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Two new day-flying species of *Agrotis* Ochseneimer (Lepidoptera: Noctuidae) from the alpine summit of the Maunakea Volcano

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Abstract

Two new endemic Hawaiian species of *Agrotis* Ochseneimer (Noctuidae) are described: *A. helela* and *A. kuamauna*. Both species are day-flying and occur at high-elevations. Observations of adult and larval morphology and biology are included, as well as illustrations of adult moths and genitalia for both sexes.

Key words: Hawai'i, Mauna Kea, Maunakea, Mauna Loa, Maunaloa, Noctuinae, morphology, taxonomy

Introduction

Agrotis Ochseneimer 1816 (Noctuidae) is a cosmopolitan genus with approximately 300 described species. Recent overviews of the genus along with diagnostic characters of the male and female genitalia are given in Lafontaine (2004) and San Blas (2014, 2015). The larvae of some species are considered major agricultural pests (San Blas 2014). As adults, the group is easily identified in Hawai'i using external characters by virtue of *Agrotis* having a large number of conspicuous spines along the tibiae and tarsal segments of all three pairs of legs (Zimmerman 1958). Additional genitalia characters for *Agrotis* listed by Lafontaine (2004) are also present in the Hawaiian species. Hawai'i has 27 species of *Agrotis*, 26 of them endemic with *A. ipsilon* being an accidental introduction (Nishida 2002, where *A. diplosticta* is listed as a separate species though it is a synonym of *A. hephaestaea*; Zimmerman 1958). A recent morphology-based phylogeny suggested that the Hawaiian species may be derived from a Neotropical colonist (San Blas 2015). Although new species of *Agrotis* are described periodically, no new species were described from Hawai'i since 1932 (Zimmerman 1958). Very little genitalic variation exists in the group, and other structural diagnostic characters are lacking as well, although some variation exists in the triangular processes of the male antennal flagellomeres (Zimmerman 1985). Wing pattern, locality information, and diet are necessary to separate the species of *Agrotis*. Two new day-flying members of *Agrotis* that are endemic to high elevation areas (2800m+) on Hawai'i Island are described in this paper.

Materials and methods

Adult moths were collected by hand with a net, or in other cases, reared from larvae. Larvae were captured with tuna (canned) baited pitfall traps that were placed throughout the Alpine Stone Desert ecosystem on Maunakea Volcano. Larvae that were medium (~20mm) to large (>23mm) were reared in a laboratory until they died or

reached maturity. Only medium to large larvae were collected because the main goal was to rear specimens to maturity. Larvae were placed in containers with ~ 2cm of volcanic ash. They were fed fresh, dead, adventive katydids (*Conocephalus saltator* (Saussure) (Tettigoniidae), between 0.5–1cm in body length) every 2–3 days and were provided with a piece of sponge as a constant source of moisture. Larvae were fed *C. saltator* because they were readily available outside the rearing lab where no insecticides are used. Larval behavior, molting, and pupation activities were documented along with general environmental conditions in the rearing location in a climate controlled laboratory at ~21°C. Genitalia were prepared and mounted on slides using the following protocol: Abdomens were soaked in simmering 10% KOH solution for one hour, genitalia were removed, stained with lignin pink and chlorazol black, soaked in a sequence of 30% ethyl alcohol, 90% ethyl alcohol, 100% isopropyl alcohol, and Euparal essence, then spread on microscope slides and mounted in Euparal (Bioquip, Rancho Dominguez, CA, USA). Digital photographs were taken with a Canon EOS-1D Mark II camera using a Microptics Digital Imaging System. Terminology for male genital characters generally follows San Blas (2015).

DNA was extracted from the legs of field-caught and loaned specimens using the standard protocol described in Qiagen's (Valencia, CA, USA) DNeasy kits. Using polymerase chain reaction (PCR), we amplified the mitochondrial gene *Cytochrome c Oxidase I* (COI) in two fragments. The primers LCO-1490 and HCO-2198 amplified 658 bp of COI; Jerry-k485 and Pat-k508 primers amplified 826 bp of the gene. For primer sequences see Appendix 1. Thermal profiles for PCR began with denaturation at 94°C (2 min) followed by 40 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30s, and elongation at 70°C for 1 min. Cycles were followed by a final extension step at 70°C (12 min). Amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and run on an ABI Prism 377 XL automated DNA sequencer. Genbank accession codes are listed in Appendix 2.

Institutional Abbreviations:

BPBM B.P. Bishop Museum, Honolulu, HI, U.S.A.

UHIM University of Hawai'i Insect Museum, Mānoa, HI, U.S.A.

Agrotis helela Medeiros, sp. nov.

Diagnosis. *Agrotis helela* is superficially most similar to *A. microreas* Meyrick. The two are conspicuously smaller than all other congeners, with average forewing lengths of approximately 20–25mm. The two species can be distinguished by the configuration of the white forewing bands: horizontal in *A. microreas* (see Walsingham, 1907b as well as Fig. 1F) and vertical in *A. helela* (Figs. 1A & 1E). The male and female genitalia of the two are indistinguishable.

Description (Figs 1A, 1E, & 2A). *Head*: Vertex and frontoclypeus greyish-brown, heavily scaled. Ocelli present. Antenna ca. 0.6–0.7x forewing length; flagellomere bipectinate in males, each segment cylindrical and without triangular processes (*sensu* Zimmerman 1958 pg 216); flagellomere filiform in female. Labial palpus greyish-brown; porrect; third segment drooping; heavily scaled, length ca. 2.5x diameter of eye; proboscis naked.

Thorax: Grey dorsally and ventrally. Legs grey with lighter scales near apex of tarsal segments; all tibiae and tarsal segments heavily spined; midleg with one pair of tibial spurs; hindlegs with two pairs. Forewing expanse 21–24 mm (n = 8), dorsal ground color dark grey to black with a few lighter scales near base; prominent whitish antemedial and postmedial bands running from costa to anal margin; faint, poorly defined broad medial band running from costa to anal margin, whitish subterminal spots present; fringe grey; grey ventrally. Hindwing greyish-brown, fringe greyish-brown; light grey ventrally.

Male genitalia (Fig 3C): Uncus thin, curved basally then straight, with fine setae along nearly entire length except at base. Tegumen truncated at apex. Valva subrectangular, long, narrow, with band of strong setae immediately basad of corona. Sacculus well defined, extending ~0.7x length of valva and terminating in a pointed apex; ampulla not swollen. Saccus narrow, U-shaped, with anterior spine-like projection. Juxta subrectangular. Aedeagus broad, cylindrical, with long internal sac (*sensu* Zimmerman 1958 pg 216), apex adorned with very short cornuti.

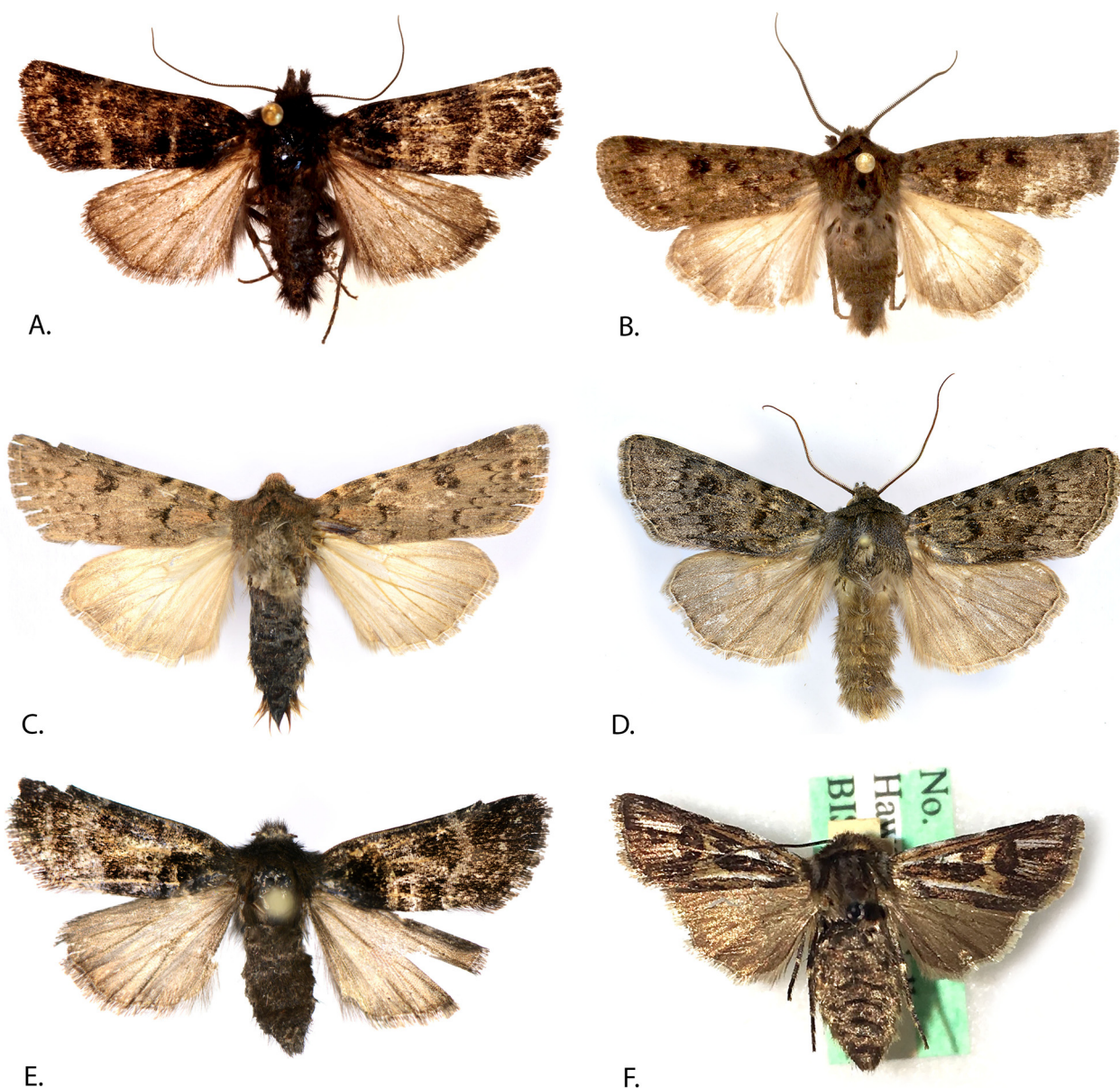


FIGURE 1. A: *A. hehela*, paratype voucher AP117, wing expanse 28.5mm. B: *A. kuamauna*, holotype voucher AP114, wing expanse 46.6mm. C: *A. kuamauna*, paratype (voucher AP114; UHIM), wing expanse 40.5mm. D: *A. epicremna* (voucher AP327; UHIM), wing expanse 48mm. E: *A. hehela*, paratype (voucher AP117; UHIM), wing expanse 24mm. F: *A. microreus* (BPBM), wing expanse 26mm.

Female genitalia (Fig 3D): Posterior apophysis 1.3x as long as anterior apophysis; ductus bursae 5x as long as anterior apophysis; corpus bursae 2.5x as long as anterior apophysis, signum absent; appendix bursae 9x as long as corpus bursae, apex globose; ductus seminalis originating laterally near corpus bursae apex.

Abdomen: dark grey with tufts of scales projecting laterally from each segment.

Larva: Unknown.

Pupa: Unknown.

Holotype: UNITED STATES: HAWAII: **Hawai'i Island**: Mauna Kea, Hale Pohaku Region, 9623 ft, N19°45.835' W155°27.032': 1 ♂, 4 Jun 2014, J. Eiben (UHIM).

Paratypes: UNITED STATES: HAWAII: **Hawai'i Island**: Mauna Loa; 3360m, 15 Jun – 1 Jul 1967: 2 ♂, 10 ♀, R.T. Hansen (BPBM). Mauna Loa; 3360m, 12 Jul 1967: 2 ♀, R.T. Hansen (BPBM). Mauna Loa Station, 11,200', 1–3 Jul 1967: 1 ♂, 1 ♀, R.T. Hansen (BPBM). Mauna Kea, 3900m, 13 Jul 1967: 1 ♀, R.T. Hansen

(BPBM). Mauna Kea, 4140m, 18 Jun 1967: 1 ♀, R.T. Hansen (BPBM). Mauna Kea, 4140m, 18 Apr – 2 Jun 1967: 3 ♀, R.T. Hansen (BPBM). Mauna Kea Summit area, 10 May 2005: 1 ♂, 1 ♀, A. Prestes, (vouchers AP116 & AP117; UHIM). Maunakea, lower Halepōhaku parking lot, 9,260 ft 19.7609 -155.4561: 1 ♀, 1 Jul 2013, J. Eiben (UHIM). Maunakea, Halepōhaku region, 10,538', N19°6.312' W155°6.909', 23 Jul 2014: 1 ♂ (slide LB80), 1 ♀, J. Eiben (UHIM). Maunakea, summit region, ~13,000', 19.8194 -155.4745 batch plant parking lot, 6 Aug 2012: 1 ♀ (slides LB81 and LB81 “wings”), J. Eiben (UHIM). Maunakea, west of Halepōhaku, 5 May 2016: 1 ♀, J. Eiben (UHIM). Mauna Loa, 10,800', 19.6239 -155.5852: 1 ♂, 29 May 2005, M.J. Medeiros (UHIM). Maunakea, pu‘u south of the Very Long Baseline Array (VLBA) radio observatory, ~12,140', 19.7978 -155.4575, 8 Jul 2014: 1 ♂, J. Eiben (UHIM). Maunakea, batch plant parking lot, ~13,000' 12 Jun 2014: 1 ♀, J. Yoshina (UHIM).

Etymology. The Hawaiian term “hele lā” means to travel by day, as opposed to “hele pō,” to travel by night. A beloved Hawaiian goddesses of O‘ahu Island is Kaiona, patroness of travelers; her home is on the summit of Ka‘ala, and she is referred to as ka wahine hele lā (the woman who travels by day). *Agrotis helela*, therefore, refers to the *Agrotis* moth that flies diurnally, though only on Hawai‘i Island.

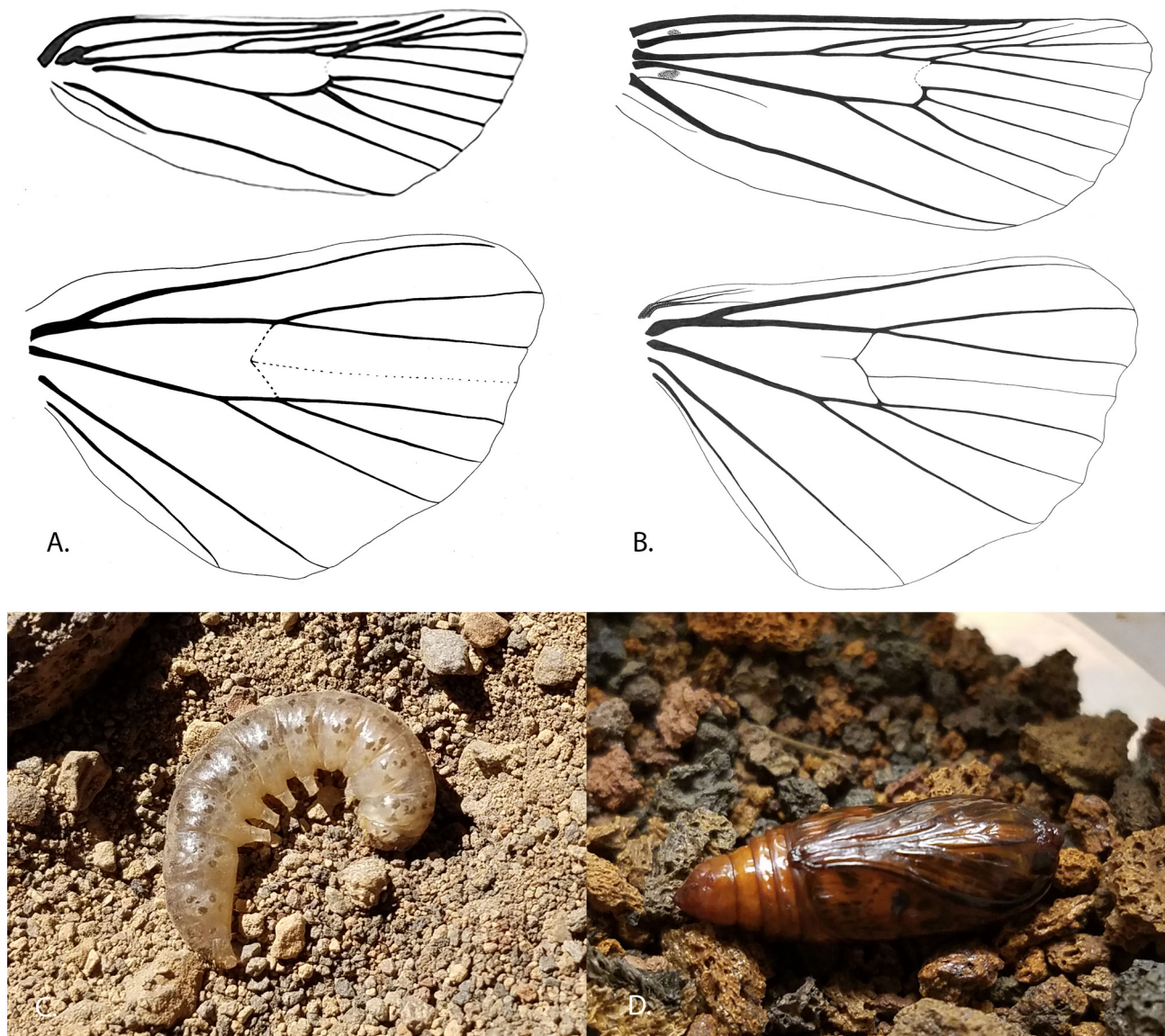


FIGURE 2. A: Wing venation of *A. helela* (from slide LB81 “wings”). B: Wing venation of *A. kuamauna* (from slide LB82 “wings”). C: *A. kuamauna* larva, length = 20mm. D: *A. kuamauna* pupa, length = 15mm.

Remarks. *A. helela* is diurnal and occurs on both Maunakea and Maunaloa volcanoes. On Maunakea, *A. helela* are commonly found flying throughout the alpine region on sunny days, even during high winds. Additionally, adults have been observed on flowers of the endemic silversword, *Argyroxiphium sanwicense* subs.

sandwicense that exist in the alpine and subalpine ecosystems on Maunakea. Although we lack information on the larval and pupal stages of this species, we hypothesize that adults occur across the alpine or subalpine region of the volcano, and may facilitate pollination of the Maunakea silversword. *Agrotis helela* joins the apparently extinct *A. microreas* Meyrick (<http://hbs.bishopmuseum.org/endangered/ext-insects.html>) as the second Hawaiian species to be conspicuously smaller than its congeners.

This *Agrotis* species is not the only diurnal member of the Agrotina subtribe of the Noctuini (Noctuidae), as several species of *Feltia*, the sister genus to *Agrotis*, are diurnal as well. These include *Feltia (Trichosilia) woodiana* (Lafontaine) and *Feltia (Trichosilia) troubridgei* Lafontaine, both from northern Yukon territory, Canada, *Feltia (Trichosilia) boreana* (Lafontaine), which ranges from Hudson Bay, Canada, to northeastern Alaska, U.S.A., and *Feltia (Trichosilia) beringiana* (Lafontaine & Kononenko), from northern Yukon, Canada, to northeastern Alaska, U.S.A., and Chukotka in northeastern Siberia, Russia. Each of these four *Feltia* species are similar in phenotype to *A. helela* in being smaller than their congeners. The small body size and diurnal flight behaviors of *A. helela* and *Feltia* species are consistent with evolutionary adaptations of arthropods that persist in alpine and high latitude environments (Somme 1989). *Specimens* AP116, AP117, and 05A54 had the COI “barcode” region sequenced with primer pairs LCO/HCO (Appendix 1) and have Genbank accession codes listed in Appendix 2.

***Agrotis kuamauna* Medeiros & Kirkpatrick, sp. nov.**

Diagnosis. *A. kuamauna* is very similar to, and possibly sister to, *A. epicremna* Meyrick; both species have acute triangular processes on each segment of the male antennal flagellomere. However, *A. epicremna* occurs on Haleakalā on Maui and is presumed to eat *Argyroxiphium sandwicense* (Zimmerman 1958) in contrast to a diet based on insects, various plants, and lichens, as observed in *A. kuamauna*; the spots on the wings of *A. kuamauna* are consistently more irregular and less well-defined at their edges than those of *A. epicremna* (see Figs. 1B, 1C, & 1D for comparison).

Description (Figs. 1B, 1C, & 2B). *Head*: Vertex and frontoclypeus mottled black to brown, heavily scaled. Ocelli present. Antenna ca. 0.7–0.8x forewing length; flagellomere bipectinate in males, each segment with acute triangular processes (*sensu* Zimmerman 1958 pg 216); flagellomere filiform in female. Labial palpus mottled black to brown; porrect; third segment drooping; heavily scaled, length ca. 2.0x diameter of eye; proboscis naked.

Thorax: Mottled black to brown dorsally and ventrally. Legs mottled black to brown with light brown scales near apex of each segment; all tibiae and tarsal segments heavily spined; midleg with one pair of tibial spurs; hindlegs with two pairs. Forewing expanse 31–40 mm (n = 15), ground color brown; pronounced black medial reniform spot present near costal margin; several smaller antimedial black spots present; black subbasal band running from costa nearly to anal margin; irregular black subterminal band present running from costa to anal margin; black terminal spots present; fringe brown; brown ventrally. Hindwing light brown dorsally and ventrally.

Male genitalia (Fig 3A): Uncus thin, straight except along base, with fine setae along nearly entire length, heavy at apex. Tegumen truncated at apex. Valva subrectangular, gradually widened on dorsal half, long, narrow, with band of strong setae immediately based of corona. Sacculus well defined, extending ~0.6x length of valva and terminating in a pointed apex, ampulla swollen at base. Saccus narrow, U-shaped, with anterior spine-like projection. Juxta subrectangular. Aedeagus broad, cylindrical, with long internal sac (*sensu* Zimmerman 1958 pg 216), apex adorned with very short cornuti.

Female genitalia (Fig 3B): Posterior apophysis similar in length to anterior apophysis; ductus bursae 1x as long as anterior apophysis; corpus bursae 0.8x as long as anterior apophysis, signum absent; appendix bursae 9x as long as corpus bursae, apex globose; ductus seminalis originating laterally very near corpus bursae apex.

Abdomen: Brown with tufts of scales projecting laterally from each segment.

Larva (Fig. 2C): Early instar larva mottled light brown to dark brown dorsally, four distinct black spots dorsally on each dorsal segment: two anterior spots near each other per segment and two posterior spots farther apart from each other. Black spots not as distinct in mature larva. Faint dorsal-medial line parallel to body, light in color. Semi-transparent ventrally, segments apparent, with sparse setae. Head capsule semicircular, dark brown, mandible strong, antennae with single setae. Each leg with a single terminal claw and long setae.

Pupa (Fig. 2D): ~15 x 5 mm, reddish brown, rounded anteriorly, tapered posterior. Brownish red, black spots on wings become visible through membrane, blackish brown before eclosion. Darker color lines the posterior of

segments 5–10, single cone on segments 6, 7, 8, black spot on segments 5–8. Cremaster small with two acute hooks; hooks diverging from each other.

Holotype: UNITED STATES: HAWAII: **Hawai‘i Island:** Mauna Kea VLBA, 12,000 ft.: 1 ♂, 8 Jan 2017, J. Kirkpatrick (UHIM).

Paratypes: UNITED STATES: HAWAII: **Hawai‘i Island:** Maunakea, 3,840m, 19.81544 -155.45583, 26 Oct 2015, pup. 9 & 11 Nov 2015, em. 1 Dec 2015: 3 ♂, J. Eiben (BPBM). Maunakea, Halepōhaku region, 3171 m, 19.77039 -155.45267, 21–27 Feb 2014, pup. 1 May 2014: 1 ♂, J. Eiben (UHIM). Maunakea, Halepōhaku region, 3171 m, 19.77028 -155.45221, 21–27 Feb 2014, em. 29 May 2014: 1 ♀, J. Eiben (BPBM). Maunakea, Halepōhaku region, 9605 ft, N19°45.836 W155°27.668 (at UV light), 28 Jun 2013: 2 ♂ (slide LB84), J. Eiben (UHIM). Maunakea, Halepōhaku Facility, 9,270ft 19°45′40.54″N 155°27′20.63″W, 7 Jun 2012: 1 ♂ (slide LB83), J. Eiben (UHIM). Maunakea MKSR, 30 Aug 2016: 1 ♀, F. Klasner (BPBM). Site ID: TH7, 26 Oct 2015, pup. 15 Nov 2015, em. 4 Dec 2015: 1 ♂, J. Eiben (UHIM). Maunakea, Pu‘uwēkiu base, 12,750ft 19.81834 -155.4599, 26 Oct 2015, pup. 13 Dec 2015, em. 1 Jan 2016: 1 ♂, J. Eiben (UHIM). Maunakea, Pu‘uwēkiu base 12,650ft, 19.81815 -155.45627, 26 Oct 2015, pup. 7 Dec 2015: 1 ♂, Dominique Zarders (UHIM). Maunakea, Pu‘uwēkiu base, 12,650ft 19.81815 -155.45627, 26 Oct 2015, pup. 12 Nov 2015, em. 1 Jan 2016: 1 ♀, J. Eiben (UHIM). Maunakea, North of Pu‘ulilinoe, 12,700ft, 19.81538 -155.45906, 26 Oct 2015, pup. 29 Nov 2015: 1 ♀, J. Eiben (UHIM). Maunakea, West Halepōhaku region, 9,500ft, N19°45.636 W155°27.683 (at UV light), 28 Jun 2013: 1 ♀ (slides LB82 and LB82 “wings”), J. Eiben (UHIM). Mauna Kea, TMT13B, 26 Sept 2008: 1 ♀, J. Eiben & Brenner, (voucher AP115; UHIM). Mauna Kea, PuusVLBA West facing slope, 17 Jul 2006, 1 ♂, D. Rubinoff & J. Eiben, (voucher AP113, UHIM). Mauna Kea, Burns cone, 14 May 2006: 1 ♂, A. Prestes, (voucher AP114; UHIM).



FIGURE 3. A: *A. kuamauna*, male genitalia (slide LB83) scale bars = 1mm. B: *A. kuamauna*, female genitalia (slide LB82) scale bar = 1mm. C: *A. helela*, male genitalia (slide LB80) scale bars = 1mm. D: *A. helela*, female genitalia (slide LB81) scale bar = 1mm.

Etymology. The Hawaiian word “kuamauna” means “spine [of the] mountain” or “mountaintop,” referring to the presence of *A. kuamauna* on the uppermost slopes of Maunakea.

Remarks. *A. kuamauna* larvae have been collected throughout the alpine and subalpine ecosystems (3000–4205m) on Maunakea including tephra rock cinder cones and glacial till substrates. Larvae appear to be associated with ash substrates which are higher in relative humidity and experience smaller changes in temperature than cinder substrates (Eiben & Rubinoff 2010). The ash substrates may reduce larval desiccation and allow for thermoregulation in this extremely dry and harsh environment, as has been observed in the co-occurring wēkiu bug (Duman & Montgomery 1991; Eiben & Rubinoff 2014). Observations made in the lab and field over a decade suggest that the larvae of this species are omnivorous and although they appear to prefer protein sources, they will also feed on plant material blown up to the alpine region (Howarth 1987); *A. kuamauna* larvae were observed to readily consume tuna and wind-deposited insects in field baited pitfall traps. A generalist carnivorous diet was observed in the laboratory for this species as well, with larvae able to successfully pupate and emerge as adults after being fed an insect-only diet. Unconfirmed field observations suggest that larvae may also feed on lichens (Duman & Montgomery 1991). Generally, larvae stop eating and lay in a supine position 2–3 days before pupation. Most larvae pupated on the surface of the ash, but a few specimens also buried their head under the ash with their abdomen erect. One larva built a loose ash case, apparently combined with salivary excretions, in which it pupated. This casing completely covered the pupa and was about 25 mm in length, 15 mm in width, and 10 mm in height. This particular specimen took about 10 days from the time it stopped eating until pupation. Eclosion for all specimens occurred about 30 days after pupation in laboratory temperatures at 20–22°C. Like *A. helela*, *A. kuamauna* is diurnal, though not small or particularly dark in color.

Discussion

These two new *Agrotis* species join several other diurnal, high-elevation moths endemic to the Hawaiian Islands, including two species of *Thyrocopa* (Xyloryctidae; Medeiros 2009), along with others such as species from *Hyposmocoma* (Cosmopterigidae), *Eudonia* (Crambidae), *Orthomecyna* (Crambidae), and *Tamsica* (Crambidae) (Medeiros, personal observations). Diurnal flight adaptations are common in species that live in alpine or tundra environments (Somme 1989). The cold night temperatures (often between 4–0°C) experienced in these environments may make flight difficult. The diurnal flight habits of these Hawaiian alpine moth species probably enable flight when temperatures are not prohibitively cold. Survival in these extreme environments also requires some degree of cold-tolerance; unidentified *Agrotis* larvae, which may have been *A. kuamauna* from Maunakea, were able to survive for 24 hours at -6.2°C in an experiment (Duman & Montgomery 1991). This experiment showed that *Agrotis* caterpillars are freeze tolerant, however, the mechanism of such tolerance is unknown. A similar new species with slightly different wing pattern may also occur on Haleakalā, Maui (specimens in UHIM), though it will be treated separately.

Acknowledgements

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APPENDIX 1. Primer pairs used in this study.

Gene	Primer Name	Sequence (5' to 3')	Reference
COI	LCO	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> 1994
COI	HCO	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> 1994
COI	Jerry	CAACAYTTATTTTGTATTTTGG	Simon <i>et al.</i> 1994
COI	Pat	ATCCATTACATATAATCTGCCATA	Simon <i>et al.</i> 1994

APPENDIX 2. GenBank accession codes.

Specimen	Species	Primer pair	GenBank accession code
AP116	<i>A. hehela</i>	LCO/HCO	MH753505
AP117	<i>A. hehela</i>	LCO/HCO	MH753506
05A54	<i>A. hehela</i>	LCO/HCO	MH753507
AP113	<i>A. kuamauna</i>	Jerry/Pat	MH753502
AP114	<i>A. kuamauna</i>	Jerry/Pat	MH753503
AP115	<i>A. kuamauna</i>	Jerry/Pat	MH753504
AP323	<i>A. epicremna</i>	Jerry/Pat	MH753497
AP324	<i>A. epicremna</i>	Jerry/Pat	MH753498
AP325	<i>A. epicremna</i>	Jerry/Pat	MH753499
AP327	<i>A. epicremna</i>	Jerry/Pat	MH753500
AP328	<i>A. epicremna</i>	Jerry/Pat	MH753501