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Notes on the genus *Chrysodeixis* Hübner, [1821] (Lepidoptera: Noctuidae) on St Helena Island

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Abstract: Until now, only a single species of *Chrysodeixis* has been reported from St Helena Island: *Chrysodeixis dalei* (Wollaston, 1879). The authors were able to confirm the existence of three species of the genus on the island: *Chrysodeixis acuta*, *C. chalcites* and *C. includens*. The barcoding of the moths and the examination of the genitalia structure have shown that *Plusia dalei* Wollaston, 1879 **syn. nov.,** is not an endemic species, but a synonym of the American *Chrysodeixis includens* (Walker, 1858).

Key words: Lepidoptera, Noctuidae, Chrysodeixis, species, St Helena Island, Ascension Island.

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INTRODUCTION

St Helena is a small, remote island of about 121 km^2, situated in the middle of the Southern Atlantic Ocean, between Africa and South America. The nearest continent is Africa, but is more than 1600 km away.

Until its discovery in 1502 by the Portuguese captain João da Nova, St Helena was uninhabited by humans. Most of the island was covered by low woodland, composed mainly of endemic arborescent Malvaceae and Asteraceae and on the highest peaks there were thickets of tree-fern. There were some areas with semi-desert vegetation, which were confined to the coast (Cronk, 1989). By the end of 16th century the island was visited quite regularly by Portuguese ships sailing back from East India. In 1589 the visiting Dutchman Jan Huyghen van Linschoten pointed out that "… in the valleyes [were] planted al sorts of fruites: which have growne there in so greagt abundance, that it is almost incredible. For it is so full of Goates, Buckes, wild Hogges, Hennes, Partridges and Doves, by thousands, so that any man that will, may hant and take them", and "… Now for fruites, as Portingall Figges, Pomgranets, Oranges, Lemons, Citrons, and such like fruites, there are so many, that growe without planting or setting, that all the valleyes are full of them", … (Thiele, 1885, in Ashmole & Ashmole, 2000). This shows that the introduction of non-indigenous plants and animals began very soon after the discovery of the island. This was continued throughout the following centuries, when the island became the property of the British East India Company and was an important stopover for ships plying between Britain and East India.

The first comprehensive report on the Lepidoptera of St Helena was published by Melliss (1875). The moths and butterflies were identified and described by F. Walker, and J. Ch. Melliss added some information about localities and abundances of the species. In 1879, Mrs. E. Wollaston reported on the Lepidoptera she found during her stay with her husband in 1875 and 1876. Further moths and butterflies were collected in the 20th century by the members of the two Belgian expeditions between 1965 and 1967, by Professor A. Loveridge between 1957 and 1980, occasionally by P. Ashmole at the end of the 20th century, and by the first author in 1995. Ashmole & Ashmole (2000) noted that there are more than 100 species of Lepidoptera on the island, of which just over 50 are endemic. This suggests that during recent centuries a large number of moth species were imported, certainly mainly accidentally. This is an ongoing process and includes the risk of introducing pest species of crops or fruits. During the 1980's a system was put in place whereby all imported fresh plant material was inspected on arrival through the only inlet to the island via shipping vessels – Jamesbay. Subsequent revisions were made to improve the system, and between 1997 and 2000, a formalized system was implemented under the St Helena Government (SHG) by way of an Integrated Pest Management Project led by Dr J. Key. During her second visit as a Pest Control Security Officer the system was further revised and formalized to include both sea and air inlets to prevent the entry of unwanted pests which could pose threats to the environment or economy of the island.

Our study is of members of the genus *Chrysodeixis* Hübner, [1821] found on St Helena Island. Until now,

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only one species had been recorded, but our studies have revealed the presence of at least three species.

MATERIALS & METHODS

The recent study is based on the material collected for the Darwin Plus Project DPLUS040 "Securing the future for St Helena's endemic invertebrates" in 2017 by the first author, his additional material from the collecting trip in 1995, and information collected about *Chrysodeixis* specimens by the Biocontrol Department of Agriculture and Natural Resources Division (ANRC), St Helena Government.

From eight specimens, genitalia dissections were undertaken following standard protocol. DNA extraction from seven specimens was performed, using a single leg from each specimen. Total genomic DNA was extracted using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek Inc., Norcross, USA) according to the manufacturer's protocol for tissue DNA, except for some smaller modifications. Instead of only two hours lysis time we performed the lysis overnight and instead of adding two times 200 µl Elution Buffer at the end, we used only 100 µl. Elution was performed twice with 100 µl Elution buffer each. A partial fragment (658 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by PCR using the primers LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hebert *et al*., 2004). Amplifications were performed in 15 µl reactions containing 7.5 µl 2x Qiagen Multiplex PCR Plus Master Mix (Qiagen, Hilden, Germany), 0.3 µM of each primer, RNase-free water and 1.5 µl template DNA. Amplification conditions were: initial PCR activation step at 95°C 5 min, 38 cycles of 30 s denaturing at 95°C, 90 s annealing at 49°C, 90 s extension at 72°C, followed by a final extension of 30 min. at 68°C. PCR products were visualized on a 1.4% agarose gel stained with Gel Red (0.1, Biotium, Hayward, USA). PCR products were purified with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Life Technologies, Darmstadt, Germany) and sequenced on an ABI3730XL sequencer using Big Dye v. 3.1 Terminator Kit (Thermo Fisher Scientific, Darmstadt, Germany) by Macrogen, Netherlands. Sequencing was performed with the same primers used for amplifying. Sequences were assembled and manually checked using Geneious 10.1.3 (Kearse *et al*., 2012) and aligned using BioEdit 7.2.5 (Hall, 1999).

A phylogenetic tree was calculated using the Maximum Likelihood method (ML) implemented in IQ-TREE 1.6.1 (Nguyen *et al.*, 2015). The best fitting model for the analysis, the Transition model, including empirical base frequencies and the FreeRate model (Yang, 1995; Soubrier *et al*., 2012), and also including the allowance for a proportion of invariable sites (*TIM2+F+I*), was obtained by ModelFinder (Kalyaanamoorthy *et al*., 2017). The ML analysis included 1,000 SH-aLRT (Guindon *et al*., 2010) and UFBoot (Ultrafast Bootstrap Approximation; Minh *et al*., 2013) replications, using all sites. SH-aLRT / UFBoot values > 70/80 % are shown on the ML tree next to the nodes concerned.

The recorded material is stored in the collection of the Museum für Naturkunde und Vorgeschichte Dessau, in the National Collection of St Helena (housed by St Helena National Trust) and in the collection of the Biocontrol Section of the Agriculture & Natural Resources Division (ANRD).

RESULTS

The phylogenetic hypothesis for nine species of *Chrysodeixis* is depicted in Figure 1, and genitalia dissections are shown in Figs 2–6, and are used to identify the taxa discussed below.

DISCUSSION

Chrysodeixis dalei (Wollaston, 1879)

This species was described by Wollaston (1879), together with three other Plusiinae (*Ctenoplusia limbirena*, *Trichoplusia orichalcea*, *T. vittata*) of African origin. Regarding the habitats and biology of *C. dalei*, Wollaston stated that the species is not uncommon in various parts of the island and at Plantation it is often seen flying around flowers of *Geranium* and *Pittosporum*. At The Barn she found the species amongst scrubwood (*Commidendrum spurium*). Concerning the origin of *C. dalei*, Wollaston was not sure and wrote "… the species (if truly indigenous) may perhaps have belonged to the now well-nigh extinct scrubwood fauna".

Because *C. dalei* remained widespread it was found by other collectors subsequently. So far, the species is known from the following localities: Plantation (1875/1876); The Barn (Wollaston, 1879), 1958, 1962/3, 1976; Varneys (Loveridge), 1967; Teutonic Hall (Belgian expedition), 1995; Longwood Gate; Mt Actaeon; Broadcasting House; Rose Hill; Bevins Gut (Karisch; all Karisch 2001), 2006; Woodcot (Mendel), 1994 – 2003; High Central Region; East Arid Area (Ashmole; after Pryce, 2017). This list of observations is, however, certainly not exhaustive.

Ashmole & Ashmole (2000) noted that *C. dalei* was also found on Ascension Island, about 1 300 km north of St Helena. We do not know the basis for this record, because Robinson & Kirke (1990) only listed *Chrysodeixis acuta* and *Ctenoplusia limbirena* as Plusiinae species for that island. According to the database held in the Conservation Center on Ascension, however, the specimens mentioned by Robinson & Kirke (1990) have been re-identified and named *C. dalei* (J. Sim, in litt., 2018). Judging from the image in Robinson & Kirke (1990) the illustrated specimen is indeed quite similar to *C. dalei*, but this still requires confirmation by examination of the specimens in the collection of The Natural History Museum in London.

In 2018 four specimens of *Chrysodeixis dalei* were provided for barcoding: 2 specimens from about 0.1 km

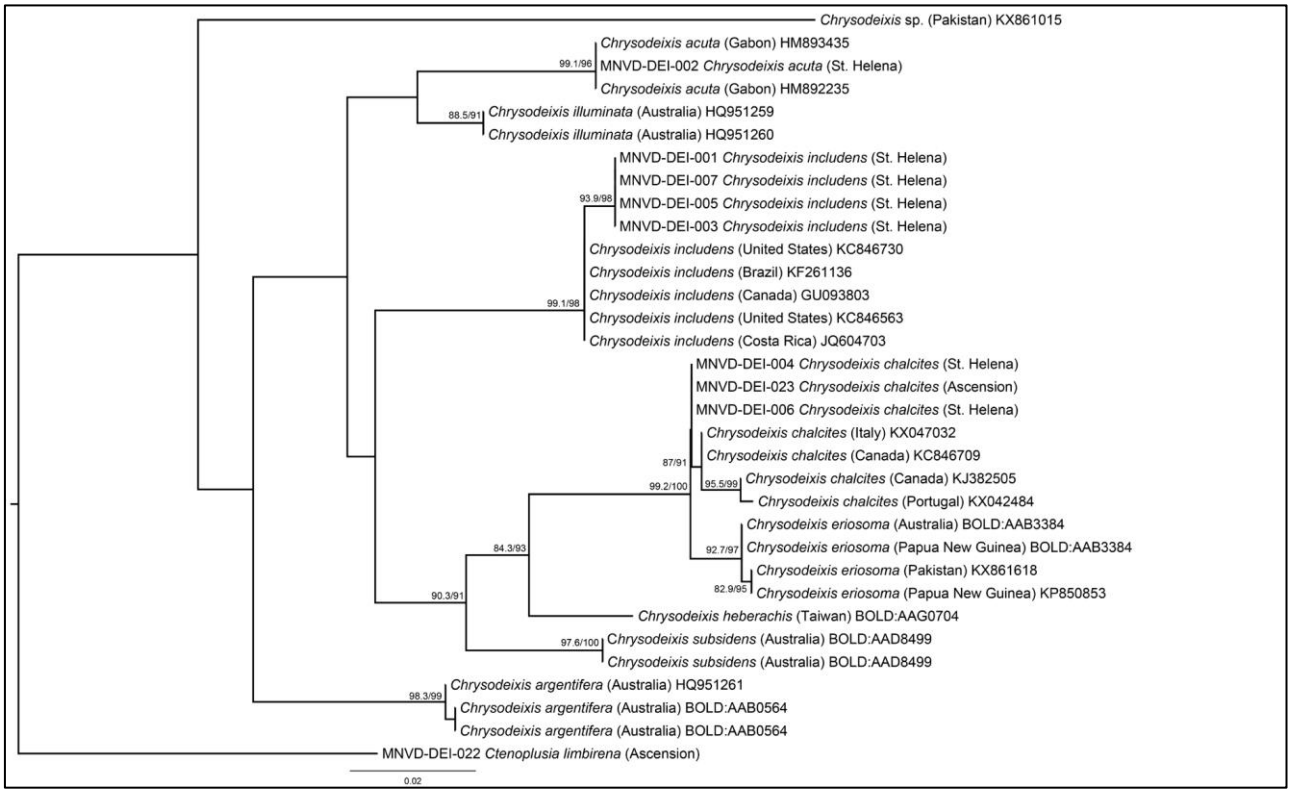


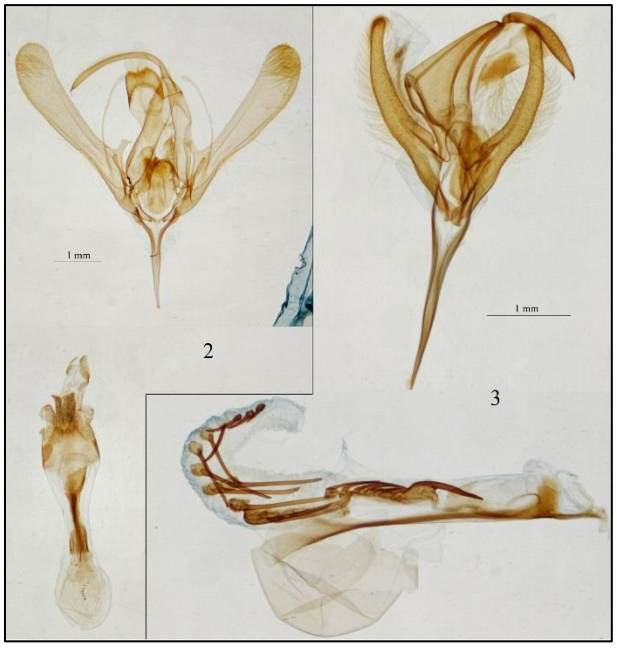
Figure 1 – Phylogenetic hypothesis for nine species of *Chrysodeixis* and, as outgroup taxa, one specimen of *Ctenoplusia limbirena* based on a maximum likelihood analysis of the barcoding section of COI based on the model TIM2+F+I. Values at the branches represent SH-aLRT / UFBoot values. The specimens used for molecular analyses are labelled with the sample ID: "MNVD-DEI-…"—unique specimen identifier — and the country of origin. The other sequences included in the analysis are taken either from GenBank (accession number is mentioned after the species name) or BOLD (BIN number is mentioned after the species name).

E of Mt Actaeon, 08.iii.2017, light trap; 1 specimen from Restauration Area at Ginger Patch on High Peak, 16.iii.2017, light trap; 1 specimen from near the chapel in Sandy Bay Valley, 19.iii.2017, light trap; all leg. T. Karisch (MNVD). No genetic differences were found between these specimens.

A comparison with publicly available sequences from GenBank and the Barcode of Life Data System (BOLD) showed a 99.7 % match with *Chrysodeixis includens* (Walker, 1858) (Fig. 1).

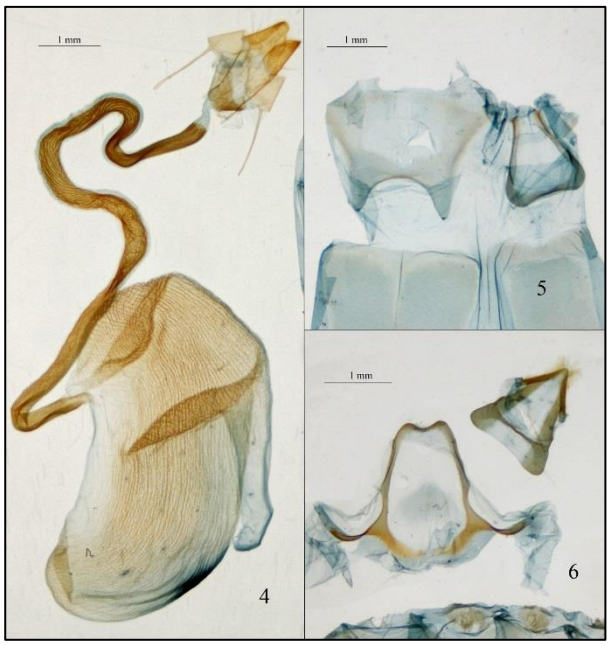
Chrysodeixis includens is a species described from Hispaniola (Santo Domingo) (Poole, 1989). It is known as Soybean Looper and considered to be a pest species on soya beans and tomatoes. However, the larva is polyphagous and feeds on a variety of Amaryllidaceae, Apiaceae, Asparagaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Lamiaceae, Malvaceae, Poaceae, Solanaceae and Verbenaceae. *C. includens* is distributed from (mainly) the eastern and southern parts of the United States, through the Caribbean and Middle America to the Tropic of Capricorn in South America. For North America *C. includens* is reported as a migratory species (EPPO, 2015).

So far, apart from *Ocrasa nostralis* (Guenée, 1854) and *Cosmopterix attenuatella* (Walker, 1864), there have been very few Lepidoptera species of American origin reported from St Helena. This is why a closer examination of the *Chrysodeixis dalei* material from St Helena was necessary in order to establish its relationship to *C. includens*.



T. Karisch

Figure 2 – Male genitalia *Chrysodeixis includens*, gen. slide 3591, Karisch; **Figure 3** – Male genitalia *Chrysodeixis chalcites*, gen. slide 3590, Karisch.



T. Karisch

Figure 4 – ♀-genitalia, *Chrysodeixis acuta*, gen. slide 3592, Karisch; **Figure 5** – ♂, sternit and tergit 8, *Chrysodeixis includens*, gen. slide 3591, Karisch; **Figure 6** – ♂, sternit and tergit 8, *Chrysodeixis chalcites*, gen. slide 3590, Karisch.

The result of this study showed that the pattern and colouration of the wings of *C. dalei* and *C. includens* are identical. Dissection of the male genitalia (Figs 2, 6), and comparison with the illustration in Brambila & Passoa (2013) and Eichlin & Cunningham (1978) revealed no differences. Therefore, we conclude that *C. dalei* cannot be considered as an endemic species of St Helena but is conspecific with *C. includens*, hence:

Plusia dalei Wollaston, 1879: 232 **syn. nov.** for *Chrysodeixis includens* (Walker, 1858).

The food plants of the larva on St Helena are still unknown, but here, fortunately, *C. includens* has not become a pest species. Nevertheless, the Biocontrol Section of the Agriculture & Natural Resources Division on St Helena holds itself responsible for ensuring that the species is not exported from the island to other countries, where it could seriously impact the cultivation of soya beans.

Chrysodeixis chalcites (Esper, 1798)

During his investigations in 2017, the first author came across specimens of a more golden *Chrysodeixis*, which he had never seen before on the island, at Ginger Patch on High Peak (16.iii.2017) and near the chapel in Sandy Bay Valley (19.iii.2017). Some of these were collected. One of these specimens was shown to the noctuid authority at The Natural History Museum, Dr. A. Zilli. He provisionally identified it as *Chrysodeixis chalcites* but advised that identification should be confirmed by genitalia dissection, which was done recently. Additionally, two specimens from near the chapel in Sandy Bay Valley, 19.iii.2017, at light, leg. T. Karisch, were provided for barcoding by the second author. After analysis and comparison with publicly available sequences from GenBank and BOLD (Fig. 1), she confirmed that both specimens were *C. chalcites* (all sequences were a 100% match). All three sequences from our analysed material (two from St Helena and one from Ascension) were 100% identical. The examination of the male genitalia (Figs 3, 6) has shown the same result. Hence, the presence of *C. chalcites* on St Helena is confirmed.

As mentioned above, *C. chalcites* has been reported from Ascension Island, and it is widespread in Africa (De Prins & De Prins, 2018). It might have reached St Helena from Ascension Island or, more likely, from South Africa. It remains uncertain when *C. chalcites* was first reported on St Helena. There is an indication of its presence on St Helena in the work of Fletcher (1910), but because he wrote, "This moth has been recorded, under about a dozen different names, …" we cannot be sure whether he was referring to *Chrysodeixis includens* rather than *C. chalcites*. However, between 1909 and 2011 no other specimens of *Chrysodeixis chalcites* were found on the island. On 16.iv.2011, Dr R. Key photographed a specimen at rest at the Salvation Army Hall in Jamestown. On 03.v.2016, a larva was collected from green bell peppers transported by the R.M.S. "St Helena". The adult moth emerged on 07.vi.2016 after having been raised in the laboratory of the ANRD.

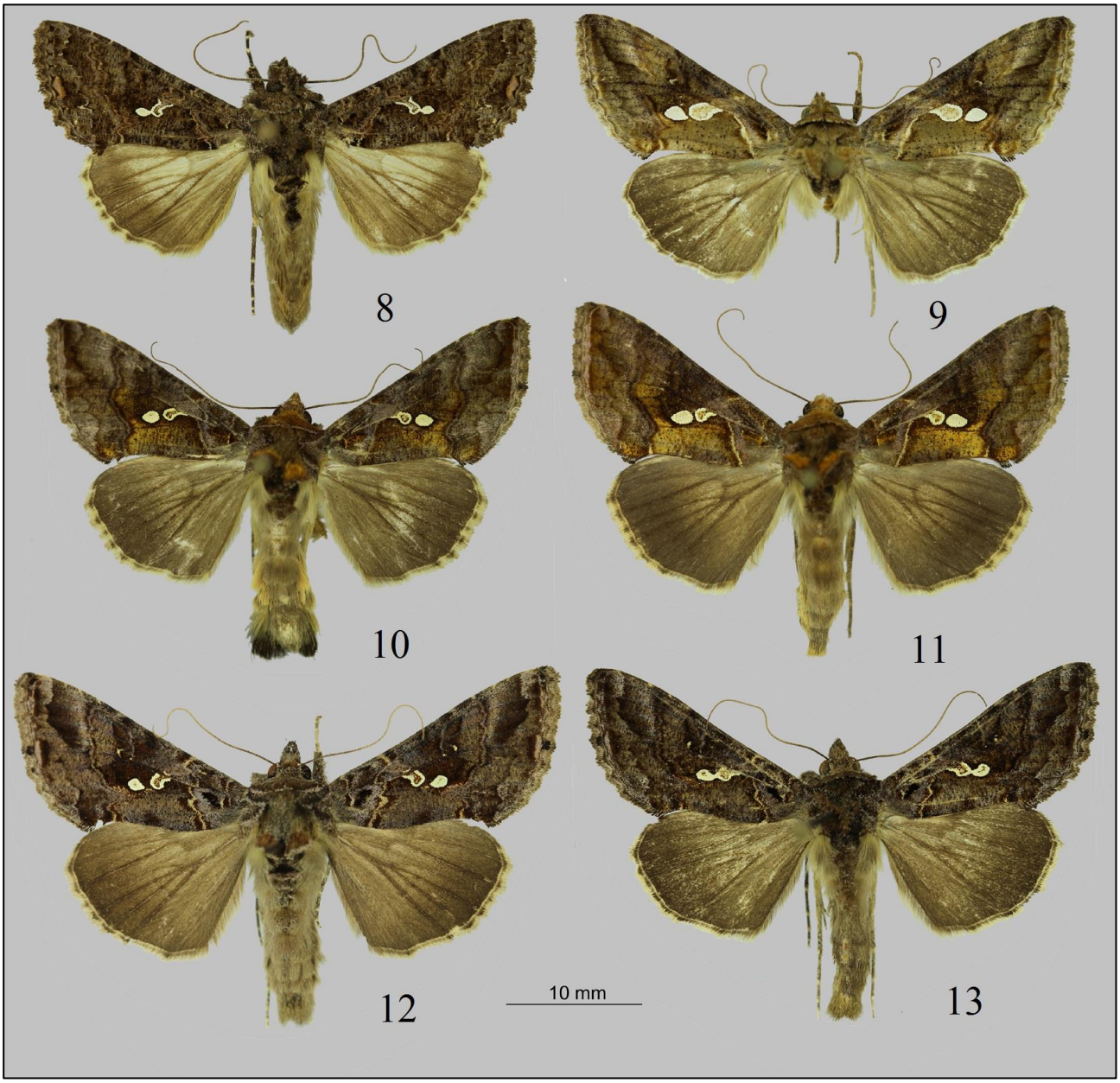
Chrysodeixis acuta (Walker, 1858)

On March 17 2017, the first author operated a light trap in the restoration area at the Ginger Patch on High Peak. In it, he found an unusual looking specimen of *Chrysodeixis* (Fig. 7), which was caught, spread, dissected and a leg provided for barcoding. The comparison of the female genitalia (Fig. 4) and the barcoding analysis (Fig. 1) confirmed the identification as *Chrysodeixis acuta* (Walker, [1858]). This is the first record of the species on St Helena.

T. Karisch

Figure 7 – *Chrysodeixis acuta* at light trap at Ginger Patch, High Peak, 16.iii.2017.

C. acuta is widely distributed in the tropical regions of Africa and Asia, and may appear as an agricultural pest locally (Goater *et al.*, 2003). So far, no damage has been



Figures 8–13 – Plusiinae from St Helena: 8. *Ctenoplusia limbirena*; 9. ♀ *Chrysodeixis acuta*; 10. ♂ *Chrysodeixis chalcites*; 11. ♀ *Chrysodeixis chalcites*; 12. ♂ *Chrysodeixis includens*; 13. ♂ *Chrysodeixis includens*.

reported from St Helena. Because barley, linseed and sorghum are not of agricultural importance on St Helena the influence of *C. acuta* on the agricultural production should remain insignificant.

Key to the species of *Chrysodeixis* on St Helena Island

A key to the *Chrysodeixis* species mentioned above is presented to enable interested people on St Helena to differentiate the species. Sometimes the identification can be difficult, in which case the genitalia should be checked. Illustrations of the genitalia are given above (Figs 2–6). For figures of the genitalia of both sexes see Goater *et al.*, 2003 (Figs 211, 281) and Eichlin & Cunningham, 1978 (Fig. 99). We include *C. limbirena* [author and date of publication?] because of its superficial similarity.

1 – copper patch present in middle part of margin of forewing .....*Ctenoplusia limbirena* (Fig. 8)
1*– copper patch absent ……..... 2
2 – with golden gloss, especially in dorsal part of forewing …....*Chrysodeixis chalcites* (Figs. 11, 12)
2*– without or with only slight golden sheen, especially in worn specimens 3
3 – large y-mark; antemedian line without tooth between cell and analis; black strigula arising in apex ..... *Chrysodeixis acuta* (Fig. 9)
3* – smaller y-mark; antemedian line with tooth between cell and analis; black strigula arising on costa before apex ..... *Chrysodeixis includens* (Figs 12, 13)

The distal part of the y-mark is twice as large in *C. chalcites* and *C. acuta* compared with that in *C. includens*. Normally, the y-mark is divided in all three species, but occasionally we found specimens of *C. includens* with attached or fused y-mark (Figs 12, 13).

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