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A new species of *Dasyhelea* (Diptera: Ceratopogonidae), mining the leaves of the floating fern *Salvinia minima* Baker

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ABSTRACT

A newly described species, *Dasyhelea mesophylla* Dominiak and Borkent, from Costa Rica is the first species in this large and diverse family known to be a leaf-miner. The species is described as third and fourth instar larvae, pupa, and male and female adults. The biology of larvae mining the floating leaves of *Salvinia minima* Baker is also described. The pupa has a distinctive, sharply pointed respiratory organ, shared with some other species of *Dasyhelea*, which pierces the surface of the leaf to breathe. The species belongs in the *grisea* group and its close relatives within that group are noted.

RESUMEN

Una especie nueva aquí descrita, *Dasyhelea mesophylla* Dominiak and Borkent, de Costa Rica, es la primera especie de esta familia grande y diversa que se sabe que es un minador de hojas. La especie se describe como larvas de tercer y cuarto estadio, pupas y adultos masculinos y femeninos. También se describe la biología de las larvas que minan las hojas flotantes de *Salvinia minima* Baker. La pupa tiene un órgano respiratorio distintivo y puntiagudo, compartido con algunas otras especies de *Dasyhelea*, que perfora la superficie de la hoja para respirar. La especie pertenece al grupo grisea y se mencionan sus parientes cercanos dentro de ese grupo.

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Introduction

One of the problems of being taxonomists working on such a large group as biting midges, with 6274 species now recognised (Borkent and Dominiak 2020; Borkent *et al.* 2022) and so many more yet to be named, is that more observations and discoveries accumulate during field work than it is possible to publish on. In 1993 the second author discovered the first instance of leaf-mining in Ceratopogonidae, of larvae of a species of *Dasyhelea* Kieffer mining the leaves of the floating aquatic fern *Salvinia minima* Baker in Costa Rica. After 30 years of sitting in storage, this new *Dasyhelea* midge is finally being described; we also provide this new *Dasyhelea* midge and providing the first detailed report of its leaf-mining behaviour, which is unique within the family Ceratopogonidae. The species and its exceptional feeding habit was briefly mentioned by Borkent and Craig

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(2001), who referred to it in their description of *Stilobezzia rabelloi* Lane pupa which uses respiratory organs to pierce roots of aquatic plants to obtain oxygen, and later also by Borkent and Spinelli (2007).

The genus *Dasyhelea*, distributed worldwide, with 628 extant species and many more not yet named, is one of the most species-rich genera within the family (Borkent and Dominiak 2020; Borkent *et al*. 2022). Members of this group are widely distributed, occur mostly in moist habitats, and are often common from lowlands to high altitudes, where they have been reported up to 4000 m a.s.l. (Borkent and Spinelli 2007).

Immature stages of *Dasyhelea* inhabit a wide variety of aquatic, semi-aquatic and terrestrial habitats, such as the littoral zone of lakes, puddles, streams, springs, swamps, peat bogs, moist soil, sap oozing from trees, wet bark, decomposing plants (Waugh and Wirth 1976; Szadziewski *et al*. 1997; Dominiak 2012), the moist, lowermost portion of otherwise dry stems of umbelliferous plants (P. Dominiak, pers. obs.), fungi (Graves and Graves 1985) and the guano from fruit bats (Vattier 1964). Phytotelmata, such as small water reservoirs formed in tree hollows, damaged tree trunks, bamboo stems, fruit shells or leaf axils, are also often used by species of *Dasyhelea* as larval habitats (Goetghebuer 1925; Johannsen 1932; Mayer 1934a, 1934b; Zilahi-Sebess 1936; Disney and Wirth 1982; Przhiboro 2005; Szadziewski and Dominiak 2006). Other examples of extreme habitats occupied by these midges are ephemeral rock pools and their artificial substitutes (Kieffer 1925; Johannsen 1932; Zilahi-Sebess 1931; Remmert 1953; Wirth 1987; Lee *et al*. 1989; Ashe *et al*. 2007), hot springs with water temperature over 50°C, pitchers of carnivorous species of *Nepenthes* L. (Johannsen 1932; Mayer 1934b; Sheppe 1975; Wirth and Beaver 1979; Lee and Chan 1985), saline soil or inland saline waters and the intertidal zone of seas (Thienemann 1925; Mayer 1934a; Wirth 1978; Szadziewski 1983).

The apneustic larvae of *Dasyhelea* are smooth and cylindrical in shape, with a rather prominent and slightly hypognathous head capsule, and with the caudal segment bearing retractile anal papillae and posterior prolegs armed with hooks and hooklets (sometimes reduced to just the hooks) (Glukhova 1979; Szadziewski *et al*. 1997). They do not swim, but crawl among the substrate (algae, mosses, liverworts or plants on water margins, detritus, etc.), making use of their mouthparts and anal hooks (Wirth 1978; Fűrst von Lieven 1998). According to Thienemann (1915) and Waugh and Wirth (1976) some species spin tubular cases in the last 4th instar but it is not a behaviour typical for all members of the genus. Although in general not much is known about the feeding habits of *Dasyhelea* larvae (Mullen and Hribar 1988), their diet consists of various unicellular algae, fungi and detritus (Zilahi-Sebess 1931; Mullen and Hribar 1988). Some species are also carnivorous (protists, rotifers, oligochaetes, early stages of larvae of chironomids and ceratopogonids) or necrophagous (larvae of mosquitoes) (Zilahi-Sebess 1931; Lee and Chan 1985; Dodson 1987; Mullen and Hribar 1988; Hribar and Denson 2008).

Pupae of at least some species of *Dasyhelea* can actively change their immersion depth by regulating the pressure of gases accumulated under their cuticle, and they use abdominal movements to slightly adjust their position (Zilahi-Sebess 1931). For species inhabiting tree sap, blankets of algae or rotting plants, larvae move towards the substrate surface before pupation takes place (Sannino and Espinosa 2004; P. Dominiak, pers. obs.).

Adults can be found in tree canopies (Thunes *et al*. 2004, 2021) or among shrubs and low vegetation near water reservoirs, and on flowers where they imbibe nectar (Waugh and Wirth 1976). *Dasyhelea* midges have been often observed on flowers of various plant species, especially of the family Apiaceae (Szadziewski *et al*. 1997), and they are known as pollinators of the cacao tree *Theobroma cacao* L. and rubber tree *Hevea brasiliensis* Muell. Arg. (Lee *et al*. 1989). Mouthparts are similarly developed in both sexes (Carter *et al*. 1921; Glukhova 1981), suggesting that females and males can actually feed on the same substrates. Unlike most other Ceratopogonidae, there are no vertebrate biters, ectoparasites or predators among *Dasyhelea* and all females lack biting mouthparts. Various species feed on nectar, sweet secretions of plants, or honeydew (Waugh and Wirth 1976; Szadziewski *et al*. 1997). However, some laboratory observations indicate that imagines of *Dasyhelea* do not need to take a meal at all (Zilahi-Sebess 1931).

The intrageneric classification of the genus *Dasyhelea* is problematic. Since comprehensive phylogenetic studies on this group of biting midges have not been undertaken, currently it is impossible to propose a natural subgeneric system. However, five subgenera arranged by Remm (1962, 1979) are traditionally used in the literature concerning the Palaearctic fauna. These are *D*. (*Dasyhelea*), *D*. (*Prokempia* Kieffer), *D*. (*Pseudoculicoides* Malloch), *D*. (*Dicryptoscena* Enderlein) and *D*. (*Sebessia* Remm). The New World's species are generally arranged in various species groups. Four of them, namely the *cincta* group, *grisea* group, *mutabilis* group and *traverae* group, were established by Wirth (1952). Later, many new species groups were proposed: the *leptobranchia* group (Waugh and Wirth 1976), for some species included previously in the *traverae* group (see Wirth 1952); the *borgmeieri* group (Wirth and Waugh 1976); and the *brevicornis* group (Grogan and Wieners 2006). Recently, Díaz *et al*. (2010) proposed the *patagonica* group for a few southern Neotropical species.

Comparison of the two systems, subgenera vs species groups, shows that the *cincta* group and the *holosericea* group are equivalent to the subgenus *D*. (*Sebessia*) (proposed by Remm 1979), the *grisea* group corresponds to the subgenus *D*. (*Dasyhelea*), and the *mutabilis* group together with the *borgmeieri* group corresponds to *D*. (*Pseudoculicoides*) (Remm 1962; Waugh and Wirth 1976).

Materials and methods

Specimens were examined, studied, measured and drawn using a Wild M3 dissecting microscope and a Zeiss Jenaval compound microscope. Photomicrographs were taken with a Canon EOS Rebel T3i camera through the Zeiss Jenaval compound microscope.

Terms for adult structures follows those used in the *Manual of Afrotropical Diptera* (Borkent 2017); and for pupae, only features differing from the generic description by Borkent (2014) are given here. Larval terms follow Díaz *et al*. (2019) and Fürst von Lieven (1998).

The abbreviations used to represent the museums cited in this text are as follows:

CNCI – Canadian National Collection of Insects, Eastern Cereal and Oilseed Research Centre, Agriculture Canada, K.W. Neatby Building, Ottawa, Ontario, K1A 0C6, Canada.
MNCR – Museo Nacional de Costa Rica, P.O. Box 749–1000, San José, Costa Rica.

The new name has been registered in ZooBank (the Official Registry of Zoological Nomenclature): urn:lsid:zoobank.org:act:0942518A-845D-4EB0-9525-9A1F6193A958.

Results

Dasyhelea mesophylla *Dominiak and Borkent, sp. nov.*

Diagnosis

The only *Dasyhelea* in the Neotropical Region with the following characteristics. *Male adult*: with a single elongate radial cell, sternite 9 straight, paramere sinusoidal, twisted and tapering to a narrow apex, fused basally with gonocoxal apodemes and forming with them an asymmetrical structure, aedeagus symmetrical with dark, well-developed anterolateral club-like projections and 2 well separated and slender posterior projections with apices that are hooked dorsolaterally. *Female adult*: with 1 elongate radial cell, frontal sclerite broader than long, with sternite 9 elongate anteriorly, conical and with a rounded apex. *Pupa*: with the respiratory organ curved and coming to a sharp point, with a double row of about 7 circular pores each (so about 14 total) restricted to the apical 0.3 of the respiratory organ, and with a slightly elongate, pointed, gradually tapering terminal process bearing 2–3 pointed tubercles laterally near its base. *Larva*: not presently diagnosable, but see taxonomic discussion.

Description

Male adult. Antenna (Figure 1(a)) dark; antennal flagellum length 0.58–0.60 mm (n = 3), antennal ratio 0.79–0.95 (n = 3). Frontal sclerite broad, with long, slender projection. Clypeus divided into two parts, with 7–11 setae distributed laterally in two rows (n = 4). Palpus (Figure 5(b)) with third palpal segment relatively stout, 44–53 μm long (n = 4); palpal ratio of the third segment 2.54–3.17 (n = 4); sensilla capitata present on segment 3 only, rather sparse and distributed closer to its outer margin. Thorax (Figure 1(b)), aside from slightly lighter lateral sclerites, dark. Scutellum yellowish, with 6–8 bristles and 1–5 smaller setae (n = 5). Wing with 1 radial cell; wing length 0.76–0.81 mm (n = 4), costal ratio 0.45–0.48 (n = 4). Halter dark. Legs (Figure 1(b)) pale with indistinct, irregular darker patches at midlength of fore- and midfemora and tibiae of all legs, and apical half of hind femur. Hind tibial comb with 5–7 spine-like setae (n = 4). Tarsal ratios: foreleg 2.1–2.3 (n = 3), midleg 2.4–2.6 (n = 3), hind leg 2.2–2.5 (n = 4). Abdominal segments 3–8 well sclerotised; sclerotisation on segments 3–5 disjunct medially. Genitalia (Figures 1(c), 5(a)). Apicolateral process of tergite 9 prominent, finger-like, with single apical seta. Cercus moderately small, with 4 setae (n = 1). Posterior margin of sternite 9 straight or with shallow excavation medially. Gonocoxite without mesoventral hook. Gonostylus nearly straight, with rounded apex, same length as or slightly longer than gonocoxite. Paramere and gonocoxal apodemes fused and forming an asymmetrical structure; paramere sinusoidal, twisted, tapering to narrow apex. Aedeagus symmetrical, with dark, well-developed anterolateral club-like projections, 2 well-separated and slender posterior projections with apices hooked dorsolaterally; anteroventral margin of aedeagus weakly sclerotised, rounded.

Female adult. Antenna (Figure 2(a)) dark; antennal flagellum length 0.52 mm, antennal ratio 0.84. Frontal sclerite broad, with long, slender projection. Clypeus divided into two parts, with about 15 setae distributed laterally in two rows. Third palpal segment relatively stout, 46 μm long; palpal ratio of the third segment 2.67; sensilla capitata as in male.

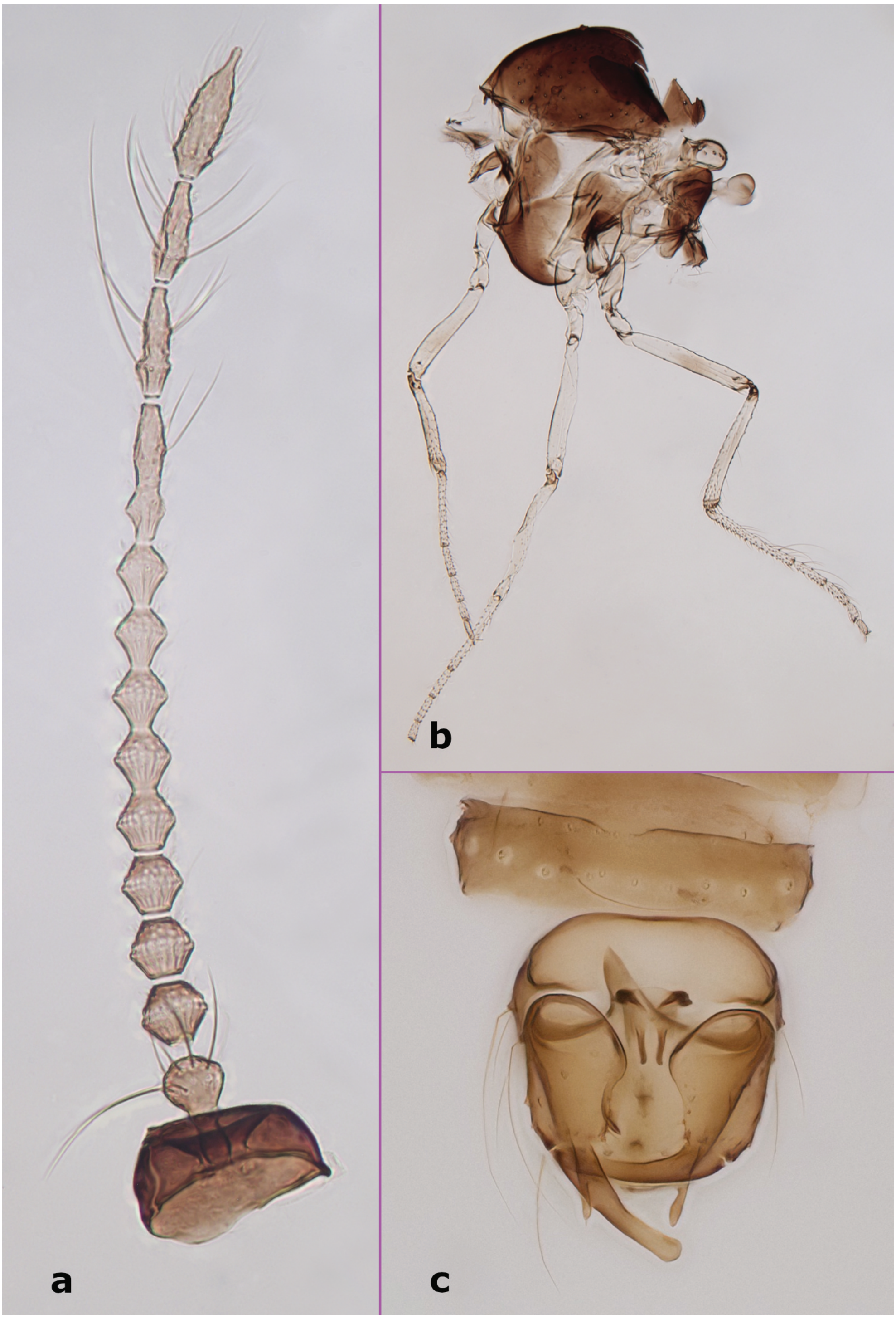

Figure 1. *Dasyhelea mesophylla*, male: (a) antenna; (b) thorax and legs, lateral view; (c) genitalia, ventral view.

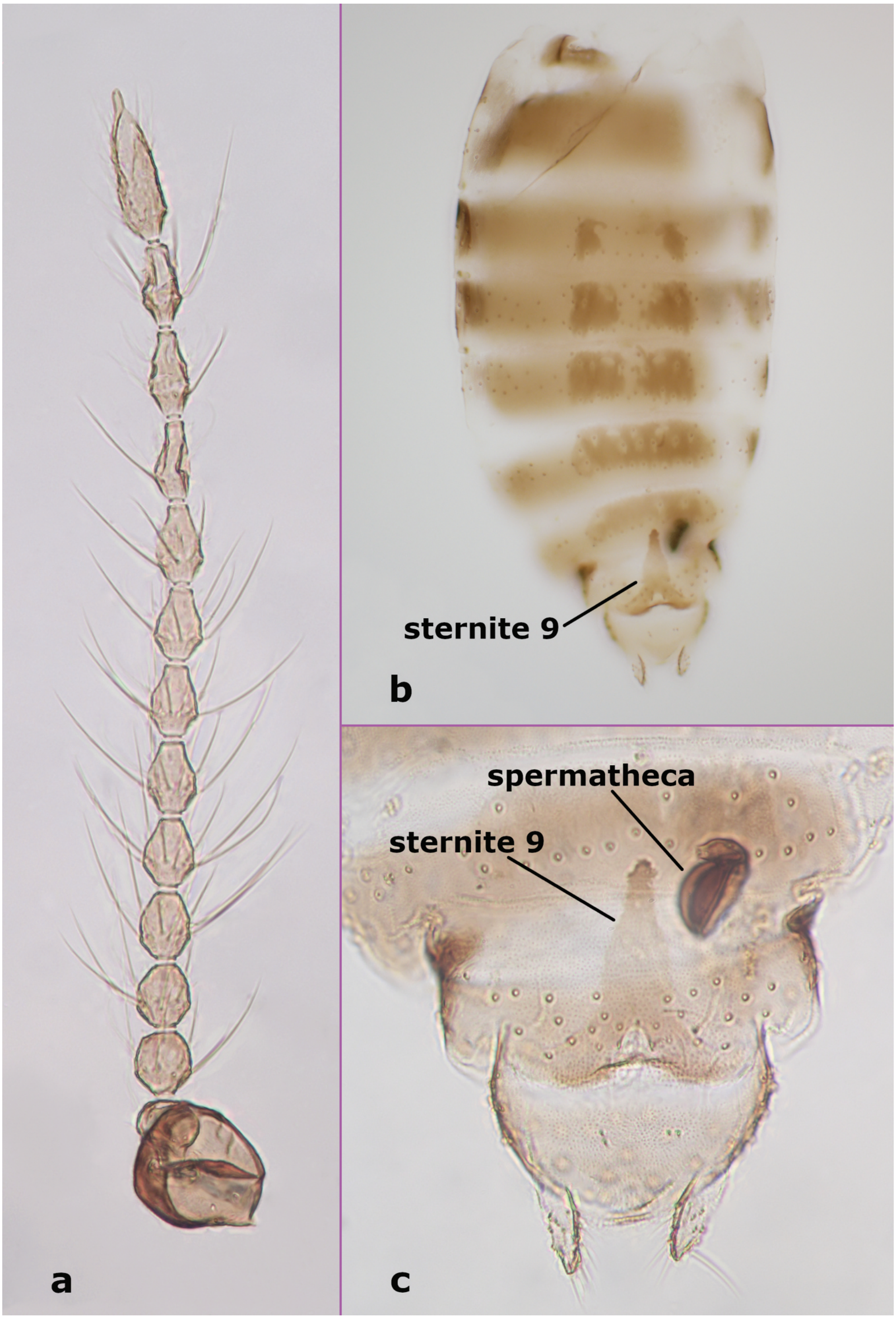

Figure 2. *Dasyhelea mesophylla*, female: (a) antenna; (b) abdomen, ventral view; (c) apex of abdomen highlighting sternite 9 and spermatheca, ventral view.

Scutellum yellowish, with 8 bristles and about 8 smaller setae. Wing with 1 radial cell; wing length 0.78 mm, costal ratio 0.50. Legs pale with indistinct, irregular darker patches at midlength of fore-, hind femora, tibiae. Hind tibial comb with 6 spine-like setae. Tarsal ratios: foreleg 2.3, midleg 2.3, hind leg 2.3. Genitalia (Figures 2(b,c), 5(c)). Abdominal segments (Figure 2(b)) 3–8 well sclerotised; sclerotisation on segments 3–5 disjunct in the middle. Sternite 9 elongate anteriorly, conical, with rounded apex (Figures 2(b), 5(c)). Spermatheca (Figure 2(c)) single, retort-shaped, length 48 µm (distorted).

***Fourth instar larva* (Figures 3(a–f), 8(b)).** Total length 1.93–3.22 mm (n = 8). Colour in life whitish, with head capsule dark brown, length 0.27–0.35 mm (n = 18), L/W 1.61–1.92 (n = 7), tapering to apex; chaetotaxy as in Figures 3(a–c), unlabelled anterior setae are uncertain, j not visible in figures but present. Labrum (Figures 3(b–c)) narrow, projecting anteriorly; anterolateral margins of head capsule extending anteriorly. Mandible (Figures 3(a,c,d)) with 3 teeth, apical 2 teeth elongate, pointed, basal tooth broad. Epipharynx (Figure 3(d)) massive, strongly sclerotised, lateral arms stout, short, dorsal comb with well-developed fringe. Hypopharynx (Figure 3(d)) well developed. Caudal segment with approximately 5–7 well-developed hooks (on each side). Other details either not visible or similar to most other *Dasyhelea* (Díaz *et al*. 2018, 2019).

***Third instar larva* (Figure 8(a)).** Similar to fourth instar. Total length 1.78–2.59 mm (n = 7). Colour in life whitish, with head capsule dark brown, length 0.20–0.23 mm (n = 8), L/W 1.62–1.74 (n = 4), tapering to apex.

***Pupa*.** Habitus as in Figures 4(a), 8(c–d). Total length 1.87–2.29 mm (n = 7). Clypeus (Figure 6(a)) with CL-1-H, CL-2-H apparently absent in most, one specimen with very tiny seta CL-2-H, otherwise the area of these sensilla represented by small section of contorted cuticle; O-2-H absent; dorsal apotome (Figure 6(b)) smooth, with DA-1-H a tiny seta, DA-2-H a campaniform sensillum. Mesonotum without short tubercles; respiratory organ (Figure 4(b)) length/width 5.3–6.3 (n = 5), curved, apex pointed, somewhat circular in cross section, with a double row of about 7 circular pores each (so about 14 total), restricted to about apical 0.3 of respiratory organ, closely abutting apically, separated more basally, outer surface with strong annulations on posterior (inner curve) for about basal 0.2–0.5 to about 0.1–0.8, without spicules, tracheal tube curved along length of respiratory organ, somewhat thicker basally, with spirals restricted to base; sensilla: anterolaterals – 1 seta, 2 campaniform sensilla; dorsal setae (Figure 6(c)) – D-1-T well anterior of D-2-T, D-3-T relatively close to D-2-T. Metathoracics (Figure 6(d)) – 2 campaniform sensilla; M-2-T, M-3-T separated (not abutting), relatively near anterior margin of metathorax. Abdomen without dark pigmentation, segments 3–8 each with some wide, shelf-like tubercles; segment 9 with terminal processes closely approximated basally, each projecting posteriorly, each with 2–3 lateral, well-developed, pointed projections, with dorsal one bearing campaniform sensillum at its base; sensilla: tergite 1 (Figure 6(d)) with 5 setae (D-2-I, D-3-I, L-1-I, L-2-I, L-3-I) and 2 campaniform sensilla (D-4-I, D-7-I); segment 4 (Figure 7(a)) with D-2-IV absent; D-4-IV, D-8-IV each on wide, low, slightly separate tubercle, D-7-IV on slightly pointed tubercle; posterior dorsal sensilla in transverse row, arranged medially to laterally: D-4-IV, D-7-IV each a campaniform sensillum, D-8-IV a short seta on wide, low tubercle, L-1-IV, L-2-IV, L-3-IV, L-4-IV each a short seta on apically

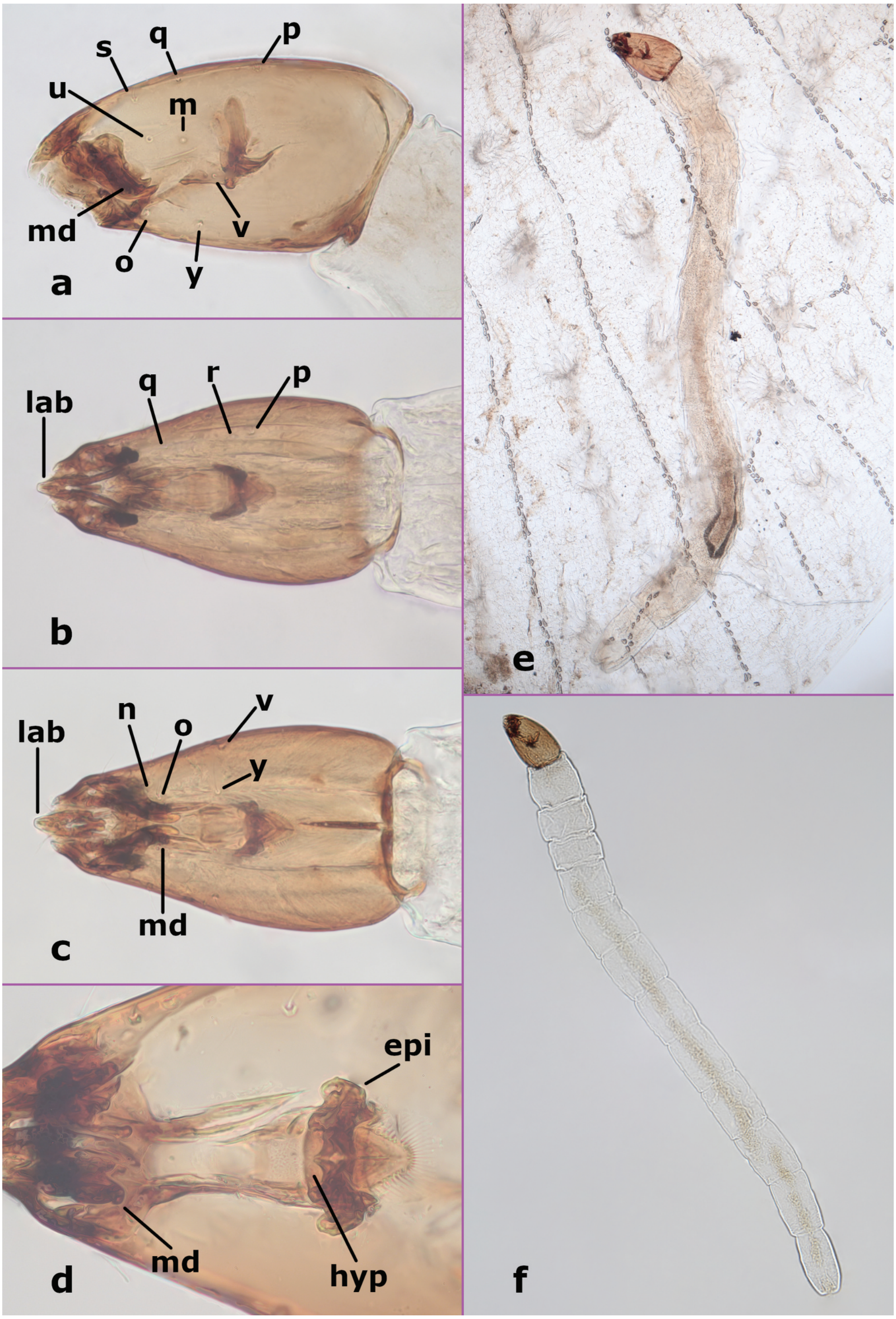

Figure 3. *Dasyhelea mesophylla*, fourth instar larva: (a) head capsule, lateral view; (b) head capsule, dorsal view; (c) head capsule, ventral view; (d) portion of head capsule, ventral view showing details of pharyngeal apparatus; (e) in situ in *Salvinia minima* leaf, (f) habitus. epi, epipharynx; hyp, hypopharynx; lab, labrum; md, mandibles.

Figure 4. *Dasyhelea mesophylla*, male pupa: (a) pupa, in ventral view, and associated fourth instar larval exuvial head capsule, in lateral view; (b) right respiratory horn, posterior view; (c) abdominal segment 9, in ventral view.

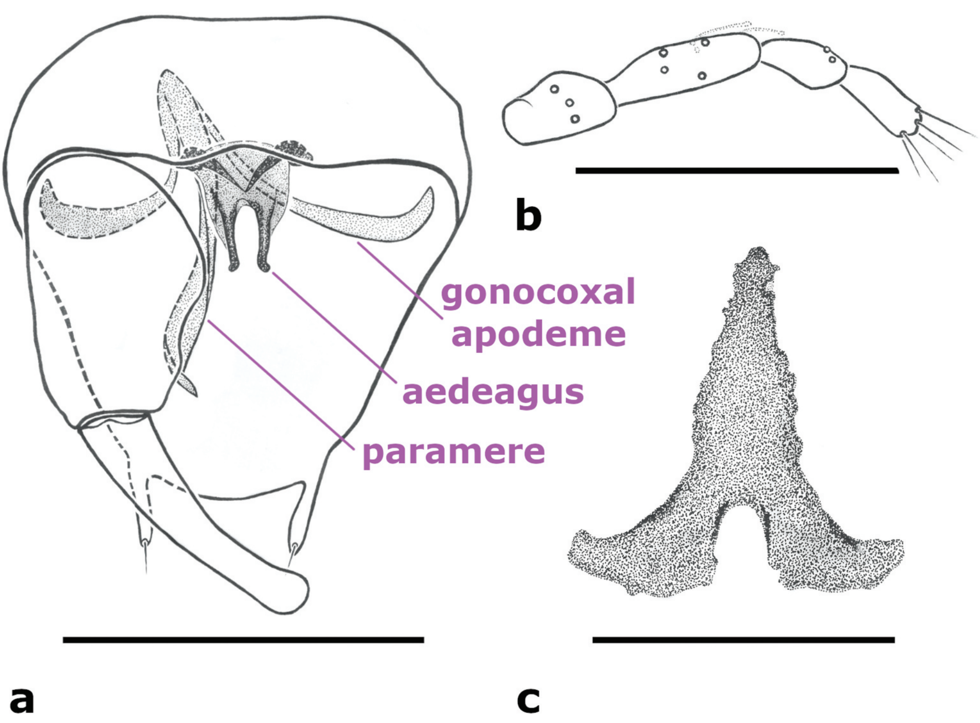

Figure 5. *Dasyhelea mesophylla*, male (a, b), female (c): (a) genitalia with right gonocoxite and gonostylus removed, ventral view; (b) left palpus, anterior view; (c) sternite 9, ventral view. Scale bars: 0.1 mm.

pointed tubercle; V-5-IV absent; V-6-IV a campaniform sensillum; V-7-IV a seta on wide, low tubercle; segment 9 (Figures 4(c), 7(b)) long, terminal process with 3–4 divisions, laterally directed tubercles curved to somewhat hook-like, posteriorly directed portion elongate, tapering to point; with only one of D-5-IX present (a campaniform sensillum), D-6-IX apparently present (difficult to discern amidst shagreen), V-1-IX, V-2-IX elongate and short seta, respectively.

Distribution and bionomics

Dasyhelea mesophylla is known only from the type locality on the west coast of Costa Rica at an altitude of about 5 m. Adult specimens were reared from larvae and pupae present in *S. minima* leaves (Figure 8) floating in a lagoon on the northern margin of Carara National Park, about 1.5 km east from highway 34 along the hiking trail. The lagoon from which this species was collected in 1993 is periodically flooded by the abutting and extremely large Tárcoles River. The larvae and pupae were common but no specific data were taken in this regard. Virtually every clump of leaves had at least one leaf being mined (or that had been previously mined). Leaves were generally in clumps of 6–12 leaves. Borkent and Craig (2001) described the pupa of *Stilobezzia rabelloi* Lane, which have piercing respiratory organs to obtain oxygen from the dangling 'roots' (actually modified leaves), from the same habitat as *D. mesophylla*.

Observations of the mining larvae and of pupae in the *S. minima* leaves were made under the dissecting microscope as follows. Several fourth instar larvae were observed in

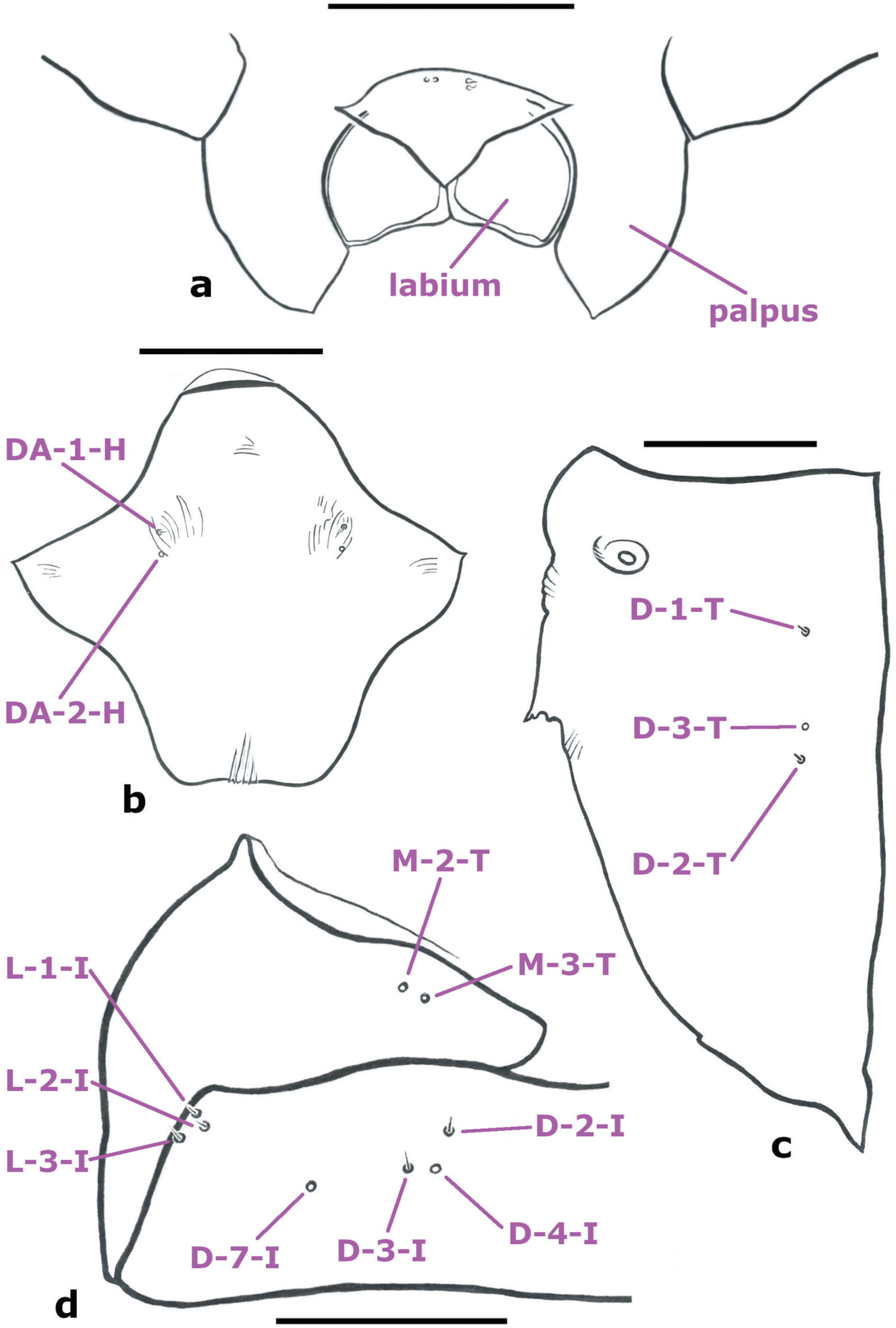

Figure 6. *Dasyhelea mesophylla*, pupa: (a) posterior portion of head, ventral view; (b) dorsal apotome, anterior view; (c) mesonotum, dorsal view; (d) metathorax and tergite 1, dorsal view. Scale bars: 0.1 mm.

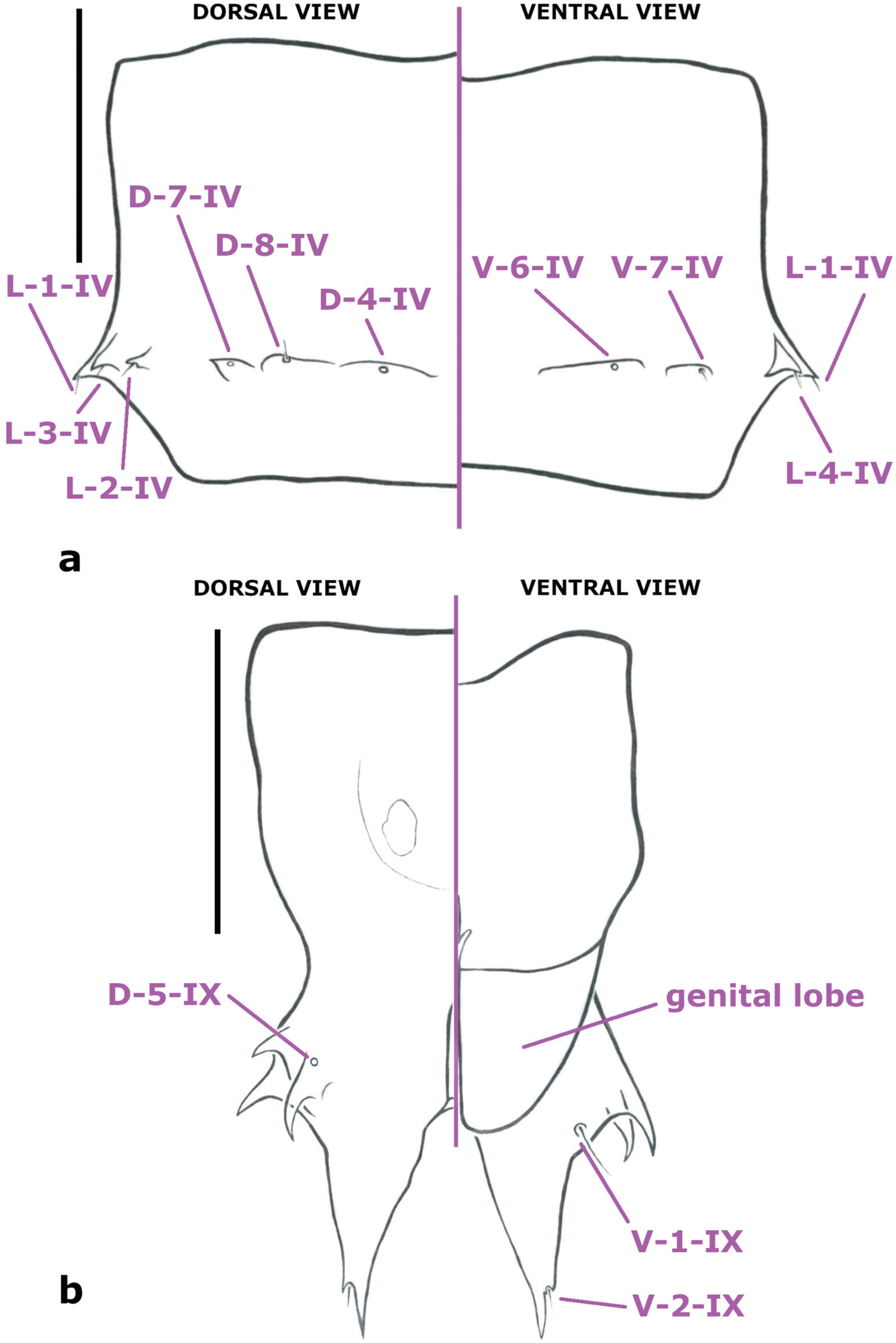

Figure 7. *Dasyhelea mesophylla*, pupa: (a) abdominal segment 4; (b) male abdominal segment 9. Scale bars: 0.1 mm.

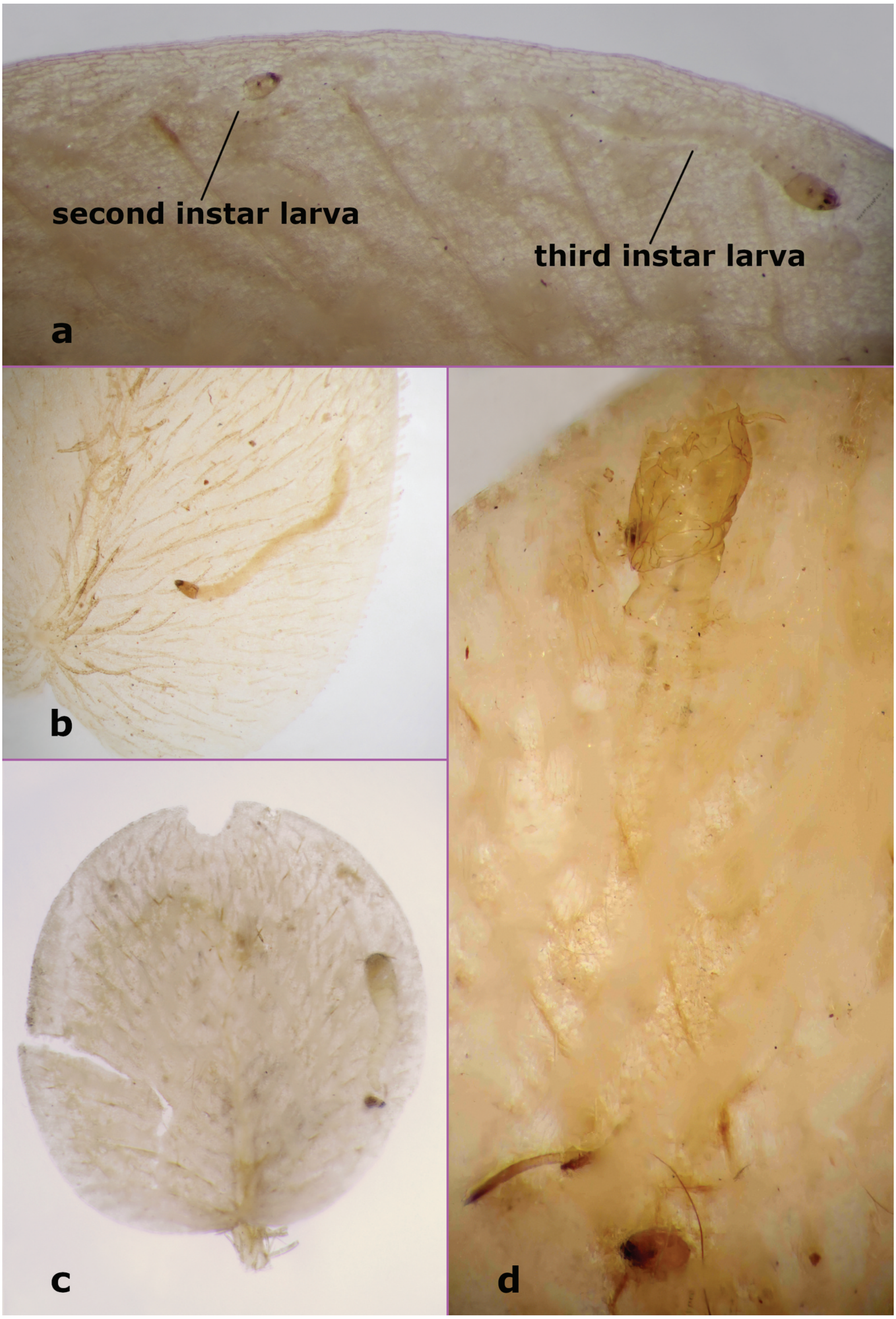

Figure 8. Immatures and exuviae of immatures of *Dasyhelea D. mesophylla* in leaves of *Salvinia minima*, all in dorsal view of leaf, other than b, taken from ventral view of leaf: (a) second and third instar larvae; (b) fourth instar larva; (c) pupa and larval exuviae in entire *Salvinia minima* leaf (for larval exuviae, only head capsule visible); (d) pupal and larval exuviae.

some detail, although many were otherwise present. The larvae actively mined the leaves, scraping at live fern tissue, had green material in their guts and produced green excrement. The narrow labrum and anterolateral margins of head capsule extending anteriorly likely facilitate the scraping of plant tissue. The mines of at least fourth instar larvae produced a bulge on the upper surface of the leaves. The frass produced by larvae was either in the mine or on the surface of the leaf. At least some larvae periodically leave the mine for the surface and then burrow back into the leaf. A few larvae were observed to move from one leaf to another. Possibly this is a means of ensuring that overcrowding does not limit any given larva which can probably move from one abutting leaf to another. One larva was observed feeding on the interior of the leaf but with about 2/3 of its body on the surface of the leaf. Another larva was seen crawling on the surface, then gnawing through the surface to the interior and, after feeding for some time, drawing the rest of the body into the leaf. Generally, when a leaf was mined, there were only 1–2 fourth instars present (aside from other earlier instar larvae), but one instance of three larvae in a single leaf was observed.

Several third instar larvae were observed mining the interior of the leaves, in an identical manner to the fourth instar larvae, but no third instars were seen on the surface of the leaves. One second instar larva was observed in a tunnel just slightly wider than its body, winding through the leaf. The mines of third and fourth instar larvae were more excavated in places, connected by swollen (leaf surface bulging at least dorsally) tunnels about the width of those larvae. First and second instar larvae seemed to be concentrated on the peripheral margins of the leaves, while third and fourth instar larvae were generally in the main body of the leaves.

On the other hand, several leaves were observed to be partially mined but without larvae, suggesting the larva had died or, more likely, had moved on to another leaf to mine there.

Two larvae were observed to pupate. One pupa was entirely encased within the mine with no direct opening to the surface, other than the insertion of its respiratory organs through the surface cuticle of the leaf. Another pupa had cut an opening near the anterior end of the pupa, but without the respiratory organs protruding. Observed again later, the pupa had stuck one respiratory organ up through the leaf cuticle, indicating the pupa only needs to obtain oxygen periodically from the surface (or can withdraw for some time if disturbed). Both pupae had their fourth instar exuviae present nearby (Figures 4(a), 8(c–d)). A number of other pupae were observed with either one or both respiratory organs protruding from the leaf.

Two emerging adults in the laboratory had free-floating pupal exuviae. The one pupa noted above that had been entirely encased by the surface of the leaf had made a jagged exit hole in the leaf, and the other appeared to escape through the hole already present. Some leaves were found with pupal exuviae with part of their abdomens still in the leaves.

It is worth noting that Forno and Bourne (1984) previously recognised an unnamed *Dasyhelea* as 'phytophagous' on *Salvinia molesta* D.S. Mitch. in Brazil. Another species, called *Dasyhelea* sp. 3 (*grisea* group), was reported by Torreias *et al*. (2013) from south-eastern Brazil, where its 'immature stages were collected in *S. auriculata*' Aubl (6). However, considering the sampling method used, the immatures (pupae only?) were most probably found among rhizomes and submerged, root-like leaves of *Salvinia*. Three males and one female were obtained but no details regarding their morphology are given by the authors (Torreias *et al.* 2013). Pelli and Barbosa (1998) mentioned *D. paulistana* Forattini and Rabello as present on *S. molesta* in Brazil, but they did not assign this species to taxa feeding on *Salvinia* or to taxa causing harm to it. The latter *Dasyhelea* species together with *D. pseudopollinosa* Díaz and Ronderos were collected from mats of *S. auriculata* and *Azolla filiculoides* Lam. in Brazil and Argentina (Díaz *et al.* 2014).

Taxonomic and phylogenetic discussion

This new species belongs to the *grisea* species group or subgenus *D.* (*Dasyhelea*) (Dominiak 2012) if following the subgeneric division proposed by Remm (1962, 1979). The presence of the strongly modified pupal respiratory organ of *D. mesophylla* is shared by a number of species in the *grisea* species group, and because it is unique within the family (Borkent 2014), we consider this a synapomorphy of these species. As such, from the limited number of *Dasyhelea* species known as pupae (Borkent 2014), we consider *D. mesophylla* to form a monophyletic group with *D. traverae* Thomsen from eastern United States, *D. pollinosa* Wirth from the western and eastern United States, *D. chani* Wirth and Linley from Florida (United States), *D. paulistana* from Argentina and Brazil, *D. pseudopollinosa* from Brazil, *D. caesia* Remm (syn. *D. lugensis* Brodskaya) from Europe, and two unnamed species from Indonesia (Sumatra, listed as *Holoconops* sp. in Mayer 1934b) and Australia (A. Borkent, pers. obs.), respectively. All of them have virtually identical respiratory organs; these are thick at the base to about midlength and taper to a sharp apex. The respiratory organs of the pupae of *D. mesophylla* are somewhat more elongate than those of the other abovementioned species of *Dasyhelea*. Although not known as immatures, male *D. unicolour* Remm and *D. stackelbergi* Remm appear very similar to those in this group of species and may be closely related.

The pupae of *D. mesophylla* stick their respiratory organs through the upper surface of the mined *S. minima* leaves to obtain oxygen from the exposed surface of the leaf, suggesting that these other species also use their respiratory organs in a similar manner. However, because at least the larvae of *D. traverae* do not appear to mine leaves (Thomsen 1935, 1937; Waugh and Wirth 1976) it is also possible that *Dasyhelea* species with such respiratory horns obtain air from water plants, similarly to *S. rabelloi*.

The piercing respiratory organs of pupae of a group of *Stilobezzia* Kieffer is clearly separately evolved, as evidenced by differences in the details of the respiratory organs compared with *Dasyhelea* as well as the phyletic distance between the two groups (Borkent and Craig 2001).

In spite of the fact that species of *Dasyhelea* are common throughout much of the world, only 62 out of 628 known species have been described as larvae (Díaz *et al.* 2013; Borkent 2014, 2016, 2018, 2019; Duan *et al.* 2019; Borkent and Dominiak 2020; Lu *et al.* 2020; Borkent *et al.* 2022). One reason is probably that larvae of *Dasyhelea* are generally morphologically conservative. However, the very narrow protruding labrum and corresponding anterolateral projections of the head capsule, likely important for scraping leaf material free to ingest, may be distinctive among those larvae which have been described so far, keeping in mind that, of the species with a piercing respiratory organ, only the larvae of *D. caesia* (Brodskaya 1995), *D. chani* (Wirth and Linley 1990) and *D. traverae* (Thomsen 1937) have been described. It is important to recognise that most descriptions of *Dasyhelea* larvae are rather superficial. Detailed comparative studies are needed of the significant differences in larvae between the numerous species of this genus. There are at least some marked differences exhibited by some taxa (eg those species living in *Nepenthes* pitcher plants in Southeast Asia which have extremely long, narrow head capsules; Tokunaga 1961; Wirth and Beaver 1979). None of the *Dasyhelea* species described from larvae or pupae other than *D. mesophylla* are known to be leaf-miners.

Types

Holotype: male adult with associated larval and pupal exuviae, on microscope slide, labelled 'HOLOTYPE *Dasyhelea mesophylla* Dominiak and Borkent', 'Costa Rica, 5 km NE, Tarcoles, C.R. 26-vii-1993, A. Borkent CD1490, CD 1490', 'Reared from larva mining Salvinia leaf' (CNCI). Allotype: female adult with associated pupal exuviae labelled as for holotype (CNCI). Paratypes: 3 males each with associated pupal exuviae, labelled as for holotype except 'Reared from pupa embedded in Salvinia leaf' (1, MNCR; 2, CNCI); 1 pupa, 19 fourth instars, 6 third instars, from type locality, 3 August 1993, in *Salvinia* leaves, CD1502 (4 fourth instars, MNCR; remainder, CNCI); 2 pupae and their associated fourth instar exuviae in *Salvinia* leaves, 2 fourth instars, 1 third instar from type locality, 3 August 1993, CD1502 (CNCI).

Derivation of specific epithet

The name *mesophylla* refers to the parenchyma between the epidermal layers of a leaf, the tissue where the larva and pupa of the new species have been found.

Conclusions

Forno and Bourne (1984) recorded an unidentified species of *Dasyhelea* as 'phytophagous' on *Salvinia*. They noted its presence only on *S. molesta* that they studied in south-eastern Brazil. Torreias *et al*. (2013) recorded the presence of an unnamed species of *Dasyhelea* in the *grisea* group from Lago Grande, Ilha da Marchantaria, Iranduba, Amazonas, Brazil, as immature stages were collected in *S. auriculata*. This suggests that there may be more leaf-mining *Dasyhelea* species or that perhaps these two records concern *D. mesophylla* which possibly is more broadly distributed in the Neotropical Region.

The discovery here of leaf-mining by a species of *Dasyhelea*, combined with the wide array of other, generally small and restricted habitats inhabited by the other 105 species known as immatures (Borkent 2014; Brahma *et al*. 2016; Díaz *et al*. 2018; Duan *et al*. 2019; Lu *et al*. 2020), suggests that there are likely further interesting, specialised habitats yet to be discovered among the remaining named and many unnamed species in this genus.

Because species of *Salvinia* can be pestiferous, occurring in huge numbers in aquatic habitats, a number of authors have examined the possibilities for biological control but have focused on larger insects (Bennett 1966; Forno and Bourne 1984; Julien 2012; Martin *et al*. 2018; Maseko *et al*. 2019; Wahl and Diaz 2019). Although their impact did not seem great, it may be valuable to study the effect of *D. mesophylla* on the productivity of floating ferns.

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