*, 2010), male genitalia of *A. proverai* are very like those of *A. fatidica* and no significant differences have been found.

Based on our observations, there are no significant differences between the two species even in female genitalia.

So the most important differences between *Agrotis proverai* and *Agrotis fatidica* remain only those of the external morphology of adults, dealt with in the previous paragraphs.

Fig. 5. *Agrotis proverai*; female genitalia. Italy, Abruzzo, Gran Sasso, Campo Pericoli, 21.VIII.2019.

The population of Central Italy, therefore, appears to be weakly differentiated, both from genetic and morphological point of view, from the Alpine populations of *Agrotis fatidica*.
The correct systematic classification of allopatric populations is one of the major themes of taxonomy because there is no standardized method for establishing whether allopatric populations represent single or different species (MUTANEN *et al.*, 2012). For the same authors, a solution to delineate the allopatric populations could be to reserve the status of species for cases in which the divergence is evident for two or more independent characters.
Following this criterion, in the authors' opinion, the taxon *proverai* is certainly valid even if its specific validity is questionable and therefore it remains doubtful whether the taxon *proverai* should be assigned a specific or subspecific rank.

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