Missing Stickman Found: the First Male of the Parthenogenetic New Zealand Phasmid Genus Acanthoxyla Uvarov, 1944 Discovered in the United Kingdom

by Paul D. Brock, Malcolm Lee, Mary Morgan-Richards & Steven A. Trewick

Introduction

Three species of New Zealand phasmids have become naturalised in the UK: Prickly Stick-insect *Acanthoxyla geisovii* since Edwardian times, Unarmed Stick-insect *Acanthoxyla inermis* since the 1920s, and Smooth Stick-insect *Clitarchus hookeri* since the 1940s. Smooth Stick-insect is only found on the Isles of Scilly, but the two *Acanthoxyla* species are found in localised populations in the south-west of England, principally within Cornwall and Devon. Around 250–300 reports of stick insects a year are now being received by Malcolm Lee, the national recorder, almost exclusively through the online reporting system set up by the Phasmid Study Group. Many of these are now also accompanied by an image enabling ready confirmation of the species. There are now thought to be just three species of *Acanthoxyla* in New Zealand following synonymy of several morphological forms (Brock & Jewell, 2015), but males have never been recorded in the genus. The whole genus is considered to be of hybrid origin and contains both diploid (with two copies of every chromosome) and triploid (with three copies of every chromosome) lineages (Morgan-Richards & Trewick, 2005; Buckley *et al*., 2008; Myers *et al*., 2013). In the light of this, males have been assumed not to exist and all populations are regarded as parthenogenetic, that is females lay viable eggs without the need for fertilisation by a male. Males of the similar looking Smooth Stick-insect are not uncommon in New Zealand. They may account for up to 50% of insects in some populations, but are absent in other populations which must breed parthenogenetically (Morgan-Richards *et al*., 2010). No male Smooth Stick-insect has ever been recorded in the UK population, which is therefore regarded as wholly parthenogenetic.

The Find

On 6 October 2016 a report was received from David Fenwick of a stick insect found that morning on the side of his partner's car outside his home in Heamoor, in the north-western part of Penzance, Cornwall. David had sent an earlier report to ML in 2013 with a clear image of a tiny stick insect found on his bathroom ceiling, which enabled it to be identified as a recently hatched Unarmed Stick-insect nymph. He has subsequently come across other nymphs within his house. The image accompanying this recent report was taken side-on, and showed a spindly looking 75mm long phasmid, with the end of the abdomen being quite a different shape to any of the naturalised stick insects previously seen. It was assumed to have been an escaped or discarded individual from a nearby phasmid enthusiast and was referred to PDB to see if he could identify the species. His quick response was to wonder whether this was a eureka moment, and *Acanthoxyla* had finally produced that missing male. A second email from David Fenwick included more close-up

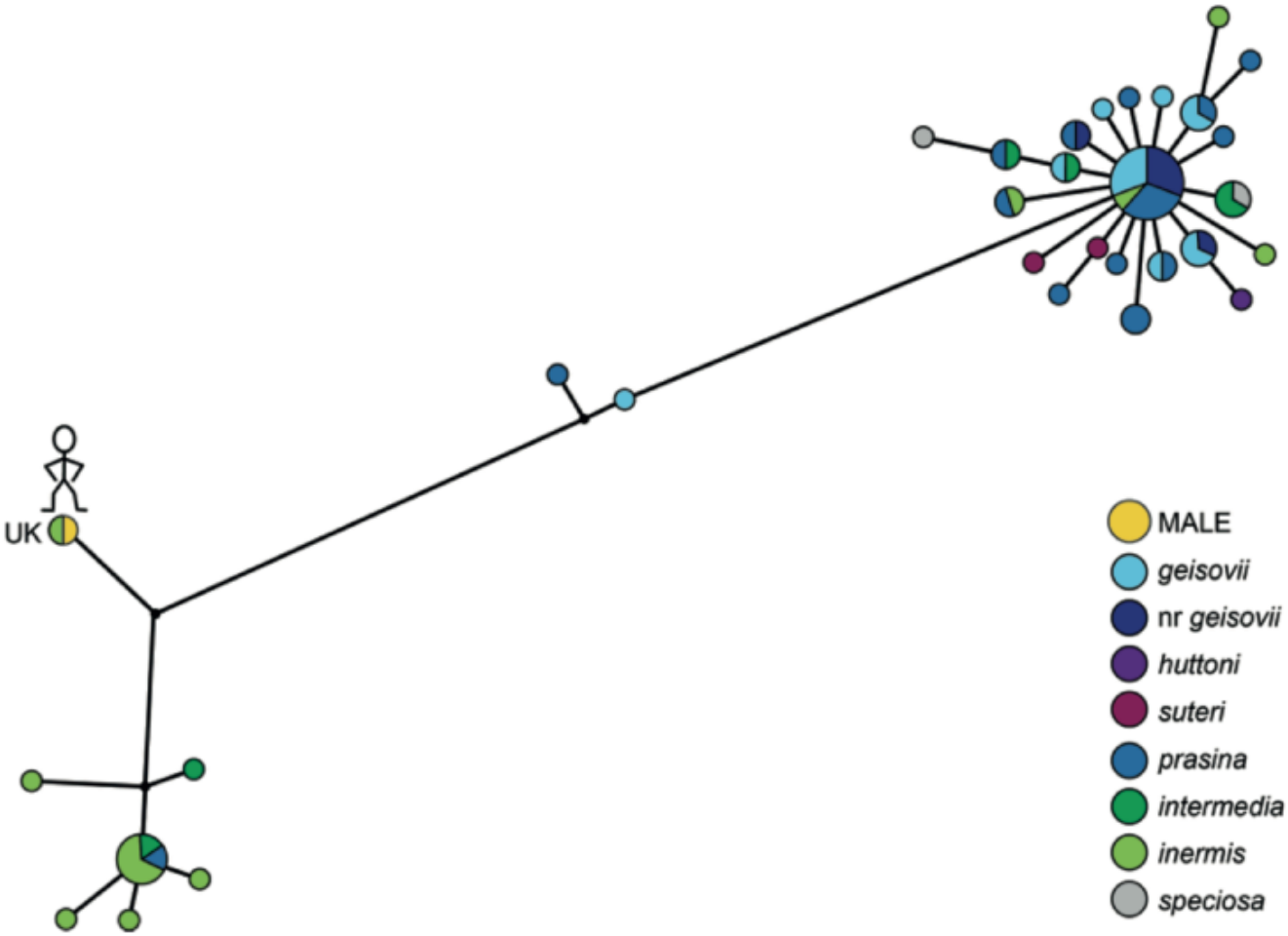


Figure 1. The above diagram shows the genetic similarity between individual *Acanthoxyla* stick-insects sampled in New Zealand and the UK. Different colours represent different *Acanthoxyla* morphological 'forms' that were sampled. All the 'forms' mentioned above are illustrated in Salmon (1991) and he considered them all to be subspecies of just one valid species; *A. prasina*, rather than the current three valid species Black-spined Stick-insect *A. prasina*, Prickly Stick-insect *A. geisovii* and Unarmed Stick-insect *A. inermis* of Brock and Jewell (2015). The longer the line (branch) the more different are the DNA sequences represented by the coloured spots. The bigger the spot the more individual stick-insects are represented. Some forms have the same DNA sequence and so spots are subdivided. Two of the 'forms' (Unarmed Stick-insect and Prickly Stick-insect) are recorded in the UK, and we found that the male (yellow) had the same sequence as a female Unarmed Stick-insect previously collected at Mevagissey, Cornwall, in 2013. Genetic similarity was assessed by comparison of partial Cytochrome Oxidase I mitochondrial DNA sequence.

pictures of his phasmid, including dorsal views of the abdomen and head. When he saw these images, PDB confirmed that, apart from a more spindly appearance and a slightly more extended black line along the top of the abdomen, this stick insect had everything to match female Unarmed Stick-insect, including red beneath the forelegs and spines beneath mid and hind femora. However, this was definitely a male with genitalia broadly similar to known males of Smooth Stick-insect.

A trip was taken by ML to Heamoor to check out the area around David Fenwick's home for more phasmids. Brambles *Rubus* spp. and other likely foodplants in nearby hedgerows and gardens were inspected for evidence of the distinctive feeding damage of phasmids, but no damage was seen and no stick insects were found. David had put the insect in a cage, so it was taken back to ML's home at Port Gaverne. An extended check of hedgerows in adjacent Port Isaac located a female and the two were put together in the hope of a pairing. There was no evidence seen of any interaction but, sadly, the male died

the following day, as did the female a few days later. In average years, October is towards the end of the annual life cycle for the naturalised phasmids so this was not unexpected. The male was preserved and has been deposited in the national collection at the Natural History Museum, London. As stick insects often do, it had shed a leg shortly after capture and fortunately David had immediately placed it in ethanol to preserve it. This leg was forwarded to New Zealand, where DNA sequencing was used to confirm with a high level of confidence that it came from an individual whose mother was an *Acanthoxyla* (Figure 1). A fragment of the DNA from the maternally inherited mitochondrial genome was sequenced and compared to DNA sequences from numerous stick-insect species. The male specimen had a sequence identical to female Unarmed Stick-insect specimens collected from the UK and very similar to many New Zealand collected individuals.

The X Chromosome and Males

In most stick insects, sex is determined by the number of X-chromosomes. Two X-chromosomes creates a female, one X-chromosome results in a male. A simple mutation during cell division can produce an egg with a missing X-chromosome, resulting in the production of rare males from parthenogenetic females. Because many *Acanthoxyla* individuals are triploid the accidental loss of a single X-chromosome is unlikely to result in a male. Most of the *Acanthoxyla* individuals identified as diploid have had few or no spines (Unarmed Stick-insect), similar to their putative paternal species (Smooth Stick-insect). A mutation resulting in the loss of an X-chromosome in a diploid lineage is much more likely to produce a son than a similar loss from a triploid individual, therefore it is of no surprise that the first *Acanthoxyla* male should have had an Unarmed Stick-insect mother.

Description of Male

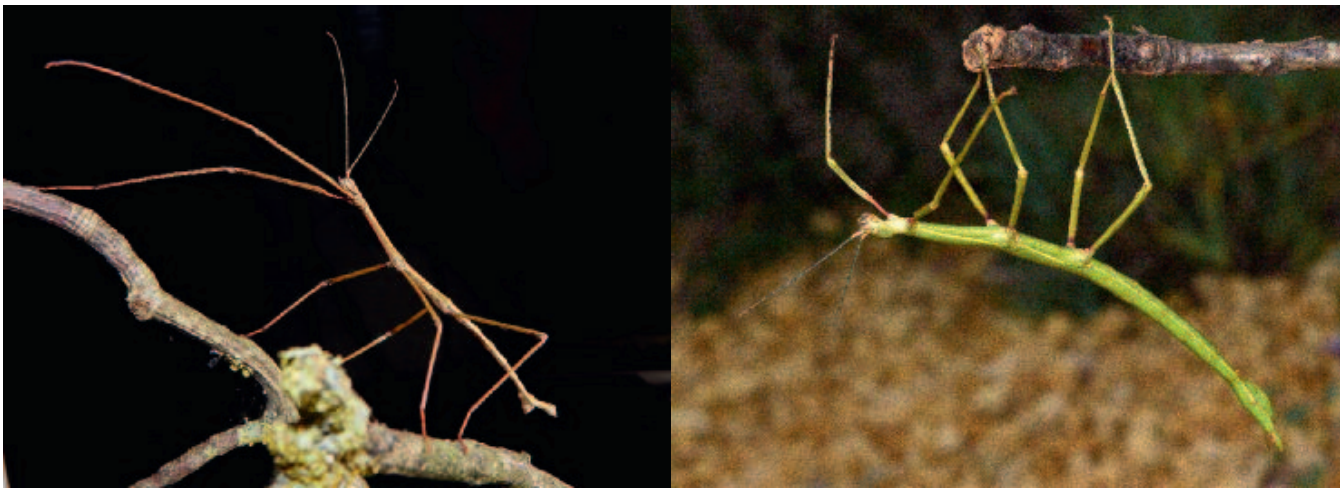
The illustrations on pp. 19 & 20 highlight various features discussed in this section, with the male images on the left and the corresponding female image on the right. These stick insects come in green or brown colour forms in the female, and it just so happens the male was brown and the female was the commoner green form.

Measurements: male: body length 75mm, head 3.6mm (width 2.2mm), antennae 28mm, pronotum 3mm, mesonotum 14mm, metanotum 10.3mm (+ median segment 3.7mm), femora: fore 22mm, mid 17mm, hind 21mm; tibiae: fore 2mm, mid 16mm, hind 20mm. Cerci 1.7mm (Natural History Museum, London (NHMUK)).

Brown with bold black longitudinal stripe on pronotum, also black marks on abdominal segments and at end of mesonotum/start of metanotum, otherwise only a faint indication of a longitudinal line.

Head: Elongate, eyes brown. Laterally behind eyes and towards hind part of segment with blackish marks. Antennae with 21 segments, basal segment elongate, segments 3 and 4 short. All segments hairy. Inner base of fore femora reddish.

Thorax: Pronotum shorter than head, with a bold central longitudinal black line. Depressed towards the top of the segment and in the centre, forming an almost rectangular area,



Adult **Unarmed Stick-insect** *Acanthoxyla inermis* (Photos: D. Fenwick (left) and P. Brock (right)).

Head and thorax of **Unarmed Stick-insect** *Acanthoxyla inermis* (Photos: D. Fenwick (left) and P. Brock (right)).

surrounded by a slightly raised area. Mesonotum and metanotum generally smooth, only very sparsely granulated. Mesonotum almost five times the length of the pronotum and the same length as the mesonotum and median segment. Upper part of mesonotum with a hint of a black line, which reappears at base. Underside reddish.

Abdomen: Elongate. Black marks prominent at the end and start of the abdominal segments 1–8. Ninth abdominal segment very elongate, the swollen subgenital plate not reaching half the length of the segment. Tiny hairs ventrally on the ninth segment. Basal part of anal (tenth) segment hairy; segment rounded at tip. Claspers broad, each clasper with five unequal stout inner black teeth. Cerci short and hairy, tapering slightly to rounded tip.

Legs: Elongate. Mid and hind femora with pair of basal spines.

Note: This sex is similar in general appearance to the female, but much more slender (normal in stick-insects). Unarmed Stick-insect is the only known *Acanthoxyla* species with a bold black line on the pronotum. The male is easily distinguished from the often less plain-looking Smooth Stick-insect male, as the latter has a more conspicuous longitudinal black line running along the body, which is sometimes broken but always on the head. It also has expanded eighth abdominal segments, lacking in *Acanthoxyla*.

End of abdomen, dorsal view of **Unarmed Stick-insect** *Acanthoxyla inermis* (Photos: D. Fenwick (left) and P. Brock (right)).

End of abdomen, lateral view of **Unarmed Stick-insect** *Acanthoxyla inermis* (Photos: D. Fenwick (left) and P. Brock (right)).

End of abdomen, ventral view of **Unarmed Stick-insect** *Acanthoxyla inermis* (Photos: D. Fenwick (left) and P. Brock (right)).

Parthenogenesis in Phasmids

The most studied parthenogenetic Phasmid is the Indian Stick-insect *Carausius morosus*. Numerous papers have been written about this species, all based on culture stocks first introduced into Europe in 1898. Crotch (1970) stated, "If the Stick Insect had been a native around Mount Ararat, Noah would have been hard put to it to find a pair of them for the Ark! In no breeding culture does the male appear more than once in a thousand adults. Some authorities give the ratio as nearer 1/10,000." PDB reared two males in about 200 insects in the 1980s. Eggs were not subjected to the very high temperatures that can result in males appearing, according to some researchers, but most rearers never see a male. It is generally believed that the male is incapable of fertilisation, although there have been no studies in India, where males may occur in some natural populations, as is the case in other *Carausius* species. Italian researchers and others have examined parthenogenesis in detail, in the mainly Mediterranean genus *Bacillus*, where males occur in some populations of *B. rossius* (for example, Scali *et al*., 2003). Other captive-bred phasmids from various countries reproduce parthenogenetically in the absence of males, but there have been no detailed,

long-term studies. But the genus *Acanthoxyla* has been a mystery up to now, uniquely with a lack of male(s) in any species, either in the wild or in captivity. The genus *Acanthoxyla* appears to be of hybrid origin, with the male of Smooth Stick-insect the putative parental species, the female unknown (Trewick *et al*., 2008).

Smooth Stick-insect *Clitarchus hookeri* (male). Waiorongomai Valley, New Zealand, 28 February 2016 (Photo: P. Brock)

There are some advantages to parthenogenesis. A single insect, or even a single egg, transported to new areas could soon lead to a viable population able to exploit this fresh habitat. This will have been a prime factor in the establishment of our UK populations. Such exploitation of new habitats is still happening, as shown by the post-2000 range expansion of Unarmed Stick-insect. A parthenogenetic population of the same size as one with equal number of males and females may theoretically produce twice as many eggs. Of course, the eggs of parthenogenetic insects may not be equally fertile, but a study on Smooth Stick-insect (Morgan-Richards *et al*., 2010) showed only marginal differences, with 74% of eggs from parthenogenetic females hatching out, compared to 85% for females from sexual populations.

In species where males occur only rarely, they may not be able to fertilize females, as is the case with cultured *Carausius morosus*. As our male died before any interaction could be observed, we do not know if it could take any part in fertilisation. The New Zealand endemic Smooth Stick-insect exhibits a geographic split between sexual and parthenogenetic populations, with sexual populations apparently restricted to New Zealand's North Island. No males have been found on South Island by recent researchers, with a pinned male specimen in the Museum of New Zealand collected by J T Salmon in 1944 near Christchurch being the sole South Island male known. This could have come about when cells in the egg of a parthenogenetic female lost an X chromosome during division, since a single male hatched out of one of the 315 eggs from a parthenogenetic female during the 2010 study by Morgan-Richards *et al*. That study also showed mated females from sexual populations gave rise to eggs that hatched out in roughly equal male to female ratio, but if such females were not allowed to mate, their unfertilized eggs gave rise to all females. Where males from sexual populations were allowed to mate with females from parthenogenetic populations, the vast majority that hatched out were females, with males representing just 1 in 40 hatchlings.

The Heamoor, Penzance Colony

In the last 10 to 15 years, the Unarmed Stick-insect has undergone a massive surge in its distribution, especially in Cornwall (see Figure 2). These stick-insects have little capacity to distribute themselves, and such a spread is undoubtedly associated with this species turning up within the grounds of several garden centres in Cornwall and Devon. As we buy their plants and take them home, stick-insect eggs, or small nymphs may tag along too. In Penzance there is an unsubstantiated report of stick-insect sightings in the town in the late 1960s, but this could relate to *Carausius morosus*, so often kept, and then discarded, by children. Such discards can occur anywhere in the UK. Apart from that anomalous one, there are 39 verified stick-insect sightings in Penzance, including Heamoor, with the first in 2003. The first for the Heamoor area itself was in 2010, so both dates are consistent with the colonies being of recent introduction.

Why the UK?

It seems a puzzle why the first male *Acanthoxyla* should turn up in a small offshoot population on the other side of the world from their native land. It may be that males are to be found in New Zealand, but their rarity means they have yet to come to the notice of researchers in this field. An indication of the likely rarity of this male comes from the

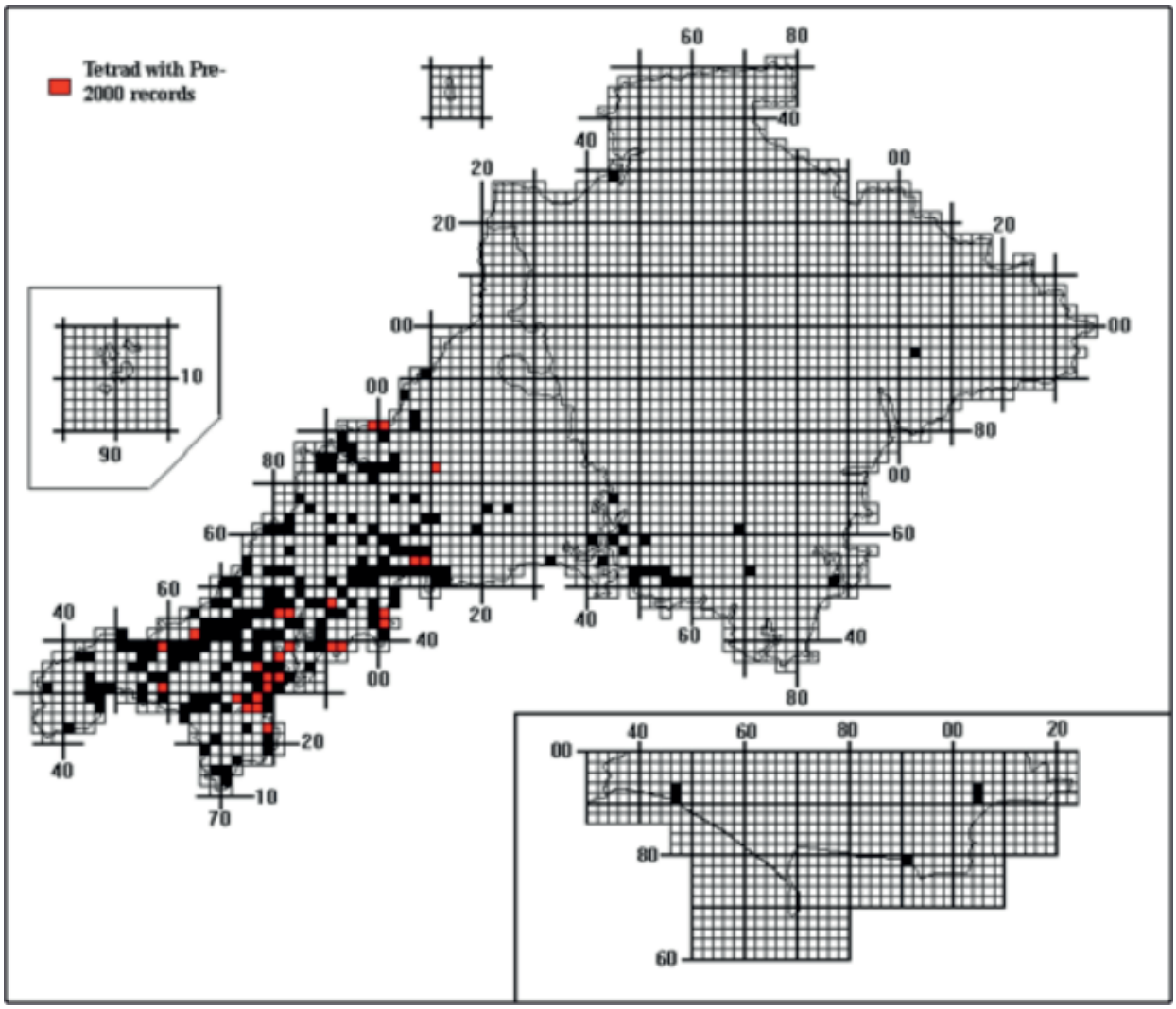


Figure 2. Unarmed Stick-insect *Acanthoxyla inermis* tetrad distribution in Cornwall, Devon & Dorset (inset) at 30 June 2015, showing original pre-2000 tetrads in red.

database kept by the national recorder. At the time of receiving David Fenwick's report, there were 2,646 separate phasmid records. Excluding records which came from the few UK recorders who regularly search for them in the field, this leaves 2,343 reports that represent truly random encounters by individuals who come across, typically, a single insect in their homes or gardens. Of those reports, 1,019 were accompanied by an image of what they had found. If we assume those images are of a single insect (around 98% are), this suggests a rarity for this male of at least one in a thousand, a similar ratio to that estimated for male *Carausius morosus* (Crotch, 1970). These masters of camouflage are so difficult to spot, even to experts, that it is unlikely researchers in New Zealand have seen anything like this number of Unarmed Stick-insect. Were a male Unarmed Stick-insect to be within a colony of the similar Smooth Stick-insect it could easily be overlooked as a male of the latter species.

No doubt this find will spur the search for *Acanthoxyla* males in New Zealand.

Acknowledgements

Our thanks go to David Fenwick for bringing this, so far unique, phasmid male to our attention, and for taking the excellent close-up images whilst the insect was still alive.

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Paul D. Brock[1], Malcolm Lee[2], Mary Morgan-Richards[3] & Steven A. Trewick[3]

[1] Scientific Associate, Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD

[2] National Phasmid Recorder, Gullrock, Port Gaverne, Port Isaac, Cornwall

[3] Ecology Group, Institute of Agriculture & Environment, Massey University, Private Bag 11-222, Palmerston North, New Zealand