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Illustrated descriptions of juvenile stages of an *Aloeides* species close to *margaretae* Tite & Dickson, including comparisons with both *A.apicalis* Tite & Dickson and *A.depicta* Tite & Dickson

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Abstract: The butterfly in this study is provisionally treated as *Aloeides margaretae* and represents observations and material from Moorreesburg, Lambert's Bay and Graafwater, in the Western Cape province. The juvenile stages illustrated herein were mostly obtained from Graafwater; although they are consistent with observations at the other localities. These juvenile stages are compared with Gowan Clark's illustrations of *A. depicta* in Tite & Dickson (1968). Some small differences in markings of the larval stages were noted, that together with a difference in the shape of the hind wing, lend some support for their separate status. A comparison is also made here between the adult stages of *A. margaretae* and *A. apicalis*. This reveals little significant or consistent difference between them in either wing shape or markings, inferring that their taxonomic relationship needs to be reviewed in a molecular study. It is surprising that Tite & Dickson did not attempt to define or question the relationship between them seeing that both taxa were described by them in the same publication and that according to their designated paratypes, their distributions overlapped. It is unlikely that species of host plant or host ant can throw any further light on their relationship as there is a dearth of such data in most descriptions of myrmecophilous juveniles, especially in the earlier publications, prompting a plea herein for lepidopterists to add observed host information to the usual collecting data and to collect samples of the host ants wherever possible.

Key words: DNO (Dorsal Nectary Organ), Monomorium fridae, juvenile stages, holotype.

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INTRODUCTION

The large genus *Aloeides* [Hübner, 1819] is in the lycaenid sub-family Aphnaeinae, which was subject of a review by Heath (1997). A molecular-based phylogeny of the subfamily was later inferred by Boyle *et al.* (2014).

The genus Aloeides was substantially revised in two key publications by Tite & Dickson (1968, 1973); subsequently a tentative phylogeny was proposed by Henning (1993) based mostly on wing markings. Tite & Dickson (1968) described Aloeides depicta on p. 383, and on the following page described apicalis as a subspecies of it. Two pages further on they described A. margaretae. Ten years later, A. depicta apicalis was treated as a full species by Dickson & Kroon (1978). Aloeides margaretae has long been confused with A. apicalis as they were both believed to occur sympatrically in the Picketberg region according to their authors (Tite & Dickson, 1968; Dickson & Kroon, 1978). Tite & Dickson (1968) listed A. apicalis paratypes from "Farm Swartberg, Piquetberg road" and also from Clanwilliam and Citrusdal. In Clark & Dickson (1971) under A. depicta they write "Subspecies apicalis Tite & Dickson seems to be confined to the western Cape,

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Copyright: This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, send a letter to Creative Commons, Second Street, Suite 300, San Francisco, California, 94105, USA, or visit: <u>http://creative.commons.org/licenses/by-nc-nd/3.0/</u> occurring from near Piketberg northwards to Little Namaqualand." Sympatry between all three taxa is also inferred in the distribution maps in Mecenero *et al.* (2013), Williams (2018) and Woodhall (2020); moreover, wing phenotypes portrayed in these publications also throw doubt on their separate status.

Juvenile stages of *A. depicta* from Port Elizabeth were illustrated in one of Gowan Clark's plates in Tite & Dickson, (1968: pl. 8). Clark & Dickson (1971) described and also illustrated them using the same plate. In a short paper, Pringle (1998) described the discovery of *A. depicta* larvae found beneath a rock at his farm near Bedford, Eastern Cape.

In this study an illustrated life history of a taxon from the Western Cape province (Fig. 1) is presented that answers to the description of *A. apicalis* as well as to *A. margaretae*; both of which seem to be closely related to *A. depicta*. Hence, in this study the relationships between these three taxa are investigated and the current taxonomy questioned.

MATERIAL AND METHODS

Photography: Photographs were taken by the first author using a Fujifilm FinePix S7000 or Olympus OM-D E-M5 camera, with an Olympus M.Zuiko Digital ED 60mm f2.8 macro lens or bellows with 20mm lens or 50mm macro lens. The digital editor used was Affinity Photo. All photographs were by the first author except the *A. apicalis* from Springbok taken by Ernest Pringle.



Figure 1 – *A. margaretae* (up + un) Graafwater.

Graafwater is a small rural community on the R364 halfway between Clanwilliam and Lambert's Bay; the locality for part of this study being 32°09'35"S, 18°36'05"E.

The Moorreesburg locality is at the top of the Swartberg Mountain, Koringberg $(33^{\circ}02'55''S, 18^{\circ}40'34''E)$. The locality 7 km south of Lambert's Bay on the east side of R365 (now fenced off) is $32^{\circ}10'32''S$, $18^{\circ}18'52''E$.

The illustration of the *A. margaretae* holotype in Tite & Dickson (1968: pl. 4 – Figs. 74 & 82) appears to be an error; it is not a photograph of the *A. margaretae* specimen labelled as holotype housed in the Natural History Museum collection, London. The latter specimen is illustrated here (Plate 1: 1a–c). The illustration of *A. apicalis* in Mecenero *et al.* (2013) resembles typical *A. depicta*, and the same specimen is shown as *A. gowani*.

The ants encountered in this study are Frida's Pharoah Ant, *Monomorium fridae* Forel, 1905 (Myrmicinae), identified by Hamish Robertson in 1997. A reference is also made herein to the ant attending *A. depicta* larvae from near Bedford, Eastern Cape: *Lepisiota capensis* (Mayr, 1862) (Formicinae). Both of these ant species were more recently referenced in Slingsby (2017).

Host plants recorded for this insect during the study period were:

Aspalathus spinosa L. (Fabaceae) Aspalathus spinescens Thunb. (Fabaceae) Wiborgia fusca Thunb. (Fabaceae) Hermannia trifurca L. (Malvaceae) Roepera teretifolia (Schltr.) Beier & Thulin (Zygophyllaceae) Atriplex bolusii C.H. Wright (Amaranthaceae) Wiborgia fusca was identified by John Manning (South African National Biodiversity Institute) and subsequently referenced in Palgrave (1992).

Juvenile stages: Methods employed to induce females to oviposit and how to locate juvenile stages in the field have been described in Heath & Claassens (2000).

Six of the early instar larvae illustrated in this study were reared in captivity from the egg, without ants but larvae were mostly collected from the veld and studied in captivity together with their own host ants and host plants.

RESULTS

During a visit to the Swartberg Mountain north of Moorreesburg in 1997 the two authors discovered *Aloeides* larvae and a pupa beneath some flat rocks that were beside an *Aspalathus spinosa* plant. These juveniles were attended by small but very aggressive ants; later identified as *Monomorium fridae* (Fig. 2). The ants did not behave in the same placid way that *Lepisiota capensis* (Mayr) ants do, nor were the larvae inside the ants nest. Following an ant trail, the actual nest was discovered about four metres away; it was subterranean and a mound of fine debris surrounded the entrance.



Figure 2 – Host ant (Monomorium fridae).

The juvenile stages reared to adults as well as free flying adults were provisionally identified as *A. margaretae* bearing in mind that paratypes of *A. apicalis* as well as *A. margaretae* were deemed to occur in this locality according to Tite & Dickson (1968).

Over subsequent years numerous visits were made to a locality 7 km south of Lambert's Bay that was at the time believed to be the type locality for *A. margaretae*. Regrettably, this locality has now been fenced due to the upgrading of the road. Opportunistically, at this locality, juvenile stages of *Aloeides* were often discovered beneath their host plants and always vigorously attended by *Monomorium fridae* ants. Adults raised from these larvae and pupae together with free-flying adults were treated as *A. margaretae*. Species of *Roepera* and *Atriplex* species were among the host plants noted.

More recently, at various localities along the R364, both *A. arida* Tite & Dickson and *A. margaretae* occurred commonly. Juvenile stages of *A. margaretae* were also found beneath species of *Aspalathus* and on one occasion, of *Hermannia*.

A closer study of this butterfly was made at a locality close to Graafwater where larvae were discovered in the sand or gravel at the foot of an *Aspalathus* stem. In September 2013 a female was observed ovipositing directly on the sand, beside a stem of a *Wiborgia fusca* shrub (Fig. 3).



Figure 3 – A host plant (*Wiborgia fusca*).

Further searches revealed that *W. fusca* is widely used in the region as a host plant for this butterfly. In all of the above cases *Monomorium fridae* was the species of host ant. At this point it was realised that the shape of the forewing apex was not consistent among the males encountered. The outer margin varied between very slightly concave or straight, to slightly convex (Plate 1); similarly, the shape of the hind wing outer margin was inconsistent.

A female of this butterfly was captured at Graafwater on 13 October 2017 for the purpose of obtaining some eggs. She was induced to oviposit in the presence of three *Monomorium fridae* ants and a few stems from one of the host plants, *Wiborgia fusca*. One of the resulting eggs was photographed (Fig. 4), measuring: 0.92 mm diameter x 0.54 mm high. Six eggs were obtained and retained until they hatched; this period averaged 18 days. The first instar larva was then photographed (Fig. 5).



Figure 4 – Egg.

Figure 5 – 1st instar larva.

In all the localities studied, larvae were never found within the main *M. fridae* ant nest; instead they congregated on or beneath the hostplant or beneath nearby rocks attended by a small group of ants. First, 3rd, 4th, 6th and final instars are shown here (Figs 5-11). First to 3rd instar larvae have been found individually during the day on fresh shoots near the base of the host plant attended by ants (Fig. 12). The more mature larvae (4th to final instar) are gregarious and several may be found sheltering together during the day. Feeding for the more mature larvae is confined to the evening and night. The largest final instar larva was 22.5 mm long immediately prior to pupation. From the 3rd instar, larvae possess a DNO (Dorsal Nectary Organ) but not the final instar; although the ants continued to visit the obsolete DNO and the larvae continued to feed on the host plant. On dissecting a final instar larva the DNO internal

structure was found to be in place but there was no surface opening. The total number of instars are not recorded here but referring to Clark & Dickson (1971) they can vary (for the closely related *A. depicta* reared in captivity) from six to eight instars. As with many lycaenids the colour pigment of the final instar fades a day or two before pupation; drained of pigmented markings, it takes on an insipid pale green hue (Fig. 10).

The host plant stem has a gap in the substrate of ± 3 mm around the stem for access to *Aloeides* larvae and perhaps for any Hemiptera species parasitising the roots of the host plant. Third instar (and later) larvae rest 1–2 cm beneath the sandy substrate crust, in alcoves close to the stem of the host plant, where they also pupate and the ants continually visit the pupae. The pupae were greeny-yellow at first, gradually changing to brown. One pupa was 12.5 mm long (Fig. 13); the longest almost 14 mm and the smallest, 11 mm. In cases observed in captivity the average time was 18 days pupation to ecdysis.

The *M. fridae* ants (Fig. 2) are very aggressive (Slingsby, 2017; A.H., pers. obs.). They even bite their larval charges during any disturbance, hence care may be needed when collecting or rearing late instar larvae in captivity.

Description of final instar larva

Putty-coloured (Fig. 9) with longitudinal dull maroon lines and yellow-orange spots, skin adorned with pin-like setae, some black but mostly white (Fig.14).

Head capsule (Fig. 15) black with a pair of conspicuous yellow-orange patches; densely covered in bristly setae, especially lower half and clypeus; sutures light grey. Head carapace has a fringe of setae along the anterior margin, and has a median longitudinal narrow white stripe flanked by a pair of brown bands each dominated by a small pointed protrusion anteriorly (seen from lateral perspective); these bands are flanked by a paler band, then black.

Segments 2–8 similar; two ragged longitudinal maroon stripes flank a median grey-blue dorsal stripe between them, either side of which is a broad putty-coloured band with two longitudinal maroon markings; near the centre of the band is an ill-defined but conspicuous yellow-orange patch; beyond the band is a pair of maroon ragged lines, thereafter whitish with obscure pale brown and maroon markings and a conspicuous black spiracle; below this are the whitish ventral aspects. Segment 9 is similar to above but lacks the orange patch and tapers dorsally towards its distal margin; a prominent vee-shaped fringe of black setae encasing some whitish setae and an obsolete DNO.

Comparison of juveniles with A. depicta

In a comparison of juvenile stages of *A. margaretae* in this study with *A. depicta* as described and illustrated in Clark & Dickson (1971), the following differences are noted: the final three segments are shown in Figure 16. Segment 10 is equipped with a pair of black tubercles armed at rim with spines, these house white tentacular organs tipped with specialised setae both of which can evert and withdraw (piston-like) rapidly when the larva is disturbed; on the segment 10 anterior margin a dense triangular



Figure 6 – 3rd instar larva.

Figure 7 $- 4^{\text{th}}$ instar larva.

Figure 8 – 6th instar larva.



Figure 9 - Final instar larva.

Figure 10 – Pre-pupal larva.

Figure 11 – Final instar, lateral view.



Figure $12 - 4^{th}$ instar larva with ant.

cluster of flattened, white mushroom-like specialized setae abut the obsolete DNO (Fig. 16; note: these are also present in earlier instars where the DNO is functional); between the tubercles two pale spots are surrounded and linked by maroon colouring, like a pair of spectacles; final segment semi-circular and flattened dorsally, densely covered in small mushroom-like setae, distal margin with a dense fringe of setae, anal carapace black, circular or kidney-shaped, with a rectangular maroon or brownish patch abutting its proximal margin.

The *A. margaretae* final instar larval head capsule has a pair of striking yellow-orange patches whilst the

A. depicta illustration does not. Final instar larvae and pupa of A. margaretae were longer; this can perhaps be explained as an artefact of Gowan Clark rearing them in captivity without ants as opposed to larvae collected from the veld. The pattern of markings on the last three segments of late larval instars do not match between A. margaretae material and A. depicta illustrated by Clark & Dickson (1971); also the ground colour is putty coloured as opposed to pale blue. The A. depicta larva described by Pringle (1998) also differs from Clark's in having a pair of conspicuous yellow-orange patches on its head capsule, similar to A. margaretae larvae. The ground colour noted by Pringle (1998) was also similar to A. margaretae larvae. However the host ant was Lepisiota capensis, unlike the host ants found at the Western Cape sites in this study.



Figure 13 – Pupa.



Figure 14 - Larval skin.



Figure 15 – Head capsule, final instar.



Figure 16 – Distal segments, final instar larva.

Comparison between adults (Plate 1)

In the adult butterfly, forewing outer margin shapes among this study's *A. margaretae* material varies between straight to slightly convex and hence are similar to both *apicalis* and *margaretae* type specimens. The hind wing outer margin in *A. depicta* is almost evenly convex, as opposed to almost straight in the *A. apicalis* and *A. margaretae* holotypes, and as noted by Tite & Dickson op. cit in their description of *A. margaretae*. Despite the obfuscation of the hind wing verso in both *A. depicta* and *A. apicalis* holotypes, a careful study reveals the same basic pattern of discoidal fascia and spots in all the specimens, subject to submarginal and other spots becoming obsolete in some specimens.

Conclusion

Whether *A. apicalis* and *A. margaretae* are conspecific with *A. depicta* remains equivocal as it is not known whether the differences described above are inter- or intraspecific, consistent or even relevant. However, no substantial differences between *A. apicalis* and *A. margaretae* could be found (Plate 1). The forewing shape appear to be a variable feature and the hind wing shape is uniform, as stated by Tite & Dickson (1968).

Although the life history of material described above is provisionally deemed to be that of *A. margaretae*, it could prove to be conspecific with *A. apicalis* in view of the lack of significant discriminatory characteristics. Confirmation for this view must await a molecular study. Note that *A. apicalis* would have page preference over *A. margaretae*.

A remarkable variety of host plants were recorded for this butterfly; six species, involving four families; hence it can be regarded as polyphagous. Only one species of host ant (*M. fridae*) has been recorded for these *Aloeides* at the Western Cape localities mentioned here, and so far it has not been recorded as a host for any other species. It would have been helpful if the species of host ant tending Port Elizabeth material of *A. depicta* was known but it is thought likely to be *L. capensis* as recorded from near Bedford by Pringle (1998). A duality in species of host ant is not uncommon and known to occur among some *Chrysoritis* species of the *thysbe* group (A.H., pers. obs.).

DISCUSSION

Some of the larger host plants such as *Aspalathus spinescens* and *Wiborgia fusca* are almost tree-like, having a sturdy main stem that would need to be scaled daily by the larvae. With this in mind, it may be speculated that some larvae might remain in the ground to feed on the surface of the roots. It would be interesting to discover if this does takes place among *Aloeides* species, having been observed among the *Orachrysops* Vári & Kroon (see Terblanche & Edge, 2007; Edge & Van Hamburg, 2008).

From experience in rearing *Aloeides* larvae in captivity it should be noted that their long-term welfare requires either no ant contact at all or the right species of host ant to be present all the time. With some ant species this may mean that they should come from the exact same colony the larvae were found with; otherwise the larvae may get badly bitten due to the ants detecting alien pheromones. This was a serious problem during the study due to the aggressiveness of *M. fridae* ants.

The life history of *A. depicta* described in Clark & Dickson (1971) was based on larvae "reared on" *Aspalathus* species, but no data was given on natural host plants and none at all on host ants. It is assumed that Clark reared the juvenile stages without host ants; otherwise he would have mentioned it in some of his life history accounts. As this butterfly species is polyphagous, the species of host ant is a more useful taxonomic trait. When

lepidopterists collect specimens it would be scientifically useful if the species of host ant was also sampled, and the circumstances recorded. This can sometimes be achieved through patient observation of the female and if oviposition is observed, much can be gleaned e.g. some species have been observed to oviposit in or on sand, such as *A. damarensis* (Trimen), *A. aranda* (Wallengren) and *A. barklyi* (Trimen) (A.H., pers obs.).

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Dr Andre C. Claassens R.I.P. was a friend and colleague for over 30 years. He was also a mentor on ant associations with lycaenid butterflies; he is greatly missed.

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Plate 1 – *Aloeides* holotype males: 1a,b,c – *A. margaretae* holotype male (South of Lambert's Bay); 2a,b,c – *A. apicalis* holotype male (O'Kiep, Northern Cape); 3a,b,c – *A. depicta* holotype male (Uitvlugt; Uitvlugt is the name of a farm 42 km NW of Humansdorp, Eastern Cape). Forms of *A. margaretae* & *A. apicalis*: 4a,b – *A. margaretae* (Graafwater, Western Cape); 5a,b – *A. margaretae* (Lambert's Bay); 7a,b – *A. margaretae* (7 km S. of Lambert's Bay); 8a,b – *A. margaretae* (Swartberg, N. of Moorreesburg); 9a,b – *A. apicalis* (Springbok, Northern Cape).