Thisbe silvestre sp. nov. (Lepidoptera: Riodinidae): a new myrmecophilous butterfly from the Brazilian Atlantic Forest

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Abstract

This paper describes a new species of Thisbe Hübner from the Atlantic Forest of south-eastern Brazil. Both adult and immature morphology are described and illustrated, and the natural history, behaviour and symbiotic interactions with ants (myrmecophily) are documented. This new species, T. silvestre sp. nov., is known only from the type locality at Parque Nacional do Caparaó, Minas Gerais state, Brazil. Due to the isolation of the known population, specialised host plant use, restricted geographic distribution and high degree of specificity in a vulnerable habitat, T. silvestre is considered threatened. Thus, monitoring this population is urgently needed, as well as the search for other populations of this butterfly in potential localities in order to evaluate its conservation status.

Key words ant-organ, conservation, mutualism, Nymphidiini, Riodinidae, trophobiosis.

INTRODUCTION

Butterfly species richness is extraordinarily high in the Neotropics, when compared with other biogeographic regions (Heppner 1991; Robbins 1993). The family Riodinidae is remarkably diverse, with approximately 1300 described species (Callaghan & Lamas 2004). Adult riodinids are notable for their morphological variation and for being usually rare. In general, their populations are located in ‘ideal’ microhabitats, being temporal and spatially scarce, even in widely distributed species (Bates 1859; Callaghan 1978; Harvey 1987; d’Abera 1994; Kaminski et al. 2014). Factors such as canopy openness and vegetation structure may influence their occurrence in a given site (Callaghan 1983; Alonso 2005). Furthermore, some species may present vertical stratification, spending much of their adult life in the tree canopy, only occasionally descending to the understory and thus consequently being only rarely recorded (DeVries 1997; Hall & Willmott 2010; Greve et al. 2013).

In the second half of the 19th century there was an increase in the rate of species descriptions, and most Riodinidae taxonomic diversity was described in this period (see discussion in Hall 1999), followed by a period of stability with little taxonomic novelties. This scenario has changed in the last 30 years, and a number of new species have been recently described (e.g. Brévignon 2010; Hall & Willmott 2010; Dolibaina et al. 2013, 2014; Callaghan & Nobre 2014; Dias et al. 2014; Siewert et al. 2014). In view of continuing new descriptions, we can assume that the current diversity of Riodinidae is underestimated and that a significant number of species are waiting to be unveiled in the coming years.

For example, the genus Thisbe Hübner includes seven species that occur from Mexico through Central and South America (Callaghan & Lamas 2004). Although there are two published phylogenetic proposals, there is still no consensus on the relationships of the species in relation to those formerly treated in Uraneis Bates (see Hall & Harvey 2001; Penz & DeVries 2001). Recently, new species have been described in this genus (Callaghan 2001; Hall & Harvey 2001), but there are still some undescribed taxa in the Atlantic Forest of Brazil (D. Dolibaina, pers. comm. 2015).

Expeditions (2012 and 2013) to a poorly sampled area of high mountains in Minas Gerais state, southeast Brazil, uncovered an undescribed species of Thisbe (Riodinidae: Nymphidiini). Here, we describe this new species, providing information about its immature stages, natural history, behaviour of adults and a discussion about its conservation status.
MATERIAL AND METHODS

Study sites and rearing

This species was studied in the narrow stream valley of a montane cloud forest (Figs 1–3) along the ‘Vale Verde’ trail (20°41′S, 41°84′W, 1200–1300 m a.s.l.) at the Parque Nacional do Caparaó, Minas Gerais state, Brazil. Behavioural observations and collections were made in the field in February 2012 and April 2013. Immature stages (eggs and larvae) were collected directly on their host plant, Croton sect. Cleodora (Euphorbiaceae) (Fig. 3). Larvae were reared in plastic containers cleaned daily, with fresh plant material provided every 2 or 3 days (following Kaminski et al. 2013). Observations and data were recorded on behaviour and development times for all stages. To observe if and when the ant-organs were functional, we placed mature larvae (fifth and sixth instar) in a rectangular plastic arena (20 × 30 × 5 cm) connected with a colony of Camponotus rufipes (Fabricius). For details of rearing of ants in captive, see Kaminski and Rodrigues (2011). Observations and data were recorded on behaviour and development times for all stages. Dry head capsules and pupal cases were retained in glass vials. Voucher specimens of the immature stages were fixed in Dietrich solution and deposited in the Museu de Zoologia ‘Adão José Cardoso’ (ZUEC), Universidade Estadual de Campinas, Campinas municipality, São Paulo state, Brazil.

Morphology

Morphological examination and measurements were made using a Zeiss® Discovery V20 stereomicroscope. To study genitalia, abdomens were detached and soaked in a test tube of hot 10% KOH solution for 5–10 min and dissections were stored in glycerol. Egg size is given as height and diameter. Larval head capsule width was measured as distance between the most external stemmata, and maximum length for both larvae and pupae correspond to the distance from the head to the posterior margin of the tenth abdominal segment in dorsal view. Measurements are given as a range. Colour patterns of immature stages were recorded using a digital camera in vivo and a stereomicroscope was used for eggs and early instars. Taxonomic nomenclature followed Callaghan and Lamas (2004). We follow Kristensen (2003) for genitalia, Downey and Allyn (1980) for eggs, Stehr (1987) for general morphology of larvae and pupae and DeVries (1988) for ant-organs.

Thisbe specimens from three public collections in Brazil were examined, including the Thisbe rupestre Callaghan type series (holotype and paratype), and additional specimens from north-east Brazil. Furthermore, we compared the new taxon...
with the Lamas collection of Neotropical butterfly type photographs at the MUSM (also available online in Warren et al. 2014), representing most currently relevant names and recognised species of Riodinidae (Callaghan & Lamas 2004).

The following collection acronyms are used: DZUP, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; UFP, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil; ZUEC, Museu de Zoologia da Universidade Estadual de Campinas, Unicamp, Campinas, São Paulo, Brazil.

RESULTS

_Thisbe silvestre_ sp. nov.


**Diagnosis**

_Thisbe silvestre_ males are similar to those of _Thisbe rupestre_, but can be easily distinguished from the latter by their larger size, the lack of blue iridescent scales on the dorsal wing surfaces and by the median white band on the dorsal hind wings (Figs 2,4), which is straight in _T. silvestre_ and convex in _T. rupestre_. In _T. silvestre_ the genitalia valvae are less robust and longer than in _T. rupestre_, and the posterior margin of uncus is rounded in _T. silvestre_ and flat in _T. rupestre_. Females (Figs 3,5) are quite distinct from _T. rupestre_, whose females lack the well-marked white band on both wing surfaces, being predominantly light brown with ochre bands (C.E.B. Nobre, pers. comm. 2014). Although, females of _T. silvestre_ are similar to those of _Thisbe irenea_ (Stoll) and _Thisbe molela_ (Hewitson), they can be easily distinguished from the latter two species by the narrower median white bands, and by the general ventral pattern, which is somewhat mottled in _T. silvestre_ but more ‘plain’ in _T. irenea_ and _T. molela_. The habitats of _T. silvestre_ and _T. rupestre_ are distinct; while _T. rupestre_ is associated with seasonal savannah and open dry environments (Callaghan 2001; Nobre et al. 2008), _T. silvestre_ was found in montane cloud forest (Fig. 1).

**Description of adults**

**Male**

Forewing length of holotype 19.0 mm; Paratypes: 19.0–21.0 mm (n = 5). **Wing shape:** Forewing semi-triangular with distal margin nearly straight; hind wing distal margin straight to a very short rounded tail at anal angle. **Dorsal surface:** Ground dark colour brown with white markings. Dorsal forewing with submarginal row of small white spots between veins, large near apex, changing to almost obsolescent white marks near anal margin; a postmedian row of five white spots forming an arch in cells R3+R5 (spot 1), R4+R5-M1 (2), M1-M2 (3), M2-M3 (4), M3-CU1 (5), spot 3 small and basally positioned in relation to remaining; some individuals present a very small white spot in cell R2-R3; a postmedian white band from Cu1 to anal margin; discal cell brown. Hind wing with row of submarginal dark brown spots barely visible between veins outlined by fine white scales; a 2.0 mm wide median white band contiguous with forewing band; vein 2A covered with conspicuous orange brown scaling in distal third. **Ventral surface:** Forewing light brown infused with pale grey scaling, pattern same as dorsal surface, with some spots outlined with light scales in basal half. Hind wing limbal area light brown with margin infused by white scales; white scales infusing into basal half of wing from median white band; irregular orange brown spots outlined with light scales in basal half; fringe

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white and brown. Head: Brown dorsally, with long scales on frons, orbit and palpi white; eyes grey in life, changing to reddish brown in museum specimens, covered with very short sparse hairs; antennae brown with white rings at shaft, a dark brown club with an orange tip; a pair of small horn-like structures on vertex covering antennae. Body: brown dorsally, beige and grey ventrally; legs light orange. Genitalia (Figs 6–8): Tegumen elongated, triangular in lateral view; vinculum broadly incomplete dorsally, forming a small narrow saccus ventrally; uncus broad, not lobed, with posterior margin rounded. Gnathos ‘C’-shaped and as long as tegumen; valvae well sclerotised, triangular in lateral view with lateral flanges, tips blunt; aedeagus long and slightly curved with cornutus; juxta narrower than aedeagus; eighth sternite semi-quadrangular without lateral projections (Fig. 8).

Female

Very similar to male, with wings more rounded and with larger white spots. Forewing length of allotype 22.0 mm; paratypes: 20.0–22.0 mm (n = 2). Wing shape: Forewing and hind wing same as male, with distal margins rounded. Dorsal surface: Ground colour dark brown with white markings same as in

Figs 6–9. Male (6–8) and female (9) genitalia of Thisbe silvestre sp. nov. (6) Lateral view; (7) ventral view; (8) eighth sternite in ventral view; (9) ventral view. Scales = 0.5 mm.

Description of immature stages

Egg (Figs 10,11): Duration 6–7 days (n = 2). Height 0.40–0.44 mm; diameter 0.65–0.66 mm (n = 3). Colour light green when laid, greenish white before hatching. General shape subcylindrical and flattened; exochorion with tiny projections and heptagonal cells. Micropylar area centred on top of anterior region.

First instar (Fig. 12): Duration 4–5 days (n = 2). Head capsule width 0.32 mm (n = 3); maximum body length 1.9 mm. Head dark brown; body yellowish green, with pairs of small reddish and white spots near abdominal spiracles; setae translucent, with brown base. Body dorsoventrally flattened

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Figs 10–21. Life history of *Thisbe silvestre* sp. nov. on *Croton* sp. (Euphorbiaceae). (10) Newly laid egg; (11) egg before hatching; (12) first instar; (13) second instar (arrow) tended by *Pheidole* worker; (14) third instar tended by *Procryptocerus* worker; (15) third instar tended by *Camponotus* sp.; (16) fourth instar; (17) fifth instar; (18) sixth (last) instar head in frontal view; (19) sixth (last) instar tended by *C. rufipes*, note the tentacle nectary organ everted (arrow); (20) pupa in frontal view, showing tubercles and flat black spot on the prothorax; (21) pupa in lateral view. Scales = 0.5 mm (10–12), and 0.3 cm (13–21), respectively. Photos (16–21) by LL Mota.
with well-delimited segments in dorsal view; long plumose setae laterally; remaining dorsal and subdorsal setae short. Openings of anterior tentacle organs (ATOs) present on metathoracic segment, and openings of tentacle nectary organs (TNOs) located on A8, but these organs are apparently not functional. Perforated cupola organs (PCOs) are located dorsally. Spiracle on the A1 segment is latero-ventral, whereas those on segments A2–A8 are in a dorsal position.

Second instar (Fig. 13): Duration 5–6 days (n = 3). Head capsule width 0.48–0.50 mm (n = 4); maximum body length 3.4 mm. Head dark brown; body grey green, with small white spots dorsally and pairs of reddish spots near spiracles; anal shield with a longitudinal dark band. Body dorsoventrally flattened with well-delimited segments in dorsal view; prothoracic shield covering almost completely covering head; ant-organs as in first instar, but functional.

Third instar (Figs 14,15): Duration 5–6 days (n = 4). Head capsule width 0.60–0.70 mm (n = 8); maximum body length 4.5 mm. General colour pattern and morphology same as second instar, but with more numerous and enlarged setae. All ant-organs present, including ATOs, TNOs, PCOs, dendritic setae and one pair of vibratory papilla on anterior border of prothoracic shield.

Fourth instar (Fig. 16): Duration 7–8 days (n = 4). Head capsule width 0.94–1.00 mm (n = 3); maximum body length 9.8 mm. Head brown with white marks; body grey green similar to third instar, but without longitudinal dark band on anal shield; prothoracic shield with two reddish bands. General morphology same as third instar, including ant-organs.

Fifth instar (Figs 17,22,23): Duration 7–8 days (n = 4). Head capsule width 1.40–1.82 mm (n = 6); maximum body length 1.48 cm. Head brown with white marks and black areas dorsally; body greyish green and covered by black and white tiny setae that confer a grainy appearance. General morphology similar to fourth instar including ant-organs, but body setae, more numerous and enlarged, including a row of flexible dendritic setae on anterior margin of prothorax.

Sixth (last) instar (Figs 18,19): Duration 7–8 days (n = 4). Head capsule width 2.00–2.46 mm (n = 3); maximum body length 2.8 cm. General colour pattern and morphology same as fifth instar.

Pupa (Figs 20,21): Duration 14–16 days (n = 3). Maximum length 1.8 cm, width at A1 0.50 cm (n = 3). Body dirty grey–greenish, with two conspicuous flat spots, black on prothorax and reddish brown on mesothorax and A1 abdominal segment. Black spiracles surrounded by white margins. Tegument entirely corrugated, with several small tubercles, including basilars on basis of wings, near ocular area and associated with abdominal spiracles in supra and subspiracular position. Body with several scattered stellate setae and clusters of papilliform setae on prothoracic and subspiracular tubercles. A silk girdle-crossing pupa over A1, near flat area. Fused A9 and A10 segments constitute ventrally flattened cremaster, which has short crochets in ventral position.

Behaviour and natural history

Adults of Thisbe silvestre sp. nov. were found along a 200 m stretch of a montane perennial stream (Fig. 1). In the first expedition, in February 2012, a single male was collected, and one female was observed in oviposition behaviour (Fig. 3) (described below). During this expedition, dozens of plants were unsuccessfully inspected in search of eggs or caterpillars. In the second expedition, in April 2013, males were observed along the same sector of the river as in the previous year, and about 20 larvae were found on the same plants inspected in 2012.

Males (n = 5) were collected at one specific sector of the river, perching on a tree about 10 m above the ground. This point was particularly suitable for collecting these butterflies, because the presence of rocks and the natural slope of the stream helped the access to the high perching site near the canopy. Throughout the day, several Riodinidae, Nymphalidae and Hesperiidae species alternated occupancy of this perch site. Males of T. silvestre appeared in the perch between 13:00 and 15:00 h and were observed chasing any butterfly that
crossed the stream. The only feeding behaviour observed was a male that visited flowers of Vernonia sp. Schreb. (Asteraceae) in the late afternoon (Fig. 2).

Oviposition behaviour was observed only once between 12:30 and 13:00 h, on Croton sp. (Euphorbiaceae). In the pre-alignment phase, the female was observed inspecting the host plant, landing above mature leaves of the host plant, and soon after, laying an isolated egg on the abaxial leaf surface (Fig. 3). The host plant is common along the riverbanks and along the access trail. Eggs and larvae were found on plants from 1.5 to 6 m in height. Larvae were found resting on the abaxial leaf surface, with the head close to the extrafloral nectaries (Figs 13–15). First to third instars larvae fed by scraping the lower surface of the leaves. After the third instar, larvae ate the entire leaf producing oblong leaf damage.

In the field, first instar was not tended by ants but from the second instar the tentacle nectary organs (TNOs) became functional, and larvae were occasionally observed being tended by ants (Figs 13–15, 19, 22, 23). A total of four ant species were recorded tending T. silvestre larvae, including one species of Camponotus Mayr, (Formicinae), one Pheidole Westwood one Procryptocerus Emery, (Myrmicinae), and one Tapinoma Foerster (Dolichoderinae) – none of which were identified to species. Larvae were commonly found without tending ants (~50%), and when tended, no more than one ant per larva was observed. Temporal turnover of tending ants was also recorded: a caterpillar was tended by Procryptocerus sp. 1 day and by Camponotus sp. the following day. In the laboratory, C. rufipes workers antennated the entire larval body, but especially the posterior region near the TNOs, as well as the anterior region where the anterior tentacle organs (ATOs) are located (Figs 22, 23). The antennation apparently stimulated the eversion of the ATOs; this eversion was observed only in larvae that were tended by ants (Fig. 23). The ATO eversion provoked clear alterations in ant behaviour, such as opening of the jaws and an increase in activity and aggressiveness.

**Etymology**

The specific epithet is masculine Latin adjective for ‘forest dweller’, alluding to the forest habitat of this species, which contrasts with the morphologically closely related Thisbe rupestre that is known to inhabit rock outcrop vegetation (‘campos rupestres’ in Portuguese), savannas and other open habitats.

**Type material**

**Holotype**: Male (Fig. 4) from Vale Verde, Parque Nacional do Caparaó, Alto Caparaó, 1300 m, Minas Gerais, Brazil. Deposited in the Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Unicamp, Campinas, São Paulo, Brazil. Labels on the holotype (four labels separated by transverse bars): / HOLOTYPUS / Vale Verde, Base Alto Caparaó, Parque Nacional do Caparaó, Alto Caparaó, MG: Brasil, 20°25'S 41°50'W, 24–25-IV-2013 – 1200–1300 m, Kaminski & Iserhard, leg / Holotypus – Thisbe silvestre / Kaminski, Iserhard & Freitas det. 2015 / ZUEC LEP 9179 /.

**Paratypes**: All same place as holotype: 1 male, 6-II-2012 (LAK – 070) (DNA voucher NS0081), Freitas, Kaminski & Iserhard leg., ZUEC LEP 9181; 3 males (LAK-173, LAK-174, LAK-175), 24–25-IV-2013 ZUEC LEP 9182, ZUEC LEP 9183, ZUEC LEP 9184; 2 females (LAK-182, LAK-183), 24–25-IV-2013, ex-larva, ZUEC LEP 9185, ZUEC LEP 9186.

**DISCUSSION**

Several aspects of the natural history of Thisbe silvestre sp. nov., including host plant use (Croton sp., Euphorbiaceae) facultative interaction with ants, the immature and adult morphology, are very similar to those described for Thisbe irenea, the type species of the genus (see DeVries 1988, 1991). This justifies the placement of the new species in the genus Thisbe. We hope that the information provided on the natural history and morphology of T. silvestre may help to clarify the taxonomy and systematics of Thisbe in the future, particularly because this species, and the phenotypically similar T. rupestre, have not been included in previous phylogenetic analyses (Hall & Harvey 2001; Penz & DeVries 2001).

Thisbe silvestre was not represented in three major scientific collections in Brazil. Because this is a large and conspicuous species, and taking into account that the Atlantic Forest is relatively well sampled for butterflies (Santos et al. 2008), it may seem surprising that this species has not been previously collected, maybe by their high perching behaviour. On the other hand, most inventories carried out in Atlantic Forest sites are very specific and localised, thus some regions of this biome are unknown for butterflies. There is a need of specific inventories to fulfil these gaps of information. However, its apparent rarity, patchiness and limited geographical distribution explain the fact that T. silvestre had remained undetected.

The mountainous area of Serra do Caparaó represents the northern limit for various butterfly species that occur in the highlands of south-eastern Brazil, including threatened butterflies such as Actinote quadra (Schaus) and Pauloglossum hydarnis (Godart) (Nymphalidae) (Freitas et al. 2014; Gomes et al. 2014). Although there are many common species of Croton in the study area, we found larvae of T. silvestre only on a single Croton species, from the Cleodora section, suggesting a high degree of host specialisation. Some Croton species in this section are recognised as having restricted geographical distributions (see Caruzo & Cordeiro 2013), suggesting that host plant specialisation might be another factor explaining the narrow geographic distribution of T. silvestre.

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Although the present data are not to evaluate the conservation status of this species, the fact that only a single population is known in the Atlantic Forest suggests a restricted geographic distribution (one important criterion to categorise endangered species as threatened under the criteria of the IUCN 2012). Accordingly, as suggested for another endangered species in the same area (see Freitas et al. 2014), conservation management actions should take the conservation status of this species into account. This includes the reduction of human disturbance to low levels in the area where the species occurs (e.g. tourism and visitation), especially along the 200 m sector where adults and larvae were observed as being common in April. Other actions include reduced vegetation management, scheduled cleaning of the edges and trails and restricted use of fire by visitors. Besides management actions, it is crucial to search for additional populations of *T. silvestre* in areas of suitable habitat, such as the region of Serra do Brigadeiro, in Minas Gerais state.

The present study exemplifies how much there is still to be discovered in a threatened biome like the Atlantic Forest, considered a hotspot of diversity in the world. If the current rates of habitat loss are not diminished, it is likely that many species will become extinct even before they are recognised.

**ACKNOWLEDGEMENTS**

We thank Waldomiro de Paula Lopes, who facilitated the work in the Parque Nacional do Caparaó; ICMBio for providing the research permits (SISBIO n° 10802-5); and Ricardo Campos, Karla Yotoko and the Field Ecology Course of ‘Universidade Federal de Viçosa’ for the logistic support. We thank Luísa L. Mota who kindly took some beautiful photos; Ken Walker, Keith Willmott and Roger Vila for critically reading the manuscript; Maria B. R. Caruzo for host plant identification; Thamara Zacca and Diego R. Dolibaina for providing pictures and dissections of types deposited in the DZUP; and Carlos E. B. Nobre for sharing unpublished data on *Thisbe rapu stre*. LAK was supported by FAPESP (10/51340-8), CNPq (163119/2013-9) and CAPES (3200-14-0). CAI thanks B. Nobre for sharing unpublished data on *Juditha felsina* (Riodinidae). This publication is part of the RedeLep fellowship 302585/2011-7) and the National Science Foundation (grant 2011/08433-8). A VLF was supported by CNPq (fellowship 563332/2010-7) and CAPES (3200-14-0). CAI thanks LAK was supported by FAPESP (10/51340-8), CNPq (fellowship 302585/2011-7) and CAPES (3200-14-0). CAI thanks

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Accepted for publication 25 May 2015.