Article

Morphology and feeding biology of larvae of the South African endemic water beetle genus *Prosthetops* Waterhouse, 1879 (Coleoptera: Hydraenidae)

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Abstract

The larvae of three species of the South African endemic water beetle genus *Prosthetops* Waterhouse, 1879 are described and illustrated for the first time. The second and third instar larvae of *Prosthetops nitens* (Péringuey, 1892), as well as third instar larva of *P. megacephalus* (Boheman, 1851) and *P. wolfbergensis* Bilton, 2013, are treated here. These three species share a combination of morphological and chaetotaxic characters that can be used for positive identification of larvae of this genus: head capsule with pores FC1 present, second antennomeres with two well-developed distal sensory appendages, maxillary seta Cdo1 very reduced, thoracic subprimary setae Dd' absent, subprimary setae Dc' minute and inserted on the boundary between pretergal and tergal areas, urogomphi moderately separated at the base, anal lobe with well-developed dorsal, lateral and ventral plates, and a lack of anal hooks. Some insights on the gut-contents of the larva of *P. wolfbergensis* are offered and an unusual urogomphal malformation observed in a larva of *P. nitens* is also described and illustrated. These are the first described larvae of the subfamily Prosthetopinae, and their morphology is compared to that of other known hydraenid larvae.

Key words: Minute moss beetles, South Africa, preimaginal stages, morphology, rockpools, teratology, gut contents

Introduction

Prosthetops Waterhouse, 1879 is an endemic South African hydraenid genus (Perkins & Balfour-Browne 1994, Perkins 2008, Bilton 2013) and the type genus of the subfamily Prosthetopinae Perkins, 1994. As defined by Perkins & Balfour-Browne (1994) and Bilton (2013) *Prosthetops* was used exclusively for a group of seven relatively large species restricted to rockpools in mountainous areas of the Cape, including the largest known hydraenid, *P. wolfbergensis* Bilton, 2013, which reaches 4.2 mm in length. More recent molecular work has demonstrated that *Pterosthetops* Perkins & Balfour-Browne, 1994 is paraphyletic with respect to *Prosthetops sensu* Bilton (2013) and necessitated a redefinition of *Prosthetops*, which now contains 21 species. Members of this genus as currently defined exploit two highly specialized aquatic habitats, quite different from the lotic environments inhabited by most other members of the family. According to Bilton *et al*. (2022), the genus *Prosthetops* has experienced several habitat shifts during its evolution, which must have affected both adults and larvae. Approximately two-thirds of the described species occupy what are apparently ancestral madicolous habitats (Bilton 2014, Bilton *et al*. 2022), whilst the rest are restricted to montane rockpools (Bilton 2013, Bilton *et al*. 2022). Fieldwork on rockpools carried out by the second author in the Cape Region (Bilton 2013) resulted in the discovery of *P. wolfbergensis*, as well as new distributional and biological information on other members of the genus inhabiting these microhabitats. Although Bilton's study focused on adults, he also discussed larvae collected with them. In the present work, we intend to expand the information provided to date, including descriptions of the larvae of three different species: *Prosthetops megacephalus* (Boheman, 1851), *P. nitens* (Péringuey, 1892), and *P. wolfbergensis.*

Bertrand (1962, 1972) reported the presence of larvae in the British Museum, collected by A.D. Harrison

in the Great Berg River (South Africa), in association with adults of an undescribed hydraenid belonging to the "*Prosthetops* group." He also (1962) describes larvae he apparently collected from Assegaibos, a tributary of the Great Berg. Among the characters listed and illustrated by Bertrand for what he termed "curious larvae" (Bertrand 1972: 366) are a wide free labrum, mandibles with a spatuliform prostheca, maxillae with fimbriate galea, long lateral filaments on the first eight abdominal segments, two long (unsegmented?) urogomphi, and a short, rounded tenth abdominal segment. The limited information provided by Bertrand does not allow for a conclusive assessment, casting doubt on the true identity of this larva, even at family level. In fact, with these characters, particularly the long filaments on the abdomen, it seems possible that this is a larva of the torridincolid genus *Delevea* Reichardt, 1976 (see Beutel *et al.* 2003), perhaps *D. bertrandi* Reichardt, 1976, which occurs frequently in this area. The descriptions presented here constitute the first reliable contribution to the understanding of the preimaginal stages of a member of the subfamily Prosthetopinae as currently understood.

Materials and Methods

Collecting

Larvae were collected from occupied rockpools (Fig. 1) by hand, being clearly visible crawling over rock surfaces or on the benthic sediments on which they appeared to be feeding (Figs 1b, 1c). Specimens were preserved directly in ethanol (70 or 99%) in the field.

Identification

Identification is based largely on association with adults. We acknowledge that such an approach can be inherently problematic, but note that *Prosthetops* adults are the *only* hydraenids ever found in the > 200 montane rockpools examined (DTB, *pers. obs.*). Additionally, one larva collected together with *P. wolfbergensis* adults was identified as this species on the basis of COI mtDNA sequence by the late Ignacio Ribera (Ribera, *pers. comm.* to DTB). In our study of each species, we discuss the level of confidence we can provide to the specific identity of each larval type.

Morphological study

Given the limited availability of material for two of the three studied species, particularly *P. megacephalus*, the most detailed descriptions will be based on the study of the third instar larva of *P. wolfbergensis*. Prior to their examination under a Nikon Eclipse E600 light microscope at magnifications of 40X to 400X, larvae were removed from ethanol and soaked for several hours in lactic acid. To enhance acid diffusion, some larvae were sectioned at the level of the third or fourth abdominal segment, allowing for extraction of the hindgut and distal end of the midgut. Gut contents were then permanently mounted for microscopic examination. Gut contents were qualitatively examined; no quantitative analysis was conducted.

All specimens are now preserved in ethanol, except for one larva of *P. wolfbergensis*, which is permanently slide mounted (CDUM). Three larvae of *P. wolfbergensis* are preserved in absolute ethanol; the remaining material being stored in 70% ethanol. Two measurements were recorded: total body length—measured from the anterior margin of the labrum to the posterior end of abdominal segment X, and cephalic width—maximum width of the cephalic capsule in dorsal view excluding the stemmata. Measurements were conducted using the above microscope and a micrometer.
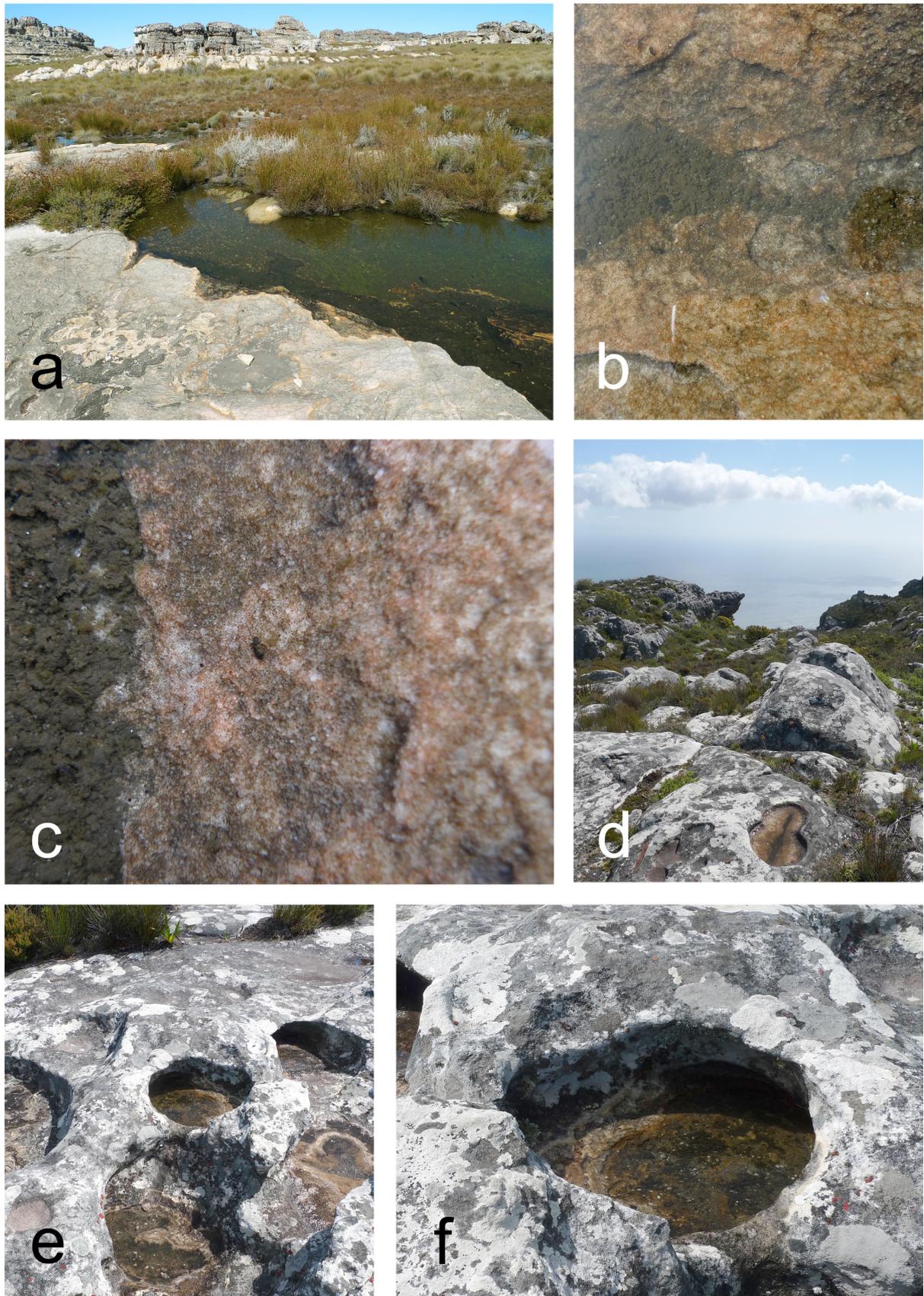
FIGURE 1. *Prosthetops* habitats in Western Cape, South Africa. a: Cederberg range, nr. Wolfberg Arch, pool with adults and larvae of *P. wolfbergensis*; b to d: Table Mountain, pool with adults of *P. megacephalus* (visible in figs b–c) and larvae of *P. megacephalus* and *P. nitens* (pool 2); e and f: Table Mountain, pool with adults and larvae of *P. nitens* (pool 1). Photos D.T. Bilton.

Chaetotaxic study

Deler-Hernández & Delgado (2017) discussed the various nomenclatural systems proposed for describing chaetotaxic patterns in some beetle larvae. Here, we follow the terminology previously employed in descriptions of hydraenid larvae (Delgado & Palma 2004, Delgado & Archangelsky 2005) and elaborated upon in detail in Deler-Hernández & Delgado (2017). Due to the particular features of one of the species studied in this work, *P. nitens*, it is important to note that in larvae of Hydraenidae, up to three different sets of setae may be present. First instars (not described in this study) bear only the primary set of setae, which follows a highly standardized number and distribution pattern. In the second and third instars, a second set of setae—the subprimary setae—is added to the primary set, also following a standardized pattern. Occasionally, and only in some species, a third set may be observed—the so-called secondary setae. When present, secondary setae may appear on the head capsule, thoracic, and/or abdominal segments, but not necessarily on all of them. Based on current knowledge, the presence of these setae on a particular tagma appears to be species-specific in Hydraenidae. However, the number and arrangement of secondary setae differ between individuals of the same species, between segments in a single specimen, and even within a particular segment, where the right and left regions may bear a different number of secondary setae! When present, secondary setae can complicate the identification of primary and subprimary setae, which are then identified by similarity of position. These secondary setae are not assigned specific names in our descriptions and will be indicated with asterisks in the figures.

Gut contents

Mid and foregut contents of a selection of the sectioned larvae indicated above were analysed. Digestive tubes were removed with the aid of two fine dissecting needles. Before light microscope examination, each sample was placed over a slide in a small drop of Hoyer's mounting medium, gently homogenized with a needle and covered with a cover slip.

Abbreviations for depository institutions

CDTB   Collection D.T. Bilton, Plymouth, UK
CDUM   Collection J.A. Delgado, University of Murcia, Spain

Results

Larva of *Prosthetops wolfbergensis* Bilton, 2013

THRID INSTAR:

MATERIAL EXAMINED. SOUTH AFRICA: 11 larvae (CDTB), 4 larvae (CDUM): "South Africa, Western Cape // Cederberg, Stadsaal Cave // Rockpools. // 21-ix-2010, D.T. Bilton leg."; 3 larvae (CDTB): "South Africa, Western Cape // Cederberg, near Stadsaal Cave // Rockpools. // 23-ix-2014, D.T. Bilton leg."; "100% ethanol".

Specific identification

In the Cederberg range of the Western Cape, *P. wolfbergensis* is sympatric only with *P. setosus* (Perkins & Balfour-Browne, 1994), where both can be found in rockpools (Bilton, 2013). *Prosthetops setosus* is much less abundant than *P. wolfbergensis* and in most pools only adults of the latter species are found. The material studied here was obtained from rockpools where only adults of *P. wolfbergensis* were observed (e.g. Fig. 1a) and one larva collected at the same time was identified as *P. wolfbergensis* based on mtDNA sequences (see above). In short, we are quite confident that the examined larvae, which appear to belong to a single species, are *P. wolfbergensis*.

Description

General aspect as in Fig. 2. Total body length: 5.2 mm ± 0.4 mm (Mean ± SD., n = 14). Body elongate and slender. Head slightly narrower than thorax, abdomen parallel-sided and slightly tapered posteriorly (Fig. 2a). More or less straight in lateral view (Fig. 2b) and subcylindrical in cross-section. Head sclerotized, reddish-brown. Thorax and abdomen sclerotized, colouration similar to head, with intersegmental areas translucent. Maximum head width: 0.67 mm ± 0.02 mm (Mean ± S.D., n = 12).

Head: Hypognathous, slightly emarginated posteriorly, elliptical in lateral view (Fig. 3c). Ecdysial line lyrate (Fig. 3a), epicranial stem short, frontal arms sinuously V-shaped, connecting distally with base of antennae. Head capsule (Fig. 3a) divided into three areas: a medial frontal area bordered by the frontal arms and two epicranial areas, located behind and laterad to the frontal arms. Gula distinct (Figs 3b, 4f: GU), narrow, closing the cephalic capsule ventrally, fused to submentum anteriorly and bordered laterally by short tentorial pits (Fig. 3b: TP). Epicranial areas each with five globose stemmata, forming a dorsolateral cluster behind the base of antennae (Figs 4c, 4b). Occipital foramen divided into two parts by tentorial bridge (Fig. 3f). Clypeus transverse, with a slightly sclerotized distal area (Fig. 4d). Clypeo-labral suture arcuate. Labrum free (Fig. 4d), semicircular. Epipharynx (Fig. 4c) rounded. Antennae (Figs 4a, 4b) three-segmented, antennomere I short (Fig. 4b: ANT I), antennomere II, 2.2 times longer than antennomere I, antennomere III slender, slightly shorter than antennomere I. Mandibles slightly asymmetrical (Figs 4h, 4i), broad at base and abruptly narrowing distally. Mesal surface of mandibular base with a mola bearing several transverse ridges of asperites and one molar tooth, retracted in this mesal area. Inner edge continuing with a conical, distally fimbriate prostheca (Fig. 4h: PR). Maxillae (Fig. 4f) with cardines trapezoidal; stipes wide and stout; malae distally divided into a galea (Fig. 4e: GA) and a lacinia, both fimbriate; Lacinia (Fig. 4e: LA) with stouter and strong lamellae; maxillary palps three-segmented, of similar length; distal segment with a long and slender digitiform sensory appendage (Fig. 4f: SD) located medially. Labium (Fig. 4f), stout, consisting of three sclerites. Submentum (Fig. 4f: SMNT) trapezoidal, slightly concave basally, mentum (Fig. 4f: MNT) subquadrate and prementum (Fig. 4f: PMNT) triangular; ligula (Fig. 4f: LG) shorter than labial palps, apex bilobed and globose; labial palps two-segmented, directly articulated with prementum. Hypopharynx (Fig. 4g) simple, membranous, clothed with short spicules directed inwards. Cervical region, membranous, with two small but conspicuous elliptical sclerotized areas (Fig. 3a: CE).

Tentorium (Figs 3e, 3f): consisting of a pair of posterior tentorial arms (Figs 3e, 3f: POR) originating at the posterobasal region of the head capsule, connected by a short tentorial bridge (Fig. 3f: TB) and two anterior arms (Figs 3e, 3f: ATA) extending forward to reach the sides of epistomal ridge (Figs 3e, 3f: ER). Anterior arms wide, dorsolateraly compressed, slightly twisted and strongly united distally to epistomal ridge. Epistomal ridge wide and well developed. A dorsal tentorial arm extends dorsally from each anterior arm (Figs 3e, 3f: DTA). Dorsal arms slender, slightly enlarged and rounded distally, not reaching the epicranium. Tentorial bridge (Fig. 3f: TB) short. Posterior tentorial arms (Figs 3e, 3f: PTA) strong, externally marked by short tentorial pits (Fig. 3b: TP) and basally connected with postoccipital ridge (Figs 3e, 3f: POR) and subgenal ridges (Fig. 3f: SR).

Thorax: Prothorax similar in width to meso- and metathorax (Fig. 2a). Dorsal regions with a single sclerite well-developed in all three segments, each with an evident median ecdysial line (Figs 2a, 5a) and divided into three longitudinal areas: an anterior weakly sclerotized area (pretergal area), a more sclerotized middle area and a posterior area (postergal area), similarly sclerotized to that of the pretergal area. Lateral regions each with two connate sclerites (Fig. 5b): a prehypopleuron or episternum (Fig. 5b: area around setae Prehy) forming a sclerotized lobe anterior to coxal cavity and a posthypopleuron or epimeron (Fig. 5b: area around seta Pohy 1). Prothorax also laterally with a third small sclerite on each side, located on a small, conical tubercle and bearing a spiracle (Fig. 5c: SP). Mesothoracic spiracles non-functional, reduced to small tubercles, almost indistinguishable. Ventral region (Fig. 5b) of each thoracic segment consisting of three small sclerites: two lateral presternites (Fig. 5b: areas around setae Pr) and a median sternite, divided into a feebly sclerotized anterior area (only well developed in prothorax) and a more sclerotized posterior plate (Fig. 5b: areas around setae St). Legs well-developed, long, five-segmented.

FIGURE 2. Third instar larva of *Prosthetops wolfbergensis*. a: Dorsal view, b: Lateral view. Scale bar: 1.0 mm.
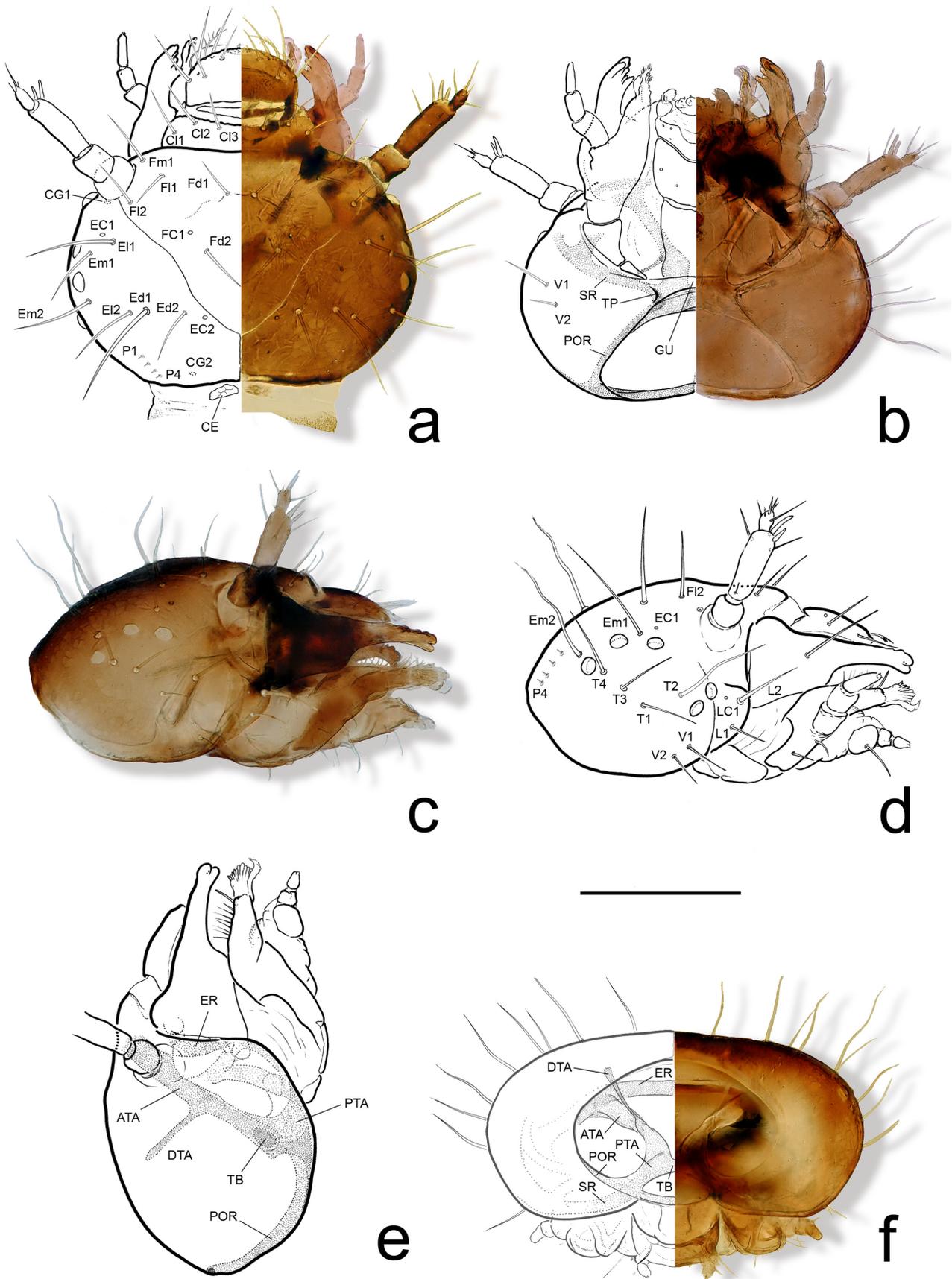
FIGURE 3. Third instar larva of *Prosthetops wolfbergensis*. a: Head, dorsal view; b: Head, ventral view; c–d: Head, lateral view; e: Tentorium, lateral view; f: Tentorium, posterior view. Scale bar: 0.3 mm.
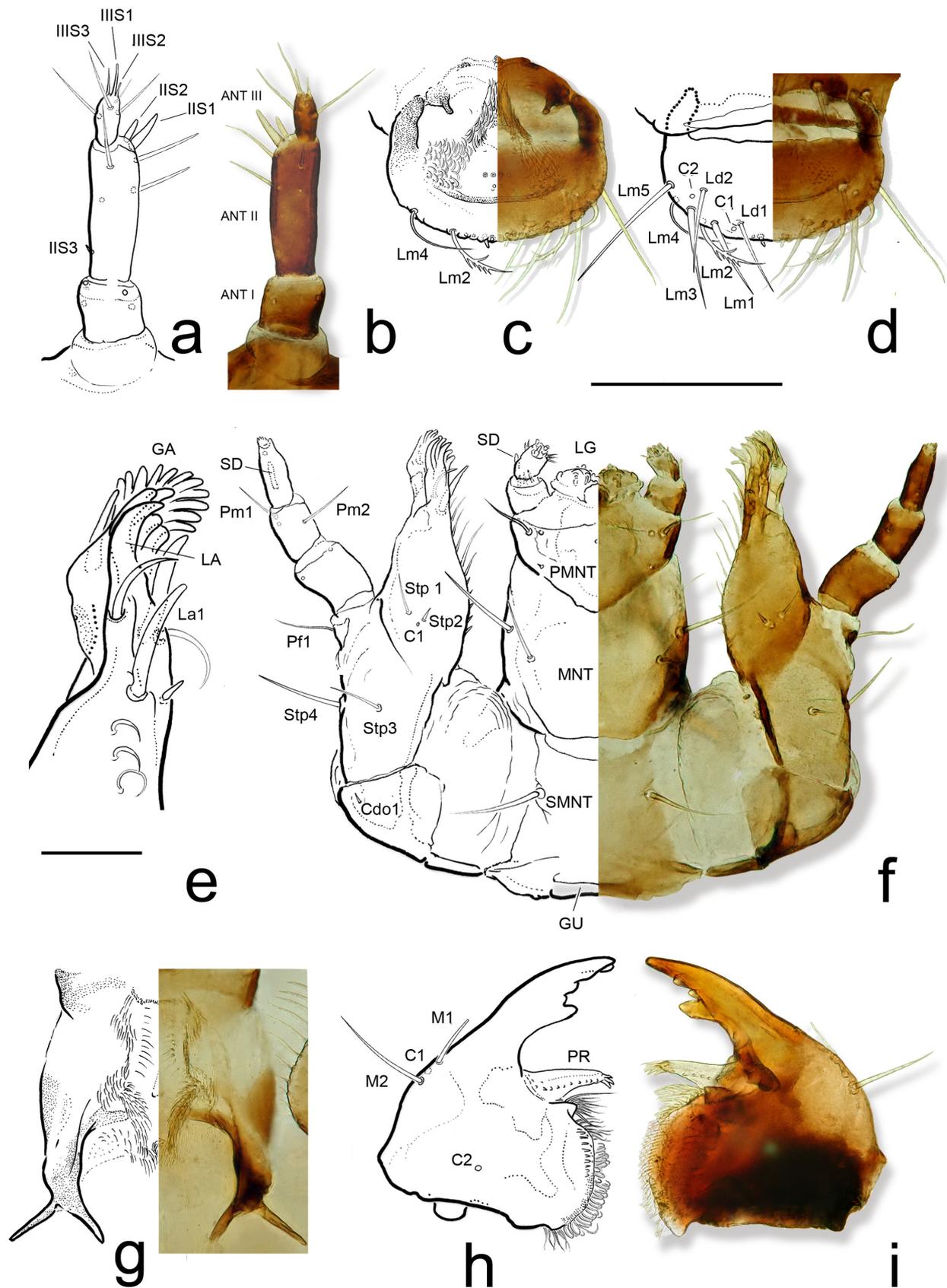
FIGURE 4. Mouthparts and cephalic appendages of third instar larva of *Prosthetops wolfbergensis*. a: Left antenna, dorsal view; b: Right antenna, dorsal view; c: Labrum, dorsal view; d: Epipharynx, ventral view; e: Detail of distal end of right maxilla, ventral view; f: Maxillae and labium, ventral view; g: Hypopharynx, ventral view; h: Left mandible, dorsal view; i: Right mandible, dorsal view. Scale bars: 0.3 mm (a–d and f–i), 0.05 mm (e).
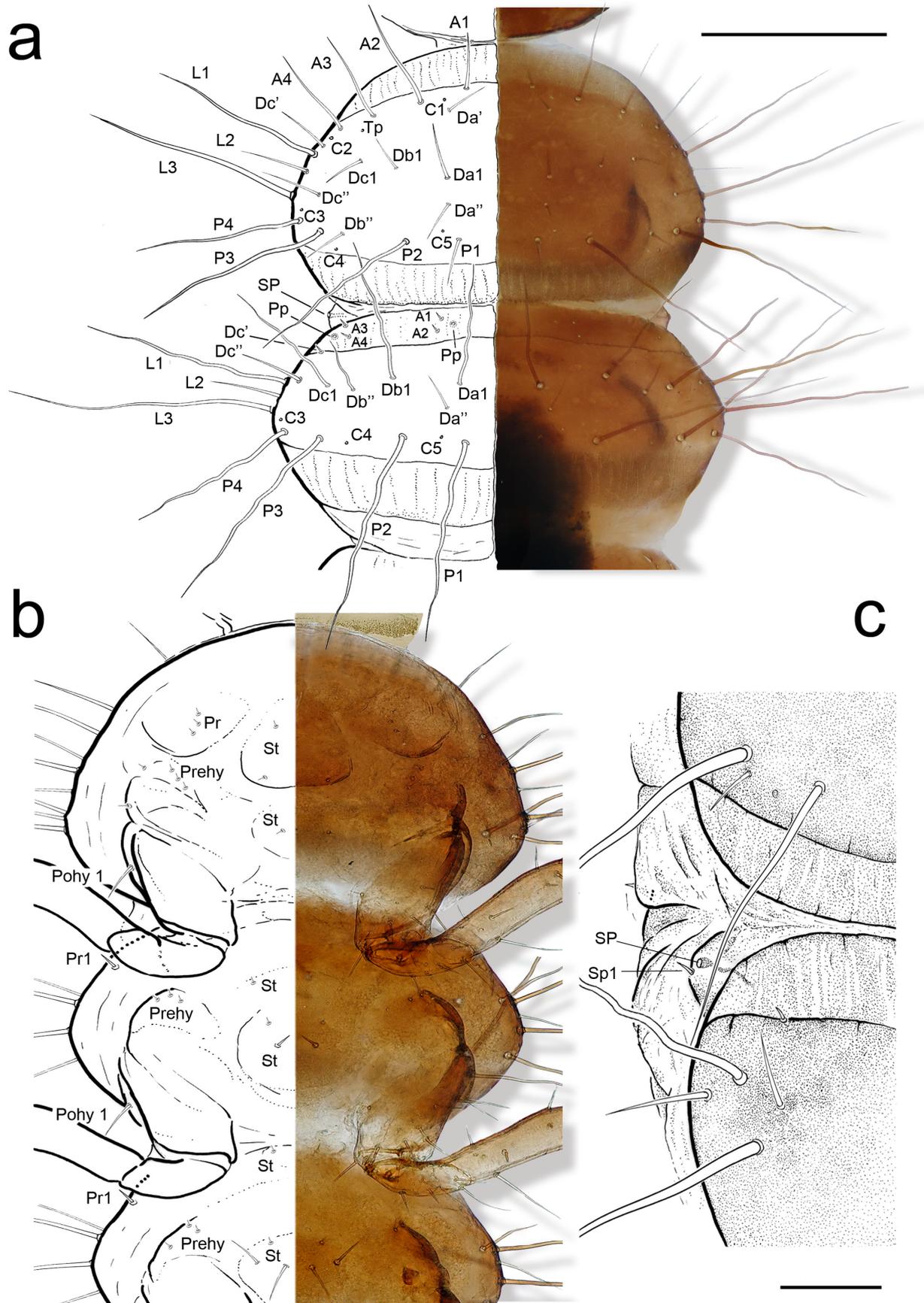
FIGURE 5. Third instar larva of *Prosthetops wolfbergensis*. a: Pro and mesonotum, dorsal view; b: Thorax, ventral view, c: Detail of mesothoracic spiracular tubercle, dorsal view. Scale bars: 0.3 mm (a, b), 0.1 mm (c).
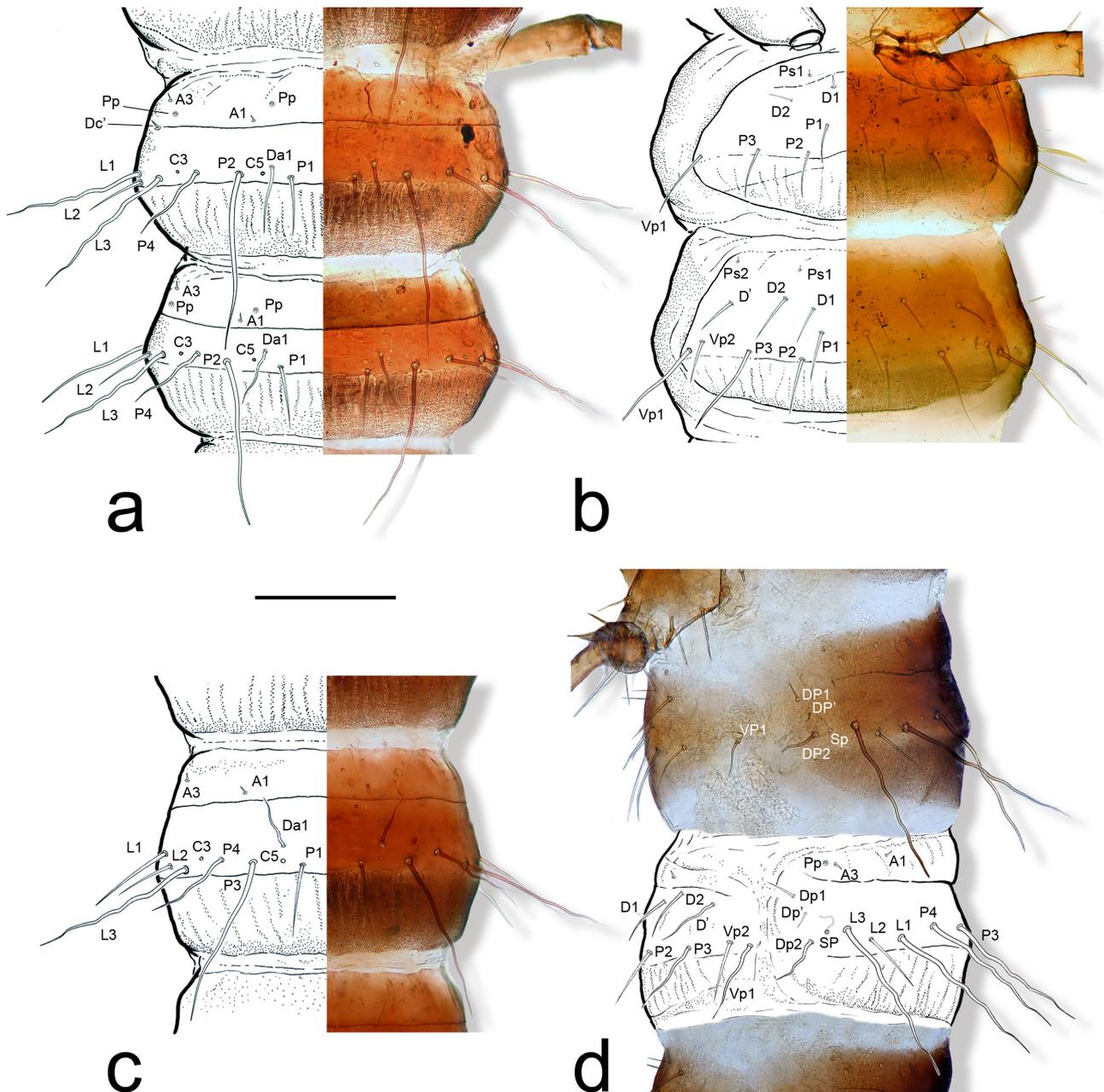
FIGURE 6. Third instar larva of *Prosthetops wolfbergensis.* a: First and second abdominal segments, dorsal view; b: First and second abdominal segments, ventral view; c: Fifth abdominal segment, dorsal view; d: First and second abdominal segments, lateral view. Scale bar: 0.3 mm.

Abdomen: 10-segmented, about 2.5x the length of the thorax (when segments completely distended); segments I to VIII (Figs 6–7), each with a well sclerotized single dorsal plate, longer than wide and delimited, anterior and posteriorly, by the two less sclerotized areas—pretergal and postergal areas—as described in thorax; postergal area considerably wider than the median sclerotized area; the relative width of these sclerotized areas are similar from abdominal segments I to VIII (Figs 6a, 6c, 6d, 7a). As in the other known mature larvae of the family, the small lateral dorsopleural and ventropleural sclerites—present as separate plates in instars I and II—fuse with the dorsal and ventral sclerotized areas of their respective segments. Lateral regions are then formed by a thin membranous middle zone (Fig. 7a: segments IV–V), flanked, above and below, by more strongly sclerotized areas, which are structurally continuous, respectively, with the dorsal and ventral sclerites of that segment. On the dorsolateral regions of segments I to VIII a small spiracular opening can be observed (Figs 6d and 7a: SP). The abdominal ventral region is also formed by a single sclerotized plate flanked anterior and posteriorly by two less sclerotized
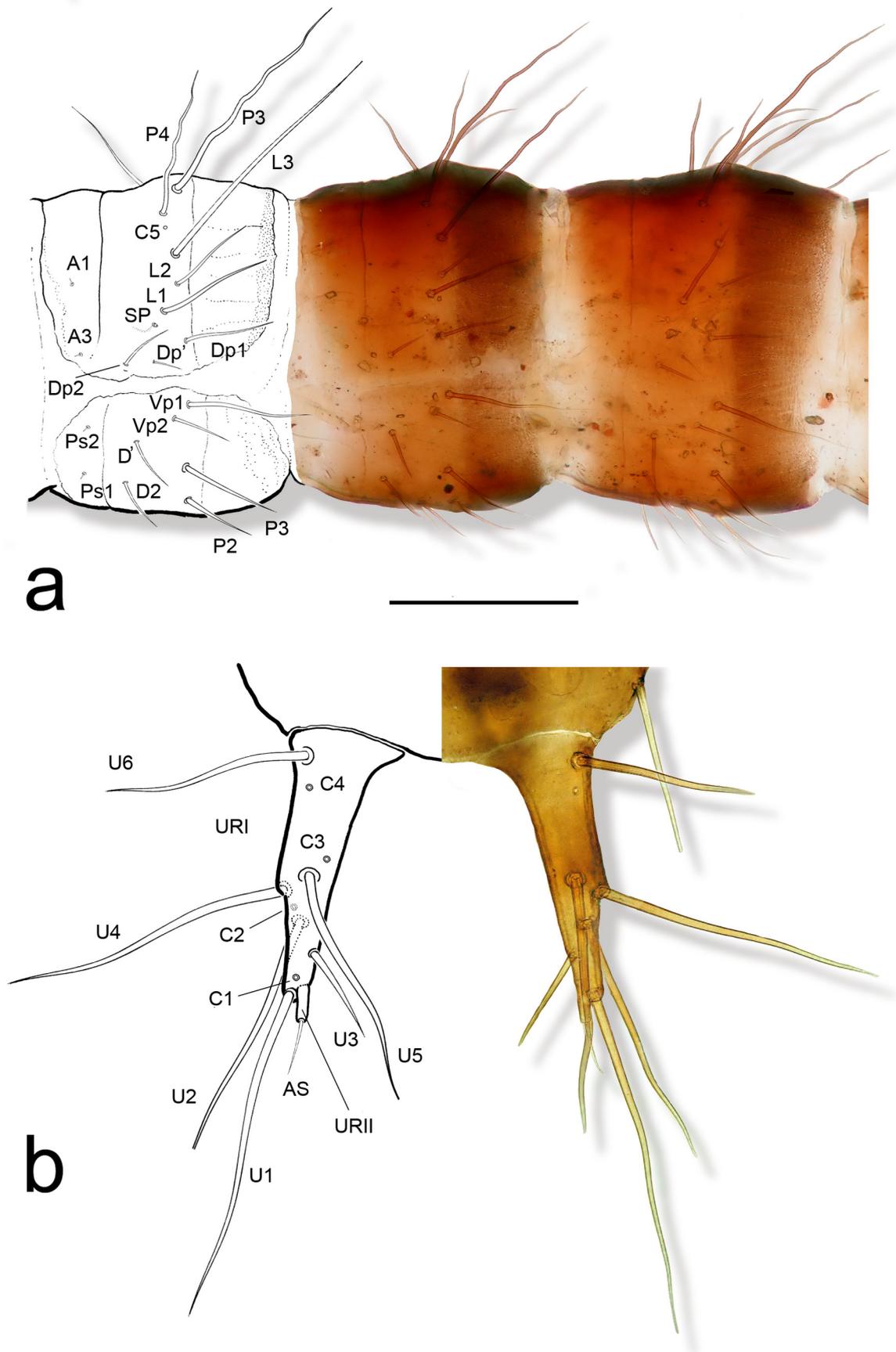
FIGURE 7. Third instar larva of *Prosthetops wolfbergensis.* a: Abdominal segments IV to VI, lateral view; B: Urogomphi, dorsal view. Scale bar: 0.3 mm.
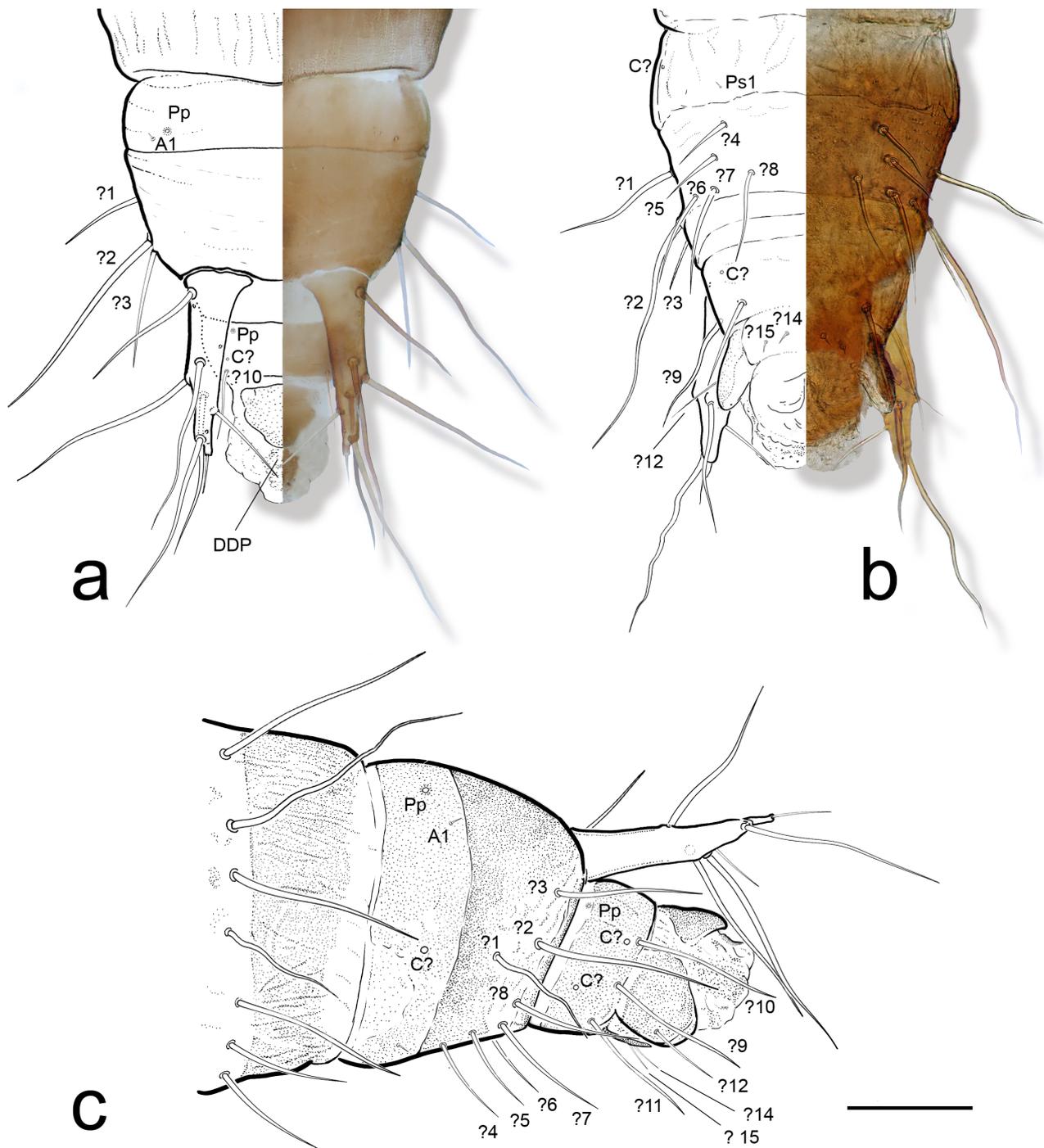
FIGURE 8. Abdominal segments IX and X of third instar larva of *Prosthetops wolfbergensis.* a: Dorsal view; b: Ventral view; c: Lateral view. Scale bar: 0.3 mm.

areas—the presternal and poststernal areas, respectively. The median sclerotized area is, in this case, wider than the presternal and poststernal areas (Fig. 6b). From segment V to VI downwards—with slight variation depending on the specimen studied—dorsal and ventral plates fuse forming a single sclerotized ring (Fig. 7a: segment VI). Laterodorsally, abdominal segment IX (Fig. 8) bears a pair of two-segmented urogomphi (Fig. 7b), not fused to the tergal region of this segment; the urogomphal bases are markedly separated from each other. Urogomphi (Figs 7b, 8) with segment I (Fig. 7b: URI) elongate, conical; segment II (Fig. 7b: URII) short, cylindrical and with a short apical seta (Fig. 7b: AS). Abdominal segment X (Fig. 8) visible from above, also forming a sclerotized ring, associated with a globose distal region—the anal lobe or pygopod. This shows a remarkable structure. In previously described

hydraenid larvae, the anal lobe is a globose, membranous structure with a small, single ventral sclerotized plate, on which some tiny setae and pores are observed. However, in *Prosthetops wolfbergensis* it appears organized as a typical abdominal segment—dorsal, lateral and ventral plates. Surprisingly, the two lateral and the ventral plates bear well developed setae (see below). The anal lobe is terminated distally by a membranous area without anal hooks. In some specimens, there is appears to be a small, additional, dorsal sclerotized plate (Fig. 8a: DDP).

Larval vestiture (Chaetotaxy)

Head: Chaetotaxy of head capsule as in Figs 3a, 3b and 3d. Head with primary and subprimary setae, without secondary setae. Frontal area (Fig. 3a) on each side with a pore (Fig. 3a: FC1) and five setae: two frontal dorsal setae (Fig. 3a: Fd1, Fd2), two frontal lateral setae (Fig. 3a: Fl1, Fl2) and one frontal marginal seta (Fig. 3a: Fm1). Clypeus with three setae on each side (Fig. 3a: Cl1–Cl3). Epicranial areas each with four pores (Fig. 3a: EC1, EC2, CG1, CG2) and 10 setae: a row of four minute posterior setae (Fig. 3a: P1–P4), two epicranial dorsal setae (Fig. 3a: Ed1, Ed2), two epicranial lateral setae (Fig. 3a: El1, El2) and two epicranial marginal setae (Fig. 3a: Em1, Em2); all setae well developed. Temporal areas, each with four setae (Fig. 3d: T1–T4), T2 longest. Lateral regions, each with two setae (Fig. 3d: L1, L2) and one pore (Fig. 3d: LC1). Ventral regions, each with two setae (Fig. 3b: V1, V2). Labrum (Figs 4c, 4d) with two pores (Fig. 4d: C1, C2) and seven setae on each side: two discal setae (Fig. 4d: Ld1, Ld2) and five marginal setae (Fig. 4d: Lm1–Lm5). Setae Lm2 and Lm4 located ventrolaterally, Lm2 distally pectinate (Fig. 4c: Lm2). Epipharynx as in Fig. 4c. Antennae as in Figs 4a and 2b; antennomere I with four pores (Fig. 4a); antennomere II with three long setae, and three solenidia (Fig. 4a: IIS1–IIS3), solenidia IIS1 and IIS2 well developed, inserted distally; IIIS3 minute, basally located; antennomere III, with four setae and three apical solenidia (IIIS1–IIIS3); solenidia IIIS1 and IIIS3 of similar length, IIIS2 shorter. Mandibles (Figs 4h, 4i) with two pores (Fig. 4h: C1, C2) and two setae (Fig. 4h: M1, M2), M1 short, slightly curved or sinuous in some individuals, projected anteriorly and adpressed against the distal mandibular branch. Maxillae (Figs 4e, 4f): Cardo with an extremely reduced seta (Fig. 4f: Cdo1); stipes with four setae (Fig. 4f: Stp1–Stp4) and one pore (Fig. 4f: C1); palpifer with a long seta (Fig. 4f: Pf1); segment II of the maxillary palps with two setae (Fig. 4f: Pm1–2); lacinia with one conspicuous falcate seta (Fig. 4e: La1).

Thorax: Only with primary and subprimary setae, without secondary setae. Tergal area of pronotum with six thoracic pores (Fig. 5a: C1–C5, Tp), and 19 setae on each side. Primary setae: four in anterior row A (Fig. 5a: A1–A4), A1 slightly shorter; three in lateral row L (Fig. 5a: L1–L3), L2 short; four in posterior row P (Fig. 5a: P1–P4) and three discal setae (Fig. 5a: Da1, Db1, Dc1). Five discal subprimary setae (Fig. 5a: Da', Da'', Db'', Dc', Dc''). Subprimary discal setae Db' absent. Chaetotaxy of ventral region of prothorax as in Fig. 5b: Prothoracic presternites, each with three minute setae (Fig. 5b: Pr); sternite with two pairs of minute setae on anterior sclerotized plate and a single pair on posterior plate (Fig. 5b: St); Prothoracic prehypopleurites, each with five minute setae (Fig. 5b: Prehy), and posthypopleurites with a well developed seta (Fig. 5b: Pohy1). Mesonotum with five pores and 18 setae on each side: Pretergal areas with four minute setae (Fig. 5a: A1–A4) and two pores (Fig. 5a: Pp). Tergal area of mesonotum with three pores (Fig. 5a: C3–C5) and 14 setae: three primary discal setae (Fig. 5a: Da1, Db1, Dc1); four discal subprimary setae (Fig. 5a: Da'', Db'', Dc' and Dc''), setae Db' absent—as in pronotum, Da' now absent and Dc' minute; three lateral setae in row L (Fig. 5a: L1–L3) and four posterior setae in row P (Fig. 5a: P1–P4). Ventral region of mesothorax: Presternites each with one seta (Fig. 5b: Pr1), more developed than the minute prothoracic setae and with only two pairs of setae on the sternal medial plate (Fig. 5b: St); Prehypopleurites, each with three small setae (Fig. 5b: Prehy) and posthypopleurites, each with a single well developed seta (Fig. 5b: Pohy1). Lateral mesothoracic spiracular tubercles with a short seta (Fig. 5c: Sp1). Chaetotaxy of metanotum very similar to that of mesonotum, with only some small variations: Pretergal region without pair of setae A4 and sternal area with a pair of minute anterior sternal setae and three pairs of setae (a pair minute and two pairs well developed) on the posterior sclerotized sternal area (Fig. 5b: St). Metathoracic spiracular setae absent.

Abdomen: Chaetotaxy of dorsal regions of segments I–VIII as in Figs 6 and 7. Only with primary and subprimary setae, without secondary setae. Abdominal tergite I with number of setae and pores reduced with respect to meso and metathoracic tergites. Pretergal setae A2, A4 plus tergal setae and pores Da', Da'', Db1, Db'', Dc1, Dc'', P3 and C4 absent; pretergal areas with the same pairs of pores as on meso and metathorax (Fig. 6a). Subprimary setae Dc' clearly visible and very reduced. Lateral dorsopleural sclerites of abdominal segment I fused with the dorsal

sclerotized areas of this segment, each bearing three pairs of setae (Fig. 6d: Dp1, Dp2, Dp'). Ventropleural sclerites fused with the sclerotized sternal area of segment I, each bearing a single seta (Fig. 6d: Vp1). Ventral region of segment I with seven pairs of setae: a pair of presternal setae (Fig. 6b: Ps1), two pairs of discal setae (Fig. 6b: D1, D2), three pairs of posterior setae (Fig. 6b: P1–P3) and the pair of ventropleural setae mentioned above (Fig. 6b: Vp1). Dorsal regions of abdominal segments II to VIII with similar chaetotaxy to abdominal segment I, except by the absence of the discal pair of setae Dc' (Figs 6a and 6c). Dorsopleural sclerites of segments II–VIII fused with tergal plates of their respective segment, with the same chaetotaxy as segment I (Fig. 6d: DP1, DP2, DP'). Abdominal sternites II–V (Figs 6b, 6d), with 10 setae on each side: two presternal setae (Fig. 6b: Ps1, Ps2), three discal setae (Fig. 6b: D1, D2, D'), three posterior setae (Fig. 6b: P1–P3) and two ventropleural setae (Fig. 6b: Vp1, Vp2). Abdominal dorsolateral regions of segments VI–VIII with same chaetotaxy as preceding segments (Fig. 7a). Abdominal ventrolateral regions of segments VI–VIII with similar chaetotaxy to preceding, except for the absence of the pair of presternal setae Ps2. Dorsal region of abdominal segment IX (Fig. 8a) with a minute seta on each side of the pretergal area (Fig. 8a: A1) and one pore (Fig. 8a: Pp); lateroventral and ventral regions of this segment with eight pairs of setae of difficult homology (Fig. 8b: ?1–?8); presternal area with a pair of minute setae (Fig. 8b: Ps1) and a pair of pores (Fig. 8b: C?). Urogomphal segment I (Fig. 7b: URI) with four pores (Fig. 7b: C1–C4) and six setae (Fig. 7b: U1–U6), U3 short and U6 inserted close to the urogomphal base. Dorsal region of abdominal segment X with a pair of pretergal pores (Figs 8a, 8c: Pp) and a pair of setae and a pair of pores in the tergal area of difficult homology (Figs 8a, 8c: ?9, C?), lateroventral areas with two pairs of setae and a pair of pores also of difficult homology (Figs 8a, 8c: ?10, ?11, C?). Lateral sclerotized plates of anal lobe each with a single seta (Figs 8b, 8c, 9c: ?12) and ventral plate with two pairs of setae (Figs 8b, 8c, 9d: ?14, ?15).

Larva of *Prosthetops megacephalus* (Boheman, 1851)

THIRD INSTAR:

EXAMINED MATERIAL: 2 larvae (CDTB): "South Africa, Western Cape // Table Mountain Nat. Park // Below Maclear's Beacon // rockpools. Pool 2. // 24-ix-2010, D.T. Bilton leg".

Specific identification

According to Bilton (2013), *P. megacephalus* is a common species on Table Mountain, particularly in rockpools and occasionally in streams. In the area, *P. nitens* is the only other species occupying rockpools (Figs 1b to 1d). In Pool 2 (see material examined, Fig. 1d), only adults of *P. megacephalus* were collected (Figs 1b, 1c) and *P. nitens* was not observed. Therefore, all the larvae found there were preliminarily associated with *P. megacephalus*. A detailed examination revealed the presence of two larval forms in Pool 2, however, indicating the presence of larvae from both species and the need to associate each type with a specific species. The rationale for this association is as follows: According to Bilton (2013) and Bilton *et al.* (2022), *P. wolfbergensis* belongs to the *megacephalus* group proposed by Perkins & Balfour-Browne (1994), *P. nitens* being more distantly related. Of the two larval morphologies in Pool 2, one is very similar to the larva of *P. wolfbergensis*, whilst the other, whilst similar, presents clear differences. In the absence of DNA sequence data it seems more reasonable to associate larvae similar to *P. wolfbergensis* with *P. megacephalus* and consider those that are different as *P. nitens*.

Description

Total body length: 4.5 mm ± 0 mm (Mean ± SD., n = 2). Maximum head width: 0.54 mm ± 0 mm (Mean ± SD., n = 2). Morphologically, identical to the previously described larvae but clearly smaller. As in *P. wolfbergensis*, the anal lobe presents four well sclerotized areas and lacks anal hooks.
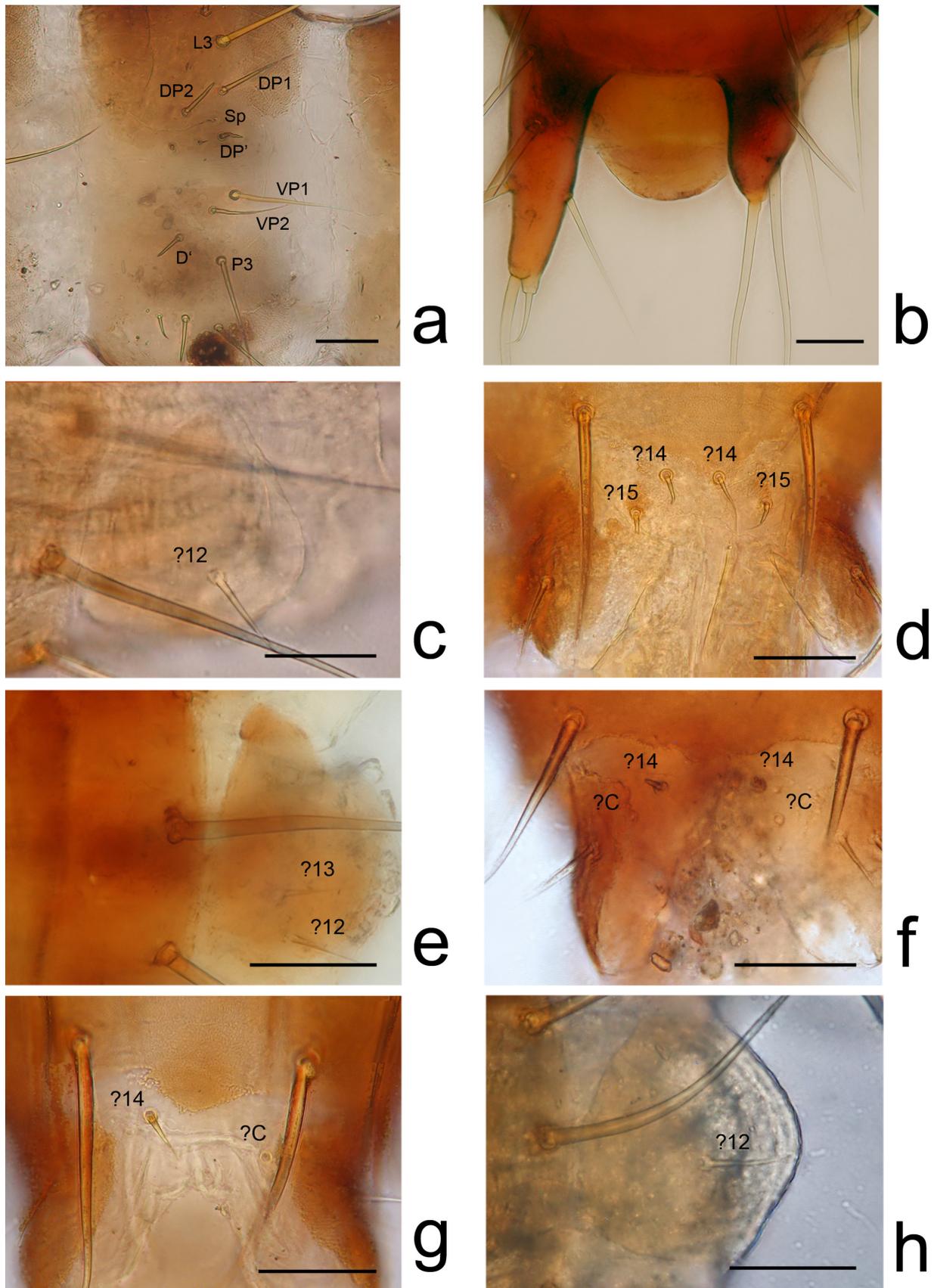
FIGURE 9. a: Second instar larva of *P. nitens*, third abdominal segment, lateral view; b: Urogomphi of a teratological third instar larva of *P. nitens*, dorsal view; c: Anal lobe of third instar larva of *P. wolfbergensis*, lateral plate; d: Anal lobe of third instar larva of *P. wolfbergensis*, ventral plate; e: Anal lobe of third instar larva of *P. nitens*, lateral plate; f and g: Anal lobe of third instar larva of *P. nitens*, ventral plates; h: Anal lobe of third instar larva of *P. megacephalus*, lateral plate. Scale bars: 0.3 mm.

Larval vestiture (Chaetotaxy)

Chaetotaxy identical to that of previous third instar larva of *P. wolfbergensis*. Only with primary and subprimary setae, without secondary setae on head, thorax and abdominal segments. Pro, meso and metanotum without discal setae Db' (Fig. 10a) and meso and metanotum with discal setae Dc' very reduced and inserted close to pretergal areas (Fig. 10a: Dc'). Setae Dc' also present on abdominal segment I (Fig. 10b: Dc'), absent from segments II to X. Anal lobes with sclerotized plates bearing a single seta each (Fig. 9 h: ?12) and two pairs of setae on ventral sclerotized plate, thus, same pattern as described for *P. wolfbergensis*.
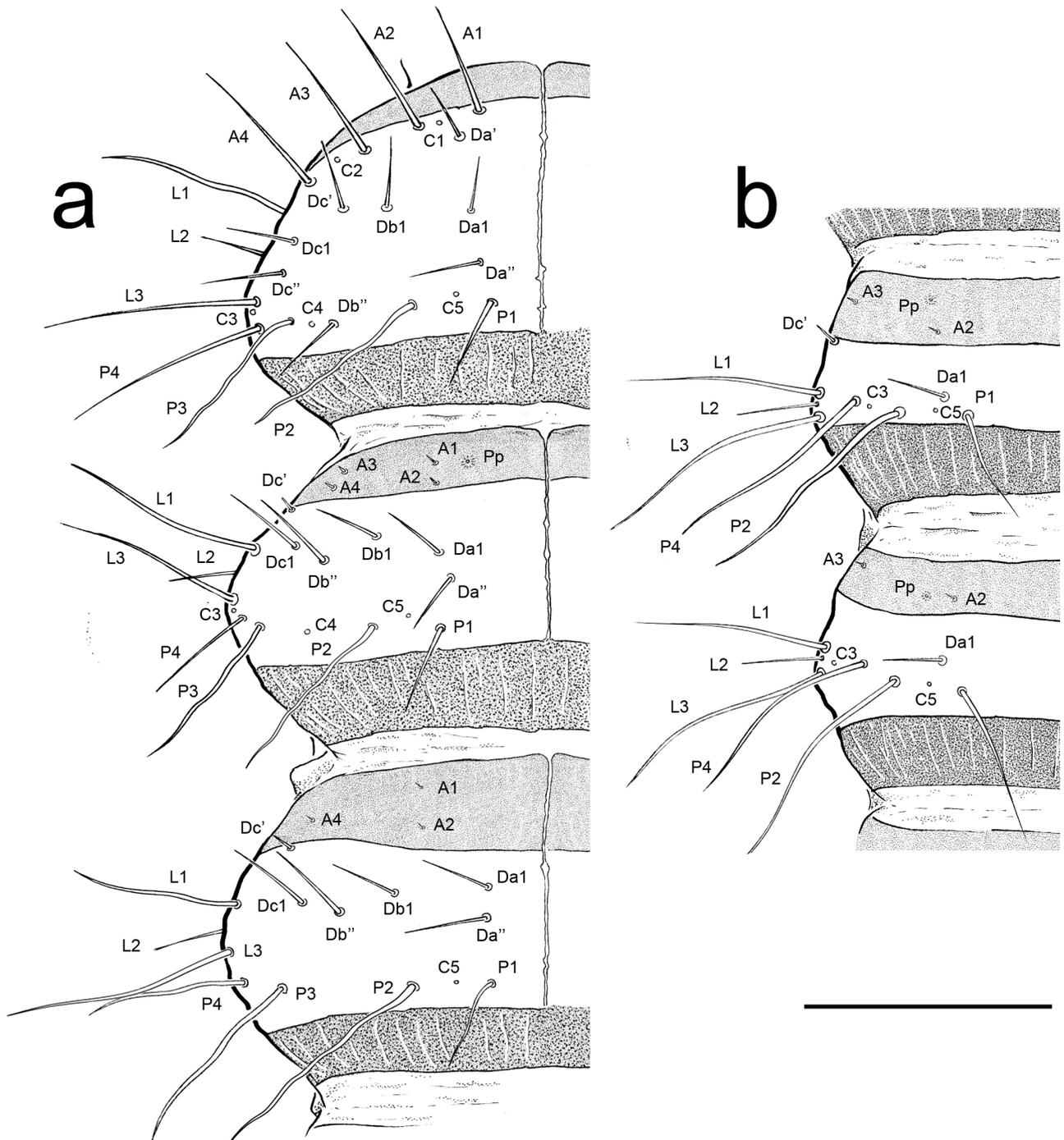
FIGURE 10. Third instar larva of *Prosthetops megacephalus*. a: Thorax, dorsal view; b: First and second abdominal segments, dorsal view. Scale bar: 0.3 mm.

Larva of *Prosthetops nitens* (Péringuey, 1892)

Specific identification

Following the logic used in the previous species association, it is concluded that the remaining larvae from Pool 2 (Fig. 1d) must be associated with *P. nitens*. Adults of *P. nitens* were collected in Pool 1 (Figs 1e, 1f), which also produced this type of larva (see material examined), supporting the proposed association.

SECOND INSTAR:

EXAMINED MATERIAL: SOUTH AFRICA: 1 larva (CDTB): "South Africa, Western Cape // Table Mountain Nat. Park // Below Maclear's Beacon // Rockpools. Pool 1. // 24-ix-2010, D.T. Bilton leg". 1 exuvia (CDTB): "South Africa, Western Cape // Table Mountain Nat. Park // Below Maclear's Beacon // Rockpools. Pool 2. // 24-ix-2010, D.T. Bilton leg".

Description

Total body length: 3.2 mm (n = 1, exuvia not useful for measurements). Maximum head width: 0.44 mm (n = 1). Except for the obvious difference in size, this second instar larva is externally very similar to that of two previous ones. However, being a second stage, it differs in some subtle details regarding the abdominal dorsolateral and ventrolateral plates, which are still present as separate plates in some segments. In *Prosthetops* this feature refers only to abdominal segments I and II. In abdominal segment III (Fig. 9a) the fusion with the dorsal and ventral sclerotized regions begins and only a slight individualization of the ventrolateral plate can be observed. In abdominal segment III dorsolateral plates are already fused with the sclerotized area of tergite III (Fig. 9a). From this segment onwards, the fusion of all lateral plates—dorsolateral and ventrolateral—is complete, and features of the abdominal lateral region in this second stage are similar to those of a third instar larva. As in *P. wolfbergensis* and *P. megacephalus*, the anal lobe presents four well sclerotized areas and lacks anal hooks.

Larval vestiture (Chaetotaxy)

Uniquely amongst the examined larvae, *P. nitens* bears secondary setae on the dorsal regions of thorax (Fig. 11c) as well as on the dorsal and ventral regions of abdomen. The number of secondary setae is not especially numerous compared to some other hydraenid larvae (Delgado, unpublished) and, as expected, the number is not constant in all areas in which they are present. In the single examined specimen three secondary pairs of setae appear on the pronotum (Fig. 11c: *) and two on the meso and metanotum. Secondary setae on the abdomen are reduced to a single pair on abdominal tergum I and are absent on all other abdominal segments. As stated in the previous paragraph, some sets of abdominal setae still appear individualized on independent sclerotized areas of the lateral regions. Abdominal segment I lacks setae Dp2. Setae Dp2 are identifiable on segments II to VIII. Thus, on the two dorsolateral areas of abdominal segments II to VIII, three setae appear, surrounding the opening of the spiracle (as in Fig. 9a: Dp1, Dp2 and Dp'). The abdominal ventrolateral plates bear two setae (as in Fig. 9a: Vp1, Vp2), the plates are still individualized in abdominal segments I and II, almost individualized in segment III (Fig. 9a) and fused into a single sclerotized ring in segments IV to VIII. Abdominal sternites each bears one or two pairs of secondary setae. The chaetotaxy of the plates of the anal lobe are slightly different to the previous described larvae. Here a pair of well-developed setae appear in each of the two lateral sclerotized plates as shown in Fig. 9e: ?13, ?12, while the ventral sclerotized area of the pygopodium bears a pair of setae and a pair of pores as shown in Fig. 9f: ?14 and ?C, respectively.
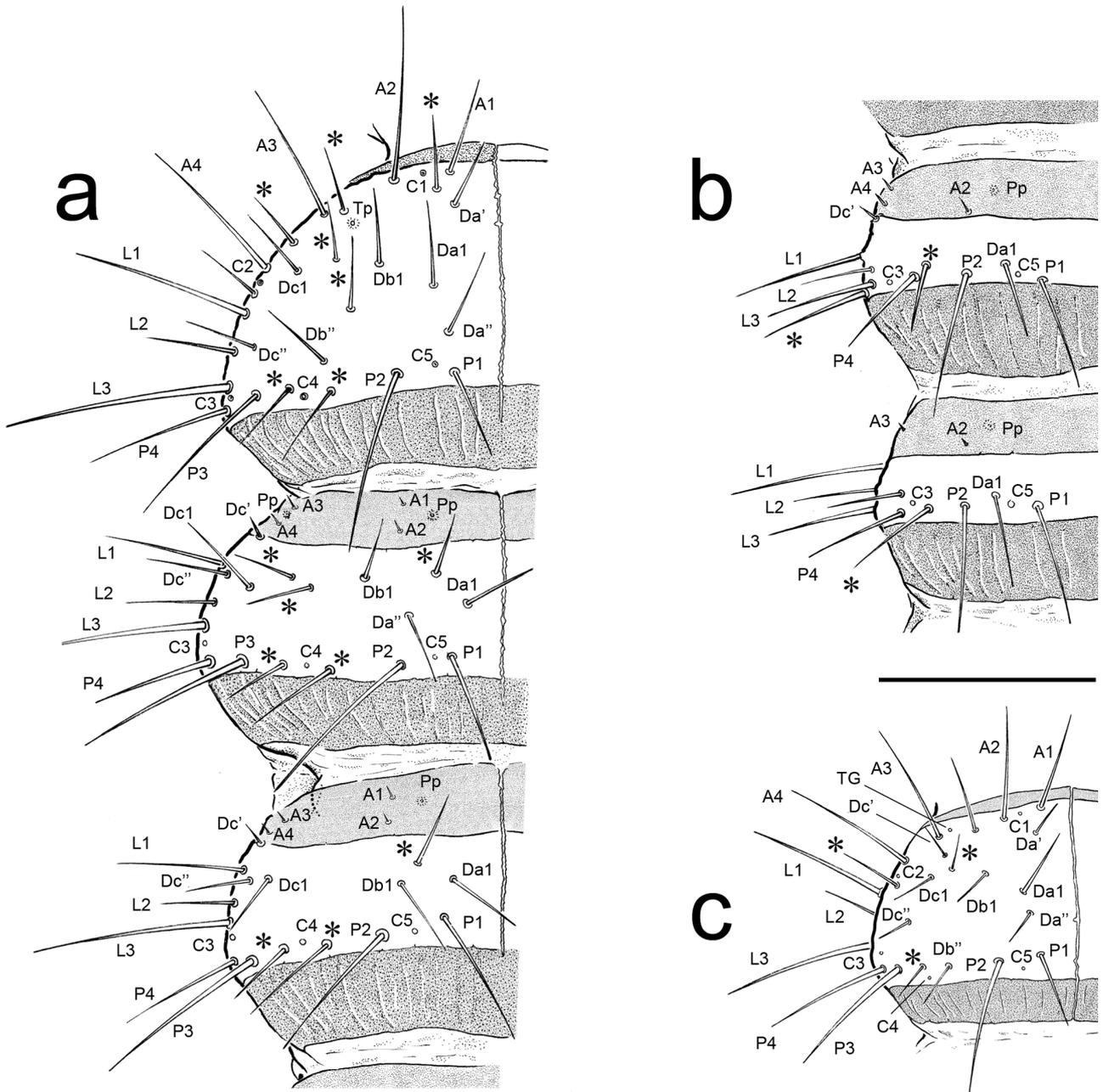
FIGURE 11. Larva of *Prosthetops nitens*. a: Thorax of third instar larva, dorsal view; b: First and second abdominal segment of third instar larva, dorsal view; c: Pronotum of second instar larva. Scale bar: 0.3 mm.

THIRD INSTAR:

EXAMINED MATERIAL: SOUTH AFRICA: 2 larvae (CDTB), 1 larva (CDUM): "South Africa, Western Cape // Table Mountain Nat. Park // Below Maclear's Beacon // Rockpools. Pool 1. // 24-ix-2010, D.T. Bilton leg". 3 larvae (CDTB), 1 larva (CDUM): "South Africa, Western Cape // Table Mountain Nat. Park // Below Maclear's Beacon // Rockpools. Pool 2. // 24-ix-2010, D.T. Bilton leg".

Description

Total body length: 4.3 ± 0.1 mm (Mean ± SD., n= 3). Maximum head width: 0.55 ± 0.02 mm (Mean ± SD., n = 6). It only differs from the second instar in the larger size, width of the head capsule and the complete fusion

of the dorsolateral and ventrolateral plates in the abdominal segments with their respective tergal and sternal sclerotized plates. Morphologically, the third instar larva of *P. nitens* is identical to that of *P. megacephalus* and only distinguishable from *P. wolfbergensis* by its smaller size.

Larval vestiture (Chaetotaxy)

The larvae of this instar continue bearing secondary setae, and the number of them apparently increases slightly compared to the second instar. On the prothorax, four to five secondary setae usually appear on each side, but are reduced to around four pairs on the mesothorax and to three pairs on the metathorax (Fig. 11a: *). On abdominal segments, secondary setae are reduced to one or two pairs on each side (Fig. 11b: *). In the examined material, secondary setae decrease in number on distal segments and are even absent on one of the sides of these segments. The chaetotaxy of the plates of the anal lobe is apparently divergent from other species and second instar *P. nitens*. Whilst the lateral sclerotized plates continue to bear a pair of long setae, as described for the second instar (Fig. 9e: ?12, ?13), the ventral sclerotized area displays a remarkable degree of variation. Two of the studied larvae follow the pattern described for the second instar: a pair of setae and a pair of pores (Fig. 9f), suggesting that this could be the common pattern for the species. In the other four larvae, a seta and/or a pore of these two pairs are absent (Fig. 9g), whilst in another examined larva, both setae are missing.

A case of teratology

To date, reported teratologies are rare in Hydraenidae and none have been described in a preimaginal stage. During morphological study of the larvae of *P. nitens*, a malformation affecting to the left urogomphus of a third instar larva was detected. This case of teratology consists of an atrophy, implying the absence of the distal half of the segment URI and, therefore, a complete lack of segment URII and its associated apical seta (Fig. 9b). The short, atrophied urogomphus bears three setae that, due to their aspect and position, seem to correspond to setae U4 to U6. It is interesting to note that, precisely in the species in which we have described a great chaetotaxic variability (presence of secondary setae and variation in poro and chaetotaxy in ventral pygopodial plates), we have also detected this case of teratology. Whether this case of malformation and the observed chaetotaxic variability are related is unclear, however.

Observations on diet

In rockpools near Stadsaal Cave in the Cederberg (Fig. 1a), Bilton (2013) recorded the presence of adults of *P. wolfbergensis* associated with some larvae grazing biofilms on the bottom of a previously desiccated rockpool, filled with water after a rainy day. Our study expands these observations with an analysis of the gut contents of some of these larvae. The predominant components of mid and hindgut contents consist of reddish particulate matter, presumably of inorganic origin, possibly composed of clay, interspersed with minute refractive mineral fragments (Fig. 12a). Significantly, a substantial number of phytoliths has also been detected (Figs 12b, 12c). Phytoliths represent minute silica bodies, formed through the decomposition of local vegetation, often associated with grasses and preserved in soil sediments (An & Xie 2022). Over an extended period, occupied rockpools have apparently served as repositories for such particles, where they have become incorporated into the benthic substrate.

Amongst material with potential nutritive value for the larvae, discernible in descending order of prevalence are, vegetal remnants, fungal spores, fungal hyphae, and pollen grains. Fungal spores are frequently encountered in clusters (Fig. 12d). The precise nature and provenance of these spores remain equivocal, likely originating from both terrestrial and aquatic fungal sources. Occasional traces of fungal hyphae have been noted, associated with vegetal remnants (see Fig. 12g). Pollen grains, unmistakably introduced via sedimentation from adjacent vegetation, must also contribute to the accumulation of organic debris lining these small water bodies (Fig. 1). Amongst the identified pollen grains are representatives from the families Malvaceae Juss., Poaceae Barnhart, Valerianaceae Batsch., the subfamily Asteroideae (Cass.) Lindl. of Asteraceae Bercht. & Presl, as well as pollen of *Asparagus* L., *Stoebe* L. (Fig. 12e) and *Myrica* L. (Fig. 12f).
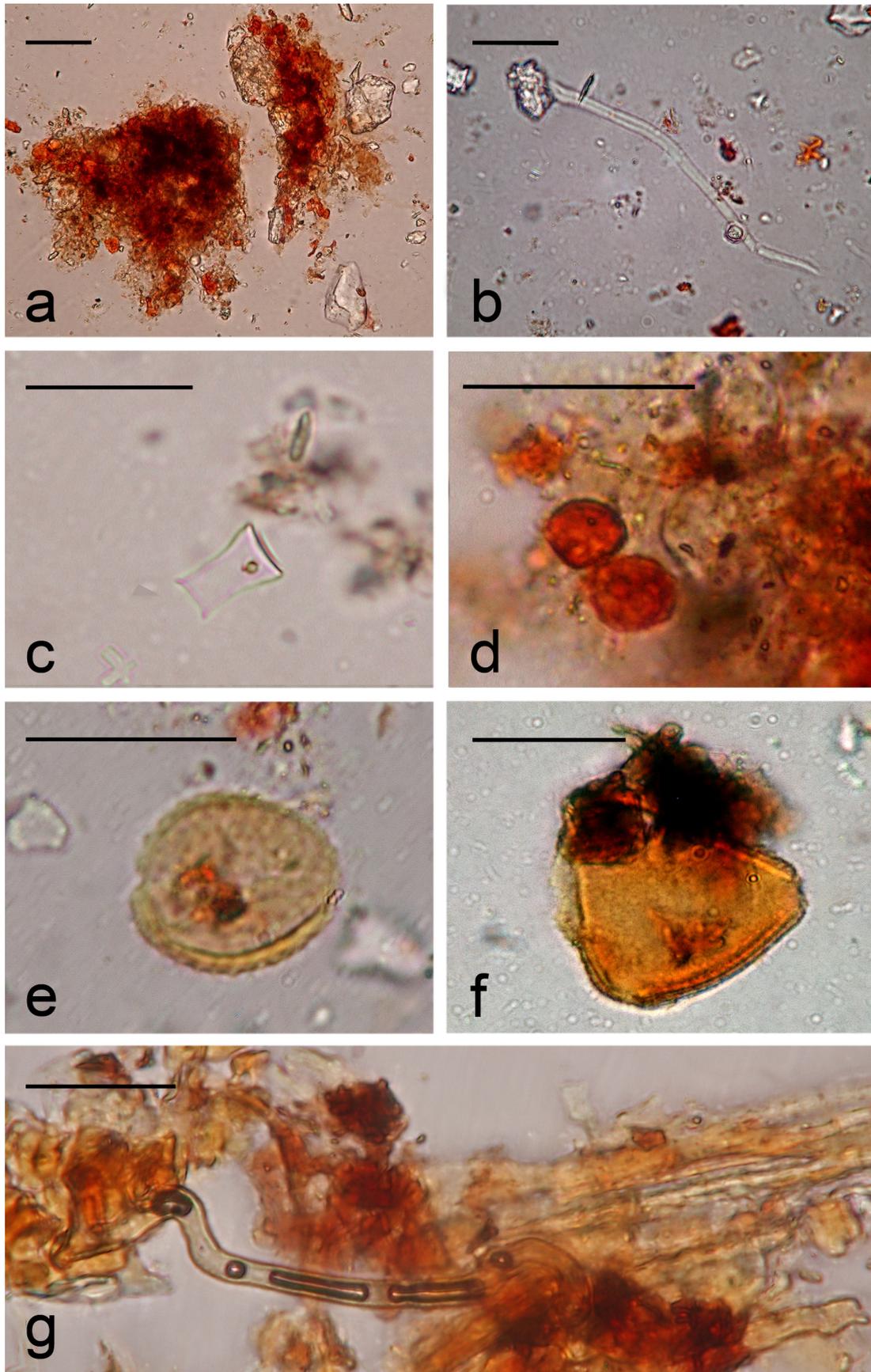
FIGURE 12. Gut contents of larvae of *Prosthetops wolfbergensis*. a: Particulate material and mineral granules; b: Prickle phytolith; c: Trapezoid phytolith; d: Fungal spores; e: Pollen grain of *Stoebe* sp.; f: Pollen grain of *Myrica*; g: Fungal hyphae and organic material. Scale bars: 30 µm.

Discussion

The *Prosthetops* larvae examined in this study exhibit a suite of morphological and chaetotaxic characters that are consistently present across all specimens, suggesting they represent fundamental features of the genus. Certain traits are unique to these larvae, whilst others are shared with described larvae of other hydraenid taxa (Delgado & Palma 1998, Delgado & Palma 2004, Deler-Hernández & Delgado 2017).

Exclusive morphological traits of the current larvae include a shortened tentorial bridge (Fig. 3f: TB), contrasting with the much longer bridge observed in other known hydraenid larvae, and an anal lobe featuring dorsal, lateral, and ventral sclerotized plates (Figs 9c, 9e, 9h); a configuration not observed in other hydraenid larvae where typically only a small ventral sclerotized area is present in this region.

Exclusive chaetotaxic features include the markedly reduced maxillary cardinal setae Cdo1 (Fig. 4f: Cdo1)—well-developed in all other known hydraenid larvae, the lack of thoracic subprimary setae Db', and substantially reduced and anteriorly located subprimary setae Dc' on the meso- and metathorax (Figs 5a, 4b Dc'). Several other setae are highly reduced or absent on the abdomen in comparison to other described larvae, such as setae Dc', already present as a reduced seta on segment I (Fig. 6a: Dc') but absent on segments II to X. Conversely, the lateral sclerotized plates of the anal lobe or pygopodium bear well-developed setae (Figs 9c, 9e, 9h); undoubtedly, a remarkably conspicuous trait. Furthermore, setae of the ventral pygopodial plate are well-developed (Figs 9d, 9f, 9g), contrasting with their extreme reduction or absence in other known larvae of Hydraenidae.

Several other noteworthy morphological traits are shared amongst larvae of the three species, but have been previously described in other hydraenid taxa. These include antennal segment II with three sensory appendages (Fig. 4a: IIS1, IIS2, IIS3), a fimbriate galea (Fig. 4e: GA), narrower dorsal tentorial branches (Figs 3e, 3f: DTA) compared to the anterior tentorial branches ATA (Figs 3e, 3f: DTA, ATA), a narrow gula (Fig. 3b: GU), and the presence of cervical sclerites (Fig. 3a: CE). The morphology of antennal segment II closely resembles that observed in *Orchymontia* Broun, 1919 (see Bilton *et al.* 2022 for synonymy of *Podaena* Ordish, 1984 with *Orchymontia*), except for the lack of a fourth basal sensory appendage. Such an observation provides potential morphological support for the relatively close relationship between *Orchymontia* and *Prosthetops* suggested by Bilton *et al.* (2022). Narrow dorsal tentorial branches (DTA) and a narrow gula are consistent features found in known *Hydraena* Kugelann, 1794 larvae, whilst cervical sclerites are commonly observed in larvae of *Ochthebius* Leach, 1815. In the distal portion of the abdomen of *Prosthetops* larvae, the urogomphi are notably separated from each other, inserted approximately 2x their width apart at their base (Fig. 7b), akin to observations in *Hydraena*, *Limnebius* Leach, 1815, and *Orchymontia*. Additionally, the absence of anal hooks (Fig. 8c) represents an intriguing feature within this genus, although it is not exclusive, as several lineages of *Ochthebius* and *Meropathus* Enderlein, 1901 also lack anal hooks.

Chaetotaxic traits of *Prosthetops* also include a mosaic of characters seen in other members of the family. Pores FC1 are present on the frontal area of the cephalic capsule (Fig. 3a: FC1), labral setae Lm2 are pectinate (Figs 4c, 4d: Lm2), mandibular setae M1 are adpressed to the mandibular body (Fig. 4h: M1), and the urogomphi possess setae U6 inserted basally and setae U5 located medially (Fig. 7b: U6, U5). The presence of cephalic pores FC1 and the setal arrangement on the urogomphi resembles that observed in *Ochthebius* larvae, whilst the orientation of mandibular setae M1 mirrors that seen in *Hydraena*. Additionally, the labral setae Lm2 exhibit a pectinate morphology, a diagnostic feature of hydraenid larvae.

In summary, the morphology and chaetotaxy of these larvae exhibit a suite of unique characters, intermingled with others shared with larvae from different lineages of Hydraenidae. Further studies of a wider range of Prosthetopinae larvae, particularly across a wide range of occupied habitats, would be useful if we are to understand the possible adaptive significance of these features.

Surprisingly little is known about the feeding habits of larval Hydraenidae. Perkins (1980) analysed the gut contents of some larvae, revealing periphyton and filamentous algae. Similar results were published by Jäch *et al* (2016). The new findings offered here are broadly in line with previous information. The heterogeneous composition of the gut contents in *Prosthetops* larvae, comprised of both inorganic and organic constituents, suggests an opportunistic feeding strategy, congruent with the observations by Bilton (2013), where both larvae and adults apparently exploit any available algal/bacterial/detrital resource within these ephemeral ecosystems. Similar conclusions were reached by McLachlan (1981) in a study of the trophic biology of dipteran larvae in Central African rockpools.

Acknowledgements

References

An, X. & Xie, B. (2022) Phytoliths from woody plants: A review. *Diversity*, 14 (339), 1–10.
https://doi.org/10.3390/d14050339

Bertrand, H. (1962) Contribution à l'étude des premiers états des Coléoptères aquatiques de la région éthiopienne (4ᵉ note). *Bulletin de l'Institut fondamental d'Afrique noire*, Sèrie A, 24 (4) 1065-1114.

Bertrand, H. (1972) *Larves et nymphes des coléoptères aquatiques du globe*. F. Paillart, France, 804 pp.

Beutel, R.G., Maddison, D.R. & Haas, A. (2003) Phylogenetic analysis of Myxophaga (Coleoptera) using larval characters. *Systematic Entomology*, 24, 171–192.
https://doi.org/10.1046/j.1365-3113.1999.00075.x

Bilton, D.T. (2013) *Prosthetops wolfbergensis* sp. nov.—a giant amongst the 'minute moss beetles, with new data on other members of the genus (Coleoptera, Hydraenidae). *Zootaxa*, 3666 (3), 345–357.
https://doi.org/10.11646/zootaxa.3666.3.5

Bilton, D.T. (2014) New species and new records of *Pterosthetops*: eumadicolous water beetles of the South African Cape (Coleoptera, Hydraenidae). *Zootaxa*, 3811(4), 438–462.
https://doi.org/10.11646/zootaxa.3811.4.2

Bilton, D.T., Jäch, M.A., Ribera, I. & Toussaint, E.F.A. (2022) Minute moss beetles in the Southern Hemisphere: Molecular phylogeny, historical biogeography and habitat shifts (Coleoptera: Hydraenidae). *Systematic Entomology*, 48, 142–162.
https://doi.org/10.1111/syen.12567

Deler-Hernández, A. & Delgado, J.A. (2017) The Hydraenidae of Cuba (Insecta: Coleoptera) II: Morphology of preimaginal stages of six species and notes on their biology. *Zootaxa*, 4238 (4), 451–498.
https://doi.org/10.11646/zootaxa.4238.4.1

Delgado, J.A. & Archangelsky, M. (2005) Description of the larval stages of *Gymnochthebius jensenhaarupi* and phylogenetic analysis of the relationships with other species of the subfamily Ochthebiinae (Coleoptera: Hydraenidae). *European Journal of Entomology*, 102 (2), 231–240.
https://doi.org/10.14411/eje.2005.036

Delgado, J.A. & Palma, R.L. (1998) Larval stages of three *Meropathus* species (Coleoptera: Hydraenidae: Ochthebiinae) from New Zealand. *New Zealand Journal of Zoology*, 25 (4), 409–419.
https://doi.org/10.1080/03014223.1998.9518164

Delgado, J.A. & Palma, R.L. (2004) Larval stages of *Podaena latipalpis* from New Zealand and phylogenetic relationships of the subfamily Orchymontiinae based on larval characters (Insecta: Coleoptera: Hydraenidae). *New Zealand Journal of Zoology*, 31: 327–242.
https://doi.org/10.1080/03014223.2004.9518386

Jäch, M.A., Beutel, R., Delgado, J.A. & Díaz, J.A. (2016) Hydraenidae. *In*: Beutel, R. & Leschen, R.A.B. (Eds.), *Handbook of Zoology. Vol. 4. 2ⁿᵈ Edition. Arthropoda Insecta. Part. 38. Coleoptera, Beetles. Vol. 1. Morphology and Systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*. De Gruyter, Berlin, pp. 316–345.

McLachlan, R.J. (1981) Food sources and foraging tactics in tropical rain pools. *Zoological Journal of the Linnean Society*, 71, 265–277.
https://doi.org/10.1111/j.1096-3642.1981.tb01133.x

Perkins, P.D. (1980) Aquatic beetles of the family Hydraenidae in the Western Hemisphere: classification, biogeography and inferred phylogeny (Insecta: Coleoptera). *Quaestiones Entomologicae*, 16, 3–554.
https://doi.org/10.5281/zenodo.6184550

Perkins, P.D. (2008) New species and new collection records of Prosthetopine water beetles from southern Africa (Coleoptera: Hydraenidae). *Zootaxa*, 1864, 1–124.
https://doi.org/10.11646/zootaxa.1864.1.1

Perkins, P.D. & Balfour-Browne, J. (1994) A contribution to the taxonomy of aquatic and humicolous beetles of the family Hydraenidae in southern Africa. *Fieldiana Zoology*, 77, 1–159.
https://doi.org/10.5962/bhl.title.3533

Turner, C.R. (2007) Water beetles associated with reservoirs on Table Mountain, Cape Town: implications for conservation. *Journal of Insect Conservation*, 11, 75–83.
https://doi.org/10.1007/s10841-006-9020-2

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