

*This contribution is published
to honor Dr. Amnon Freidberg,
a scientist, a colleague and a friend,
on the occasion of his 75th birthday.*

**The life cycle of the Afrotropical snail-killing fly
Sepedon (Parasepedon) umbrosa (Diptera: Sciomyzidae),
whose larvae are predators–parasitoids of the terrestrial snail
Subulina octona (Mollusca: Subulinidae)**

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ABSTRACT

All stages of larval development of the sciomyzid *Sepedon (Parasepedon) umbrosa* are detailed. Following a parasitoid life-style, the larvae consume, in small numbers, the terrestrial mollusc *Subulina octona* of the Subulinidae family. Some larvae attack the prey by directly penetrating the mouth of the mollusc. Details of egg laying and the duration of each immature stage are described and the results of isogenic development of three successive generations from a wild pair are presented. Comparison between the dynamic populations of adults are presented, based on captures made in Benin and in Democratic Republic of Congo. A summary of the different life cycles known for sciomyzids from the Afrotropical region is provided.

KEYWORDS: Acalypttratae, distribution, biology, immature stages, predation, successive generations, taxonomy.

RESUME

Tous les stades de développement du cycle larvaire du sciomyzide *Sepedon (Parasepedon) umbrosa* sont détaillés. Comme proie, les larves consomment en petit nombre le mollusque terrestre *Subulina octona* de la famille des Subulinidae, suivant un mode parasitoïde. Certaines larves attaquent leurs proies en pénétrant directement dans la bouche du mollusque. Sont précisés les détails de ponte des femelles, les durées de chaque stade immature ainsi que les résultats du développement isogénique de trois générations successives issues d'un couple d'adultes capturé dans la nature. Une comparaison de la dynamique des populations des adultes, basée sur des captures effectuées au Bénin et en République démocratique du Congo a été réalisée. Un récapitulatif des différents cycles de développement connus pour les sciomyzides de la région afrotropicale est indiqué.

MOTS CLES: Acalypttratae, distribution, biologie, stades immatures, générations successives.

INTRODUCTION

Currently, the family Sciomyzidae comprises 543 species in 63 genera (Vala *et al.* 2013, 2014). Recently, three new species have been added by Knutson and Deeming (Knutson *et al.* 2018). Descriptions of other species are in preparation by Vala (one in *Pherbellia* Robineau-Desvoidy, 1830, and two in *Sepedoninus* Verbeke, 1950), and by Murphy (12 new species of *Dictya* Meigen, 1803, pers. comm.). Moreover, three new synonymies have been recognised recently by Knutson *et al.* (2018.). In addition, a new monobasic genus is being described from Australian materials by Knutson and Vala.

The genus *Sepedon* Latreille, 1804 has always been dominant in the Afrotropical Region, with 41 species out of 81 known worldwide. For this region, Verbeke (1950) described three taxa of the generic rank, mainly based on the male genitalia structure: (1) subgenus *Sepedon* (*Mesosepedon*) Verbeke, 1950 (7 species) without cochleate vesicle and spiraled filaments; (2) subgenus *Sepedon* (*Parasepedon*) Verbeke, 1950 with presence of these two features (32 species); and (3) genus *Sepedomyia* Verbeke, 1950 characterized by a long antennal scape (2 species); the last taxon was subsequently considered as subgenus by Steyskal (1973), who regarded the antennal characteristic insufficient for the establishment of a new genus. Thus, all other world *Sepedon* lacking one of the above features must be placed in the subgenus *Sepedon* Latreille, 1804 *s. str.* (40 species). This arrangement has been adopted by Vala *et al.* (2013, 2014).

Our study concerns the life cycle and the biology of Afrotropical *Sepedon* (*Parasepedon*) *umbrosa* Verbeke, 1950 (Fig. 1). Before presenting new data we recapitulate here all the previous biological records regarding Afrotropical Sciomyzidae. The first studies began with the work of Knutson *et al.* (1967), where the authors described the complete life cycle of *Sepedon hispanica hispanica* Loew, 1862 and specified the feeding behaviour of larvae that are parasitoids during the first instar (L1) stage and predatory during the second (L2) and third (L3) instar stages. The authors also detailed the L3 and the pupal stage of *Sepedon* (*P.*) *ruficeps* Becker, 1923 and of *Sepedon* (*P.*) *scapularis* Adams, 1903. Then, Barraclough (1983) described all immatures of *Sepedon* (*P.*) *neavei* Steyskal, 1956 and *Sepedon* (*P.*) *testacea* Loew, 1862, whose larvae are predators of freshwater molluscs of the genera *Physa*, *Biomphalaria*, *Lymnaea* and *Planorbis*. Maharaj (1991) and Maharaj *et al.* (1992) studied the feeding behaviour of *Sepedon* (*P.*) *scapularis* and confirmed the influence of prey-size on larval predation, previously indicated for other sciomyzids by several authors such as Geckler (1971), Eckblad and Berg (1972), Barraclough (1983), Beaver (1989) and Manguin *et al.* (1988). Vala *et al.* (1995) published the life cycle of *Sepedon* (*P.*) *trichrooscelis* Speiser, 1910, whose larvae are parasitoids of hygrophilous semi-aquatic Succinidae molluscs. In addition, they mentioned the diverse types of sensillae and proposed a distribution pattern applicable to all sciomyzid larvae. Later, Knutson (2008) confirmed their parasitoid behaviour. Vala *et al.* (2000a, b) related the biology of *Sepedonella nana* Verbeke, 1950, captured

in a temporary freshwater biotope at Cocotomey, Benin. According to studies of Vala and Gbedjissi (2011), the larvae consume the small aquatic oligochaetes *Aulophorus furcatus* (Oken) of the family Naididae; in 2002 at the Fifth International Dipterology Congress in Brisbane, Australia, Vala *et al.* (2002) reported the same feeding behaviour for *Sepedon (Mesosepedon) knutsoni*, which Vala *et al.* (1994) captured in a permanent aquatic station site in Agnavo, Benin. Gbedjissi *et al.* (2003) detailed the predation of *S. (P.) ruficeps*, and Gbedjissi and Vala (2014) published its complete life cycle and biology. Recently, Agboho *et al.* (2019) described the life cycle, biology and the population dynamics of *Sepedon (Sepedomyia) nasuta* (Verbeke, 1950), from specimens collected in Benin.

MATERIALS AND METHODS

Environmental setting, collecting and rearing of insects

Adults were captured with a sweep net over low vegetation at Cocotomey and principally at Pahou, Benin. In total, 168 specimens were caught by P. Agboho from July 2014 to June 2015 for the study of the population dynamics. The station (alt. 55–60 m) is confined to marshy areas, which border the permanent lake Toho-To Dougba at 5–6 km from the Atlantic coast and are alternately dry or flooded during dry and rainy seasons. In Benin, the climate is characterized by a long rainy season from early April to mid-July, a short dry season from mid-July to mid-September, a short rainy season from mid-September to mid-November and a long dry season from mid-November to the end of March.

The station is close to the University of Abomey-Calavi and was visited fortnightly. In the laboratory, we isolated each pair of flies (one male, one female) in transparent jars (diameter 10 cm, height 15 cm) closed with a mosquito net allowing ventilation. Each jar was provided with 2–3 wooden sticks as insect supports; a glass container (diameter 3 cm, high 6 cm), with a cotton wick in its pierced lid to provide drinking water by capillary action; and various pieces of fruit, mainly banana and pineapple, and sugar as food. We examined the jars twice a day to collect newly laid eggs with a fine brush moistened with water. We transferred the eggs individually to a Petri dish (diameter 5 cm) containing wet filter paper with a few drops of Tegosept added to prevent bacterial and fungal infections. After hatching, each neonate larva (L1) was immediately moved to a similar dish with water (depth up to 4 mm) and small freshwater molluscs, small freshwater oligochaetes, or terrestrial molluscs found at the site, where the adult flies were captured. The same individual isolation procedure was repeated for subsequent larval stages (L2, L3) and pupae (without prey). All experiments were conducted under laboratory conditions, i.e. temperature 25–32 °C, relative humidity 70–85 % and a natural photoperiod LD 12:12.

Microscopy and imaging

For scanning electron microscopy, eggs and larvae were dehydrated in ethanol with increasing (70–100 %) concentrations, followed by critical point drying with CO₂ and covering with gold-palladium. The specimens were examined under a JEOL

JSM 35 microscope in the Electronic Laboratory of the University of Sciences and Technology, Montpellier, France.

Photographs of the male genitalia (Figs 6, 9) were taken by Vala during his dedicated study in 2013 at the IRSNB from Verbeke's original slide preparation of the holotype of *S. (P.) umbrosa*, and the genitalia drawings (Figs 7, 8) are given after Verbeke (1950: figs 33, 34).

Prey tested for larval rearing

For biological studies, we collected potential prey to be consumed or attacked, according to the information from the aforementioned research into Afrotropical sciomyzids. Prey items consisted mainly of the freshwater snails *Bulinus forskalii* (Ehrenberg), *Biomphalaria pfeifferi* (Krauss), the freshwater oligochaete *Aulophorus furcatus* (Oken), and the hygrophilous snail *Succinea campestris* Say living on emergent plants in the fly habitats. The sciomyzid larvae attacked none of these prey. Fortuitously, we used the little land snail *Subulina octona* (Bruguière), found in slightly damp crevices of stones or piles of old bricks stored on the site. We also gathered these molluscs in a garden, at stems bases and roots of *Portulaca grandiflora*, a widespread ornamental plant.

Acronyms of cited museums

- IRSNB – Institut royal des Sciences naturelles de Belgique, Brussels, Belgium.
- MNHN – Muséum national d'Histoire naturelle, Paris, France.
- MRAC – Musée royal de l'Afrique centrale, Tervuren, Belgium.
- NMWC – National Museum of Wales, Cardiff, United Kingdom.
- USNM – US National Museum of Natural History, Washington, DC, USA.

RESULTS AND DISCUSSION

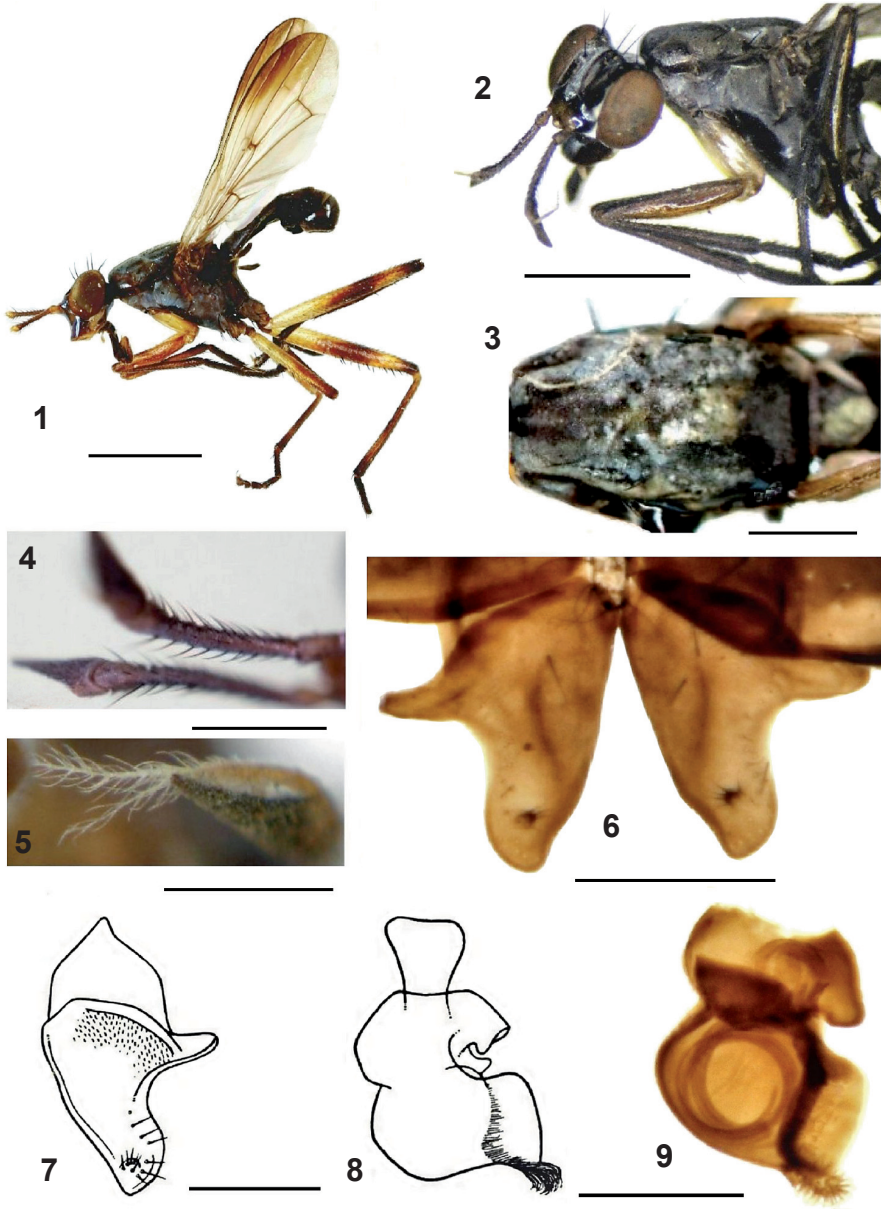
Sepedon (Parasepedon) umbrosa Verbeke, 1950

(Figs 1–9, 12–31)

Sepedon (Parasepedon) umbrosa Verbeke, 1950: 44.

Description: Adult (Figs 1–9). Body colour mainly blue-grey, legs yellowish with black parts (Figs 1–3). *Head*. Frons slightly shiny, dark yellowish in middle, with dark grey edges; black more or less elongated frontal spot, separated from eye by silvery stripe pruinosity extending to upper 1/3 of the front. Face entirely blue-black, silvery, mostly below the antennae. Lunule shiny, yellowish in middle, with blackish edges. Black ocellar triangle with 2 strong setae. Occiput shiny blue-black on its sides. Antennae (Fig. 4): scape (segment I) short, yellowish; pedicel (II) with long strong hairs, postpedicel (III) brownish, apex sharp; arista long plumose (Fig. 5), yellowish at base. Gena typically shiny blackish, narrowly yellow posteroventrally, with shiny blue-black spot between antenna and eye edge.

Thorax. Matte, black; notum with light greyish pruinosity, two light yellow median stripes, and long wide blackish stripe on each side; callus blackish brown, shiny (Fig. 3). Legs predominantly yellowish; front (I) femur dorsally blackened throughout;



Figs 1–9: Adult morphology of *Sepedon (P.) umbrosa*: (1) habitus, holotype male; (2) head and thorax, lateral view; (3) antenna; (4) arista; (5) notum, dorsal view; (6, 7) gonostyli; (8, 9) aedeagus. Scale bars: 1 mm (Figs 1, 2), 0.5 mm (Figs 3, 4), 0.05 mm (Figs 5, 9), 0.25 mm (Fig. 6), 0.1 mm (Figs 7, 8). Figs 1–6, 9, photos of J.-C. Vala, with Figs 6 and 9 based on original slides prepared by Verbeke; Figs 7, 8 from Verbeke (1950: figs 33, 34).

mid (II) femur, mostly apical half blackened; (III) hind femur with large black ring barely separated from black apical ring, 2–3 dorsal setae at apical two-thirds; tibiae I and II brownish, III blackish; tarsi I blackish, II, III brownish, apices of all tarsi yellowish. Wing light brown, frontoapical quarter darker, transverse veins shaded, apices of veins R_4 and R_5 slightly converging.

Abdomen. Dark brown, slightly shiny, faint greyish pruinosity everywhere. Male: tergite 9, gonostyli and aedeagus as in Figs 6–9, with well visible spiraled filament (Fig. 9), a characteristic *Parasepedon* feature.

Distribution: *Sepedon (P.) umbrosa* was described by Verbeke (1950) from specimens collected in the **Democratic Republic of the Congo** (DRC; then Belgian Congo): the male holotype labelled “Wombali, IX.1913 (P. Vanderlijst)” and 4 paratypes: 1♂ 1♀ “Moyen-Kwili, Leverville (P. Vanderlijst)”; 1♂ 1♀ “lac Kivu, N’Gwess (Carlier)” (all types in IRSNB). The specimens were also cited by Verbeke (1963) and Knutson & Vala (2011). Knutson *et al.* (2018: 87) erroneously noted: “Holotype (?): The specimen, examined by Knutson in 1978 in MRAC, is a female labelled “Moyen-Kwili; Leverville, [no date] P. Vanderlijst”, contrary to Verbeke’s (1950: 45) information.

Sepedon (P.) umbrosa was reported from **Côte d’Ivoire**: J.-C. Vala examined in 1987 the following specimens: 1♂ Lamto, Pauck-Quanto Po, 26.xii.1970, D. Lachaise; 1♂ Réserve du Banco, R. Paulian & C. Delamare (both MNHN). **Benin** (Fig. 10): Gbedjissi (2003) captured the species first at Kpassa (1♂ 1♀, 11.ii.1992) close to Okpara riverbank vegetation and at Bassa; then in Cocotomey on July 1999 (3♂ 3♀) in a dry habitat; Agboho searched various localities during her PhD studies and collected the species at Pahou, near Cocotomey. Among the 11 sciomyzid species reported in Benin, Vala *et al.* (1994), Gbedjissi (2003) and Agboho *et al.* (2017) considered *S. (P.) umbrosa* as one of the rarest species, sporadically collected in the country and needing more surveys to refine its distribution. Fortunately, the species is active in both dry and rainy seasons. **Nigeria**: 1♀ Lagos, Ojo, 11.viii.1974, M.A. Cornes (USNM), noted by Knutson & Vala (2011) and Knutson *et al.* (2018).

Population dynamic of Sepedon (P.) umbrosa in Benin

The population variations (Fig. 11) is based on the monthly catch of adults at Pahou in swampy areas from July 2014 to June 2015. We have not included the eight adults collected by Gbedjissi in other localities.

It appears that the species is present all year round, but at very variable levels. The maximum number of individuals has been captured from July to November, then their abundance declines from February (1) and March (0) to June (2). Low figures probably reflect the low level of the population or seasonal changes in the studied area. However, in previous years only a total of 1–6 specimens were seen at the same period. In comparison, Verbeke (1963: 66, table II) identified 301 specimens of this species collected in Garamba National Park (DRC) by H.G. De Saeger, from December 1949 to September 1952 (Fig. 11). It appears that the population

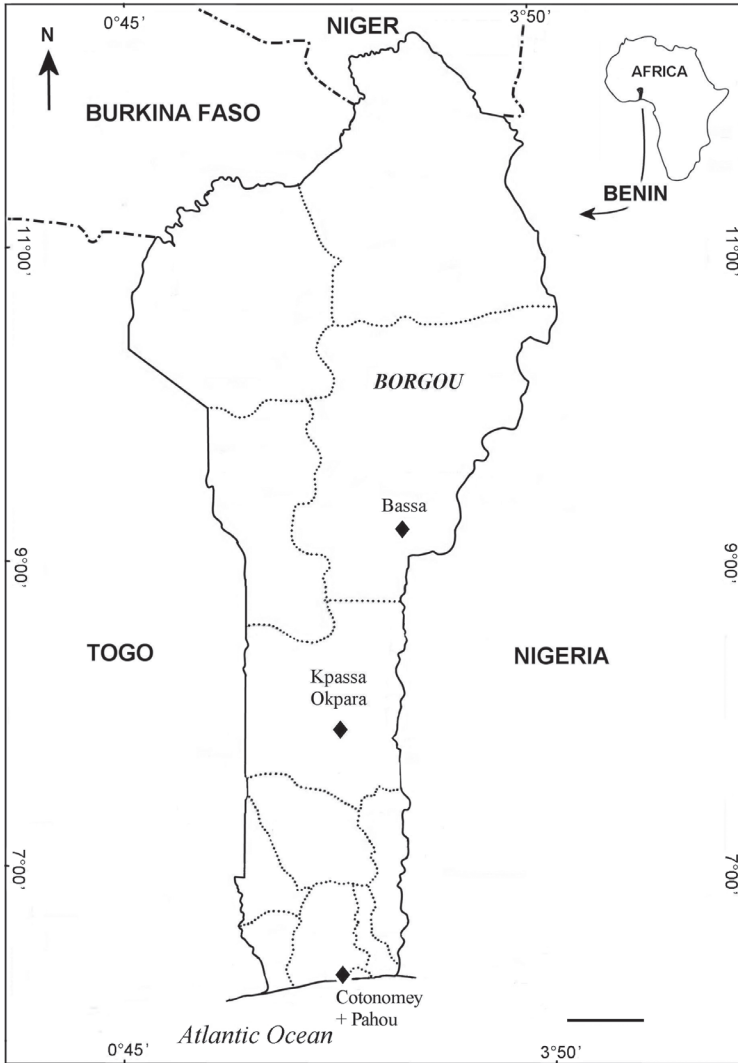


Fig. 10: Current locations known for collecting *Sepedon (P.) umbrosa* in Benin. Scale bar = 100 km.

dynamics of *S. (P.) umbrosa* observed in Benin and the DRC follow similar profiles. The maximum number of individuals is found from July to November and declines thereafter, with the minimum in April–June. Verbeke (1963: 68) wrote “It is regrettable that we do not provide any data concerning the malacological fauna of the diverse aquatic and semi-aquatic biotopes prospected ... and presumed to be abundantly populated by gastropods.”

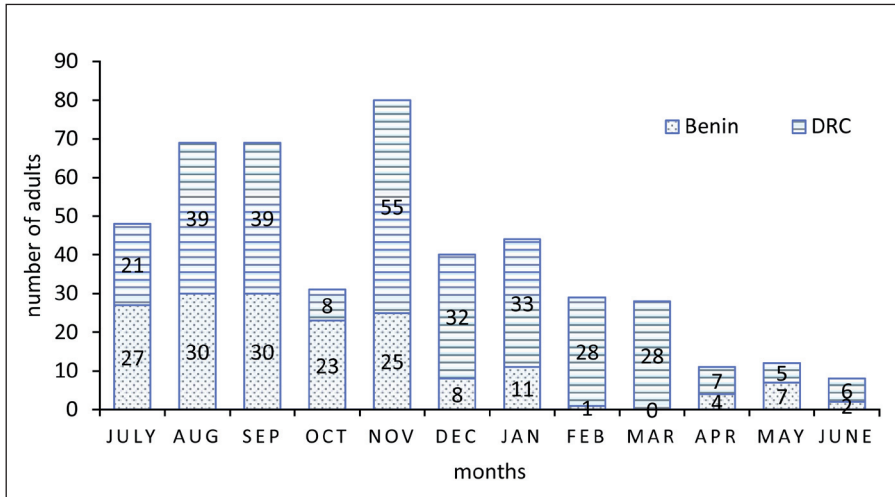
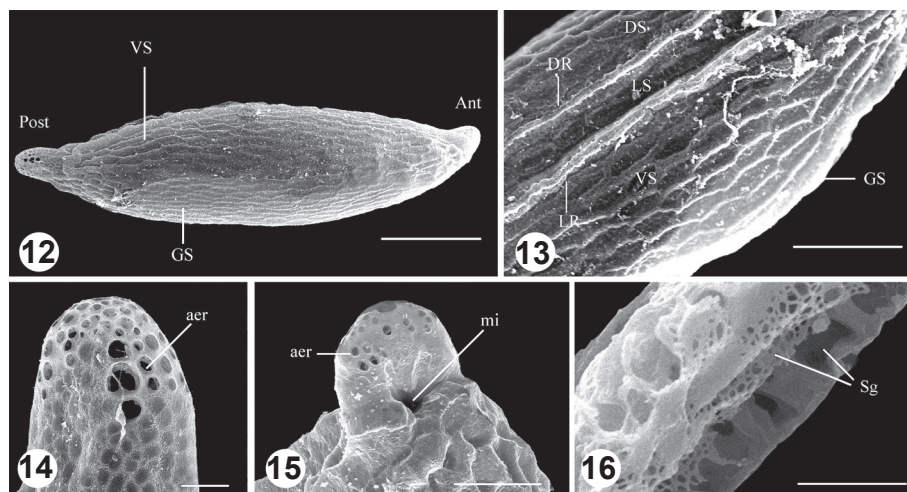


Fig. 11: Comparison between population dynamics of *Sepedon (P.) umbrosa* adults, observed in southern Benin at Pahou from July 2014 to July 2015 in our study, and data reported by Verbeke (1963) in Garamba National Park (DRC) from December 1949 to September 1952.

Immature stages

Egg (Figs 12–16). Fusiform. Length 1.00–1.17 mm, width 0.26–0.30 mm (Fig. 12). White when freshly laid, turning yellowish to greyish thereafter. Chorion reticulated, surface showing contiguous hexagonal structures and two pairs of longitudinal ridges, one dorsal (DR) and one sub-lateral (LR) (Fig. 13) separating the egg surface in four distinct areas (Figs 12, 13): a dorsal surface (DS), slightly convex, bordered by the two dorsal longitudinal ridges; a narrow lateral surface (LS) weakly spaced (0.03 mm) demarcating the dorsal and the sub-lateral ridges on each egg edge; and a large ventral surface (VS), between the two sublateral ridges, less convex, basally covered with an adhesive gelatinous glue substance (GS). Extremities rounded; the posterior (Fig. 12, Post) very prominent, dorsally upturned, punctured by several round aeropyles (Fig. 14, aer) of varying diameters, sometimes obliterated; the anterior (Fig. 12, Ant), shorter, also with many aeropyles (Fig. 15), a wide subventral micropyle (mi) dorsally surrounded by an extended arched lip. The cross section of the chorion (Fig. 16) shows the gallery system, connected with the aeropyles, bringing oxygen necessary for embryonic development.

Larvae (Figs 17–23). As for all sciomyzids, there are three larval instars (L1, L2, and L3). Body (Fig. 17) subcylindrical, subdivided in three parts: (a) head or cephalic segment (Figs 17, Hd, 18), apically bilobed, each cephalic lobe (Fig. 19, CL) showing one antennal organ (AO) and one more or less round maxillary organ (MO) with different sensillae, mouth ventrally positioned with its posterior border bearing a large postoral spinule band well visible when the larva is extended, but on the illustrations retracted into the 1st thoracic segment (Figs 18, 19); (b) thorax

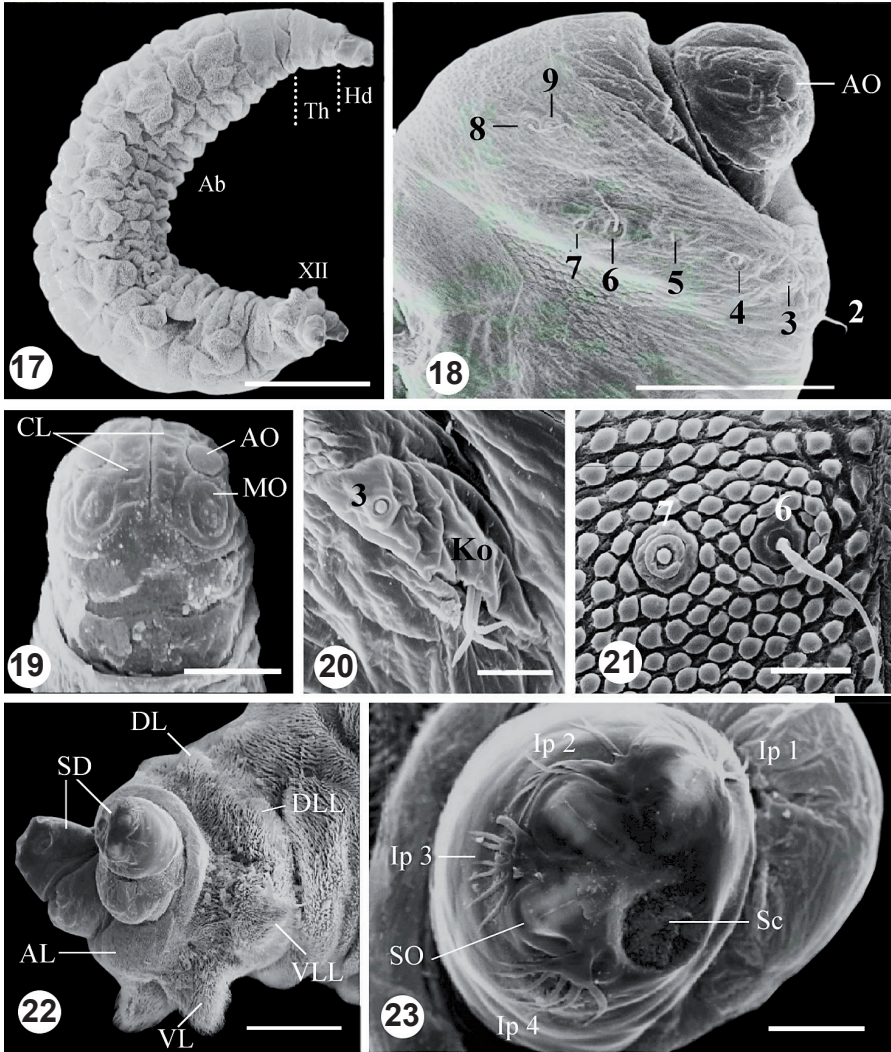


Figs 12–16: Egg of *Sepedon (P.) umbrosa*: (12) entire egg, dorsolateral view; (13) egg portion, dorsoventral view; (14) posterior extremity; (15) anterior extremity; (16) transversal section of chorion. Abbreviations: aer – aeropyle, ant – anterior extremity, DR – dorsal ridge, DS – dorsal surface, GS – gelatinous glue substance, LR – sublateral ridge, LS – lateral surface, mi – micropyle, post – posterior extremity, Sg – section gallery, VS – ventral surface. Scale bars: 200 μm (Fig. 12), 20 μm (Figs 13–15) and 5 μm (Fig. 16).

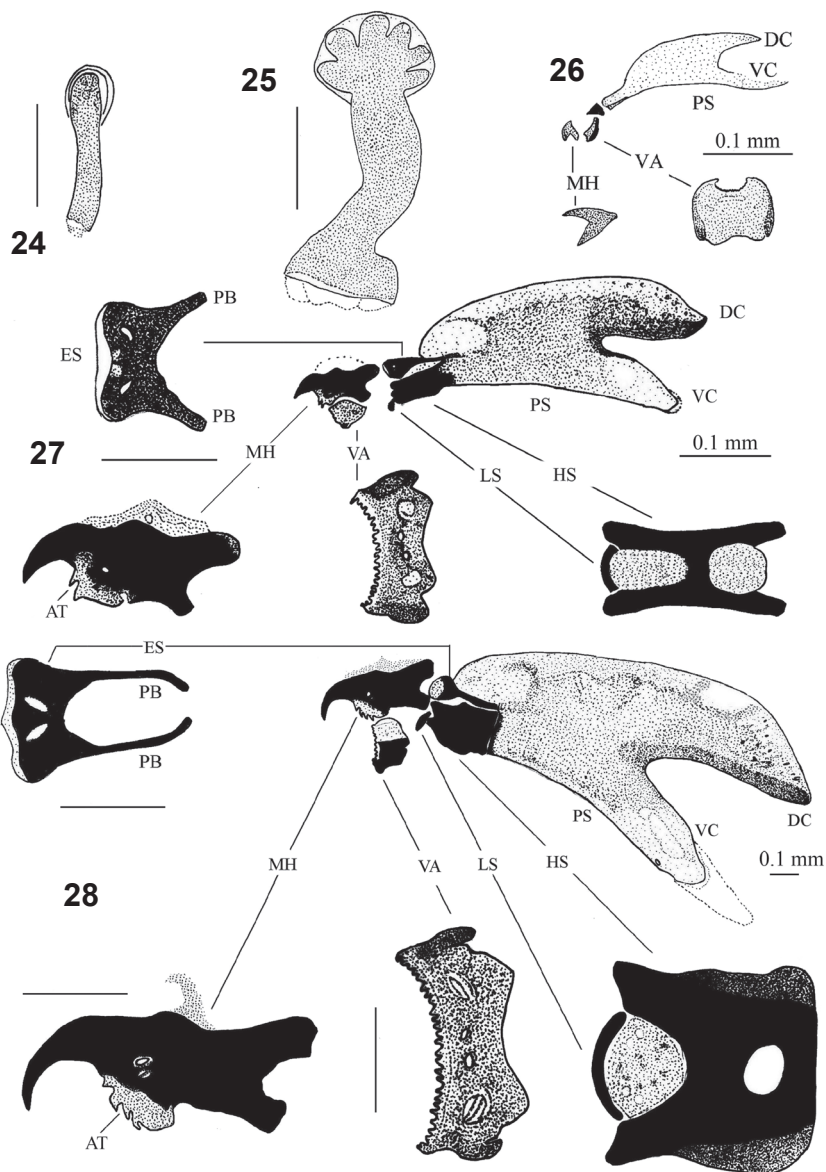
with 3 segments, each characterized by presence of one pair of ventral Keilin organs (Fig. 20, Ko), discernable by their 3 sensillae emerging from a single pit; other visible thoracic sensillae are of coeloconicum (without seta, numbers 3, 7, 9 on Figs 18, 20, 21) or trichodeum type (with seta, numbers 4, 5, 6, 8 on Figs 18, 21); and (c) abdomen (8 segments) without dorsal swimming tufts; last segment (Fig. 22), posterior disc with 4 pairs of peripheral lobes and 2 spiracular discs (Fig. 23). The morphological differences between the larval stages are shown below and described in detail for the second and third instars. In particular, only the first stadium do not have anterior spiracles (Figs 24, 25) and the stigmatic scar on the posterior disc of the last larval segment. Features of segment XII are detailed for the L2 and L3 only.

First instar larva (L1) (Fig. 26). Length 0.98–1.36 mm, greatest width 0.23–0.30 mm. Body subcylindrical, whitish, with transparent integument. Cephalopharyngeal skeleton (Fig. 26): length 0.22–0.25 mm, anterior part more sclerotized than posterior; mouthhook (MH), tiny, V-shaped, with no accessory teeth; ventral arch (VA) bilobed, lateral edges more sclerotized, anterior margin finely denticulate; hypostomal sclerite fused with pharyngeal sclerite; pharyngeal sclerite (PS) brown clear, dorsal cornu (DC) posteriorly pointed, ventral cornu (VC) large, posterior end continues as a light zone.

Second instar larva (L2) (Figs 24, 27). Length 2.0–3.0 mm, greatest width 0.45–0.71 mm. Body slightly greyish white, with transparent tegument. Anterior



Figs 17–23: Larval morphology of *Sepedon (P.) umbrosa*, based on L3: (17) entire larva, lateral view; (18) head and first thoracic segment, lateral view; (19) head, ventral view; (20) thoracic sensillae Keillin organ (#2) and coeloconic (#3); (21) thoracic sensillae #6 and #7; (22) last larval segment, abdominal XII, lateral view; (23) details of a spiracular disc. Scale bars: 1.00 mm (Fig. 17), 18 μ m (Fig. 18), 20 μ m (Figs 19–21), 200 μ m (Fig. 22) and 50 μ m (Fig. 23). Abbreviations: Ab – abdomen, AL – anal lobe, AO – antennal organ, CL – cephalic lobe, DL – dorsal lobe, DLL – dorsolateral lobe, Hd – head, Ip1–Ip4 – interspiracular processes, Ko – Keillin organ, MO – maxillary organ, Sc – stigmatic scar, SD – spiracular disc, SO – spiracle opening, Th – thorax, VL – ventral lobe, VLL – ventrolateral lobe, XII – last abdominal segment – spiracular disc, 2–9 – thoracic sensillae 2–9.

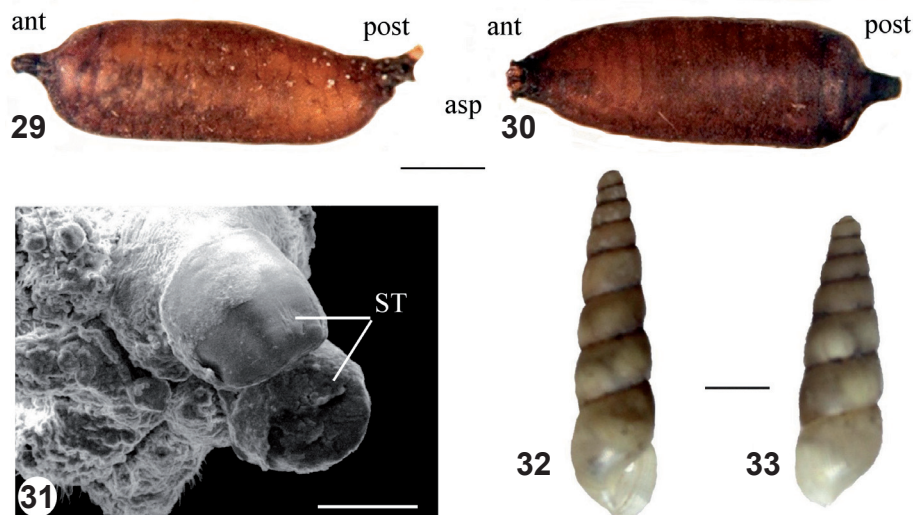


Figs 24–28: Larval morphology of *Sepedon (P.) umbrosa*: (24, 25) anterior spiracle: (24) second-instar larva; (25) third instar-larva; (26–28) the complete cephalopharyngeal skeletons with their different parts: (26) first-instar larva; (27) second-instar larva; (28) third-instar larva. Abbreviations: AT – accessory teeth, DC – dorsal cornua, ES – epistomal sclerite, HS – hypostomal sclerite, LS – lingual sclerite, MH – mouth hook, PB – parastomal bar, PS – pharyngeal sclerite, VA – ventral arch, VC – ventral cornua. Scale bars: 0.05 mm, unless indicated otherwise.

spiracle small, with 6 small papillae (Fig. 24). Cephalopharyngeal skeleton well sclerotized (Fig. 27), length 0.41–0.47 mm, light to dark brown. Mouthhook (MH) with 1 strong curved hook, pointed apex, 3 (rarely 2) accessory pointed curved teeth (AT), situated relatively far from hook base; posteriorly with 1 long dorsal and 1 shorter ventral projection. Epistomal sclerite (ES) bilobed, with 2 circular subcentral foramens fused to parastomal bars (PB). Pharyngeal sclerite (PS): dorsal cornu long, pointed (DC); ventral cornu (VC) short, clear. Ventral arch (VA) bilobed, anterior margin with 20 teeth, two foramens in the middle, lateral edges blackish, slightly discarded. Hypostomal sclerite (HS) H-shaped, fused to basal anterior part of pharyngeal sclerite. Lingual sclerite (LS) arched, inserted between hypostomal anterior arms. Segment XII (Figs 22, 23): posterior spiracular disc with 4 peripheral pairs of lateral lobes: 1 slightly conical ventral pair (VL); 1 more or less similar ventrolateral pair (VLL); 1 slightly high laterodorsal pair (DLL); 1 similar dorsal pair (DL); 2 protuberant stigmatic tubes (ST), each ending into 1 stigmatic plate. Each stigmatic plate with 1 rounded scar left by stigma of L1 (Sc), 4 short, branched tufts of interspiracular processes (Ip1–Ip4) (or float hair), and 1 elongated spiracular openings (SO). Ventrally, the last segment shows 1 weakly prominent pre-anal lobe (Fig. 22, AL).

Third instar larva (L3) (Figs 25, 28). Morphology similar to that of second instar larva. Length 6.57–9.14 mm, greatest width 1.28–1.71 mm. Initially transparent dark-grey to light grey integument at end of the stage. Anterior stigma sclerotized, with 6 wide papillae (Fig. 25). Cephalopharyngeal skeleton (Fig. 28): length 0.65–0.77 mm. Indentation index 38.88–44.23. Mouthhook (MH) dark, dorsal side raised, with strong curved hook, 4 curved conical accessory teeth (AT), 1 foramen, posterior part with elongated broad projection and thinner and shorter ventral projection. Epistomal sclerite (ES) bilobed, each lobe with 1–2 central foramens, posteriorly fused to parabasals bars (PB). Pharyngeal sclerite (PS) strongly sclerotized, dorsal cornu (DC) slightly pointed without window, ventral cornu (VC) with 1 small round hyaline window. Ventral arch (VA) bilobed, anterior margin with 20–26 teeth, 2 circular foramens, and thickened lateral edges slightly folded inwards. Hypostomal sclerite (HS): anterior edges well sclerotized, median part clear, posteriorly massive with round foramen, not fused to pharyngeal sclerite. Lingual sclerite (LS) inserted between anterior hypostomal arms, arched, just connected to hypostomal sclerite by lateral extremities. Segment XII similar to that of L2 (Figs 22, 23), except being larger, stigmatic scars result here from stigmatic openings in L2.

Pupa (Figs 29–31). Length 5.75 ± 0.37 mm, greatest width 1.73 ± 0.17 mm. Subcylindrical, matt or iridescent, not very transparent; dorsal face slightly convex and ventral face relatively straight (Fig. 29); streaked transversely, light brown to dark brown as on the dorsal view (Fig. 30). Anterior end (ant) abruptly narrowed, extending forward the body; laterally the two larval anterior spiracles (asp) visible (Fig. 30). Posterior extremity less tapered, barely exceeding to dorsal surface (Fig. 29), both prominent posterior stigmatic tubes (Fig. 31, ST). Only VL and VLL lobes of L3 are visible. Exceptionally, the pupae form inside the mollusc shell. The pupae



Figs 29–33: Pupa of *Sepedon (P.) umbrosa* (29–31) and snail prey (32, 33): (29) pupa lateral view; (30) pupa dorsal view; (31) pupa posterior end showing 2 sclerified stigmatic tubes; (32) *Subulina octona* snail, ventral (32) and dorsal (33) views. Abbreviations: ant – anterior end, asp – anterior spiracle, post – posterior end, ST – stigmatic tube. Scale bars: 1.1 mm (Figs 29, 30), 100 μ m (Fig. 31) and 3 mm (Figs 32, 33).

form outside or sometimes inside of the mollusc prey *Subulina octona* (Figs 32, 33). The description is based on 38 specimens.

Duration of immature stages and biology

The duration of the complete immature life cycle of *S. (P.) umbrosa* is 3 to 5 weeks (Fig. 34). The whole larval phase (L1–L3) lasts an average of 11.38 ± 1.76 (S.D.) days ($n=63$). The L2 stage appears to be the shortest with 2.24 ± 0.42 days and the pupal stage the longest with 8.07 ± 0.99 days.

In an additional experiment, we isolated in a breeding box one pair of wild adults captured at Cocotomey on July 7, 1999, considered as the initial generation (G0). Then, we studied the offspring as is described in the methods by following the laying and the larval development of the three successive generations (G1–G3). So, each female was isolated with a male of the same provenance. We used three females from the first generation (G1), one female from the second generation (G2) and one female from the third generation (G3). Mating begins and lasts several hours without being disturbed by human presence. For each female we give the life duration, number of ovipositions, total of eggs laid and immature stages successfully obtained. The results allow us to deduce the losses throughout larval development and the emerged male-female ratio from the pupal stage until the specimens died (Table 1). This table shows that the 3 females of the first generation (G1) lived longer (6 to 13 weeks) and laid more eggs than the females of the following generations (G2 and G3). In

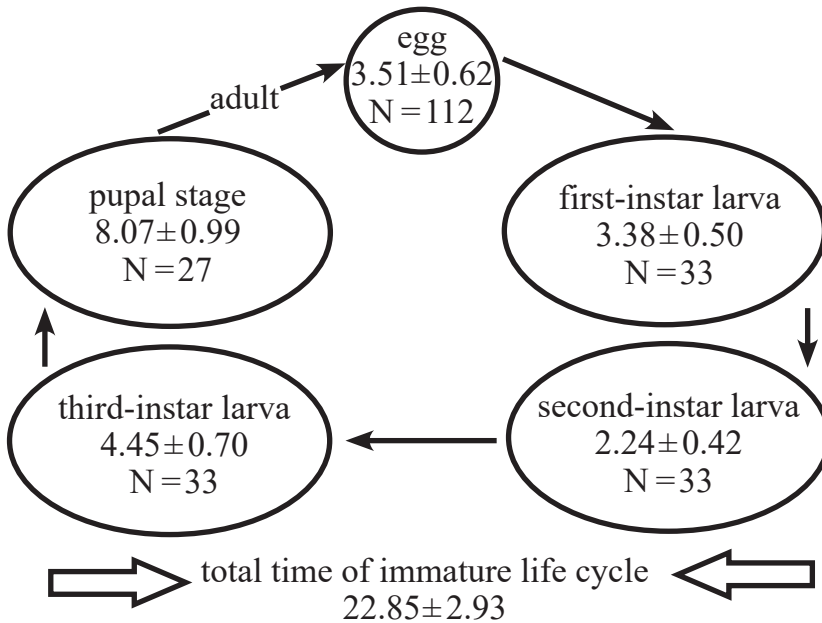


Fig. 34: *Sepedon (P.) umbrosa*, duration of the life-cycle and the development of immature stages, in days \pm S.D.

contrast, the average number of eggs laid per female is higher for females G2 and G3, even though their life-cycle is shorter, only 2 to 4 weeks. For this experiment, the preoviposition duration fluctuates from 3 to 8 days.

Larval feeding behaviour

Sepedon (P.) umbrosa larvae cannot swim when positioned in water. They have very short interspiracular processes (or float hairs). Considering this characteristic, the species would be ecologically placed among the intermediate terrestrial or semiaquatic-terrestrial larvae following classification by Vala and Gasc (1990). Despite this, we tried to grow them with the freshwater snails *Lymnaea natalensis*, *Bulinus globosus*, the small freshwater oligochaete *Aulophorus furcatus*, and even the semiterrestrial snail *Succinea* sp. that live on nearby freshwater plants. All the prey was ignored by the larvae. We also tested the terrestrial gastropod *Subulina octona* as prey (Figs 32, 33). The larvae attacked it immediately and larval development proceeded to adult emergence. In all cases, two distinct behaviours emerge from observing 32 L1 larvae tested in the presence of *Subulina octona*.

(a) Larvae, vigorously attack the mollusc at the edge of the mantle, occasionally near the pneumostome. The posterior disc always stays outside the shell without penetrating the prey. This is the case for 14 of the 32 L1 (43.75 %). Molluscs move normally with the larvae for several days. The L1 larvae spend 6–7 days in this first mollusc and moult to the second stage L2.

Table 1. Data on isogenic development of *Sepedon (P.) umbrosa* from a wild couple (G0) captured in Benin at Pahou and its successive generations (G1–G3).

| Tested female | Life-time duration | Number of laid eggs | Number of ovipositions | Mean eggs/oviposition | Number of emerged adults | |
|----------------|--------------------|---------------------|------------------------|-----------------------|--------------------------|---------|
| | | | | | Males | Females |
| Wild female G0 | 22 | 38 | 9 | 4.2 | 5 | 3 |
| Female 1. G1 | 87 | 73 | 21 | 3.4 | 4 | 3 |
| Female 2. G1 | 67 | 110 | 27 | 4.0 | 3 | 2 |
| Female 3. G1 | 41 | 73 | 15 | 4.8 | 6 | 2 |
| Female 1. G2 | 29 | 44 | 8 | 5.5 | 3 | 3 |
| Female 1. G3 | 15 | 47 | 7 | 6.7 | 2 | 1 |

(b) Some L1 larvae (18 L1, or 56.25 %) attack the mollusc by penetrating directly the mouth of the mollusc. The larvae force their way into the oral cavity even if the mollusc struggles to escape. Once installed, the larvae remain between the mouth and the oesophagus of the mollusc, their posterior disc still emerging outside. In this case, the mollusc can move with the larva, but does not feed. The larva seems to enter the mouth of the mollusc, but rather sinks into the orifice of the salivary glands, thus avoiding being crushed by the radula of the prey. The diet of the mollusc is thus hampered by the presence of the larva.

During this “intimate connection”, the prey squirms, increases mucus secretion to reject the larva, but these attempts remain unsuccessful. Sporadically, the larvae can leave the prey for a few seconds but can easily settle back in the mouth of the mollusc. The larvae stay in the prey for 5 to 7 days, which provides both a protective microhabitat by its shell and the food necessary for their growth, evidenced by the increase of their size. In all cases, the mollusc stops all displacement, retreats into the shell and eventually dies. The larvae consumed the molluscs partially or completely even under conditions where the mollusc tissues were in a liquefied state. A large number of protozoa evolve in the putrid liquid without harming the sciomyzid larvae that continue to feed and moult outside or inside the mollusc shell. If the snail is completely consumed, the larvae attack another live one.

First instar larvae attack small snails (≤ 3 mm) with ease particularly those up to 1.5 mm long. The bigger snails (10 to 15 mm) are consumed totally or partially. If the attack period occurs during mollusc reproduction, the snail eggs are an obstacle to the larval penetration. Indeed, the accumulated eggs inside the shell form a “hard” barrier almost impenetrable to the larvae and progression inside the shell is difficult when eggs are present. Naturally dead or rotting molluscs are not consumed. Several larvae, up to 3, can attack the same mollusc simultaneously at the mantle level or enter the mouth of the prey. During these multiple attacks, the mollusc remains alive for 2 to 3 days but sometimes up to 5 days. In the laboratory, it is exceptionally rare to see a mollusc moving with one L3 larva. The number of prey consumed

during the three larval stages does not exceed three molluscs. Larvae entering the mouth consume only 1 or 2 prey. Despite the general success of the attacks, some molluscs succeed in swallowing larvae. This happens when the larva is positioned upside down in the oesophagus of the prey, i.e. the posterior disk directed towards the stomach and the head to the mouth of the prey. It remains motionless and ends up being digested by the attacked prey.

DISCUSSION

Our results are the first data on the biology and immature stages of *Sepedon (P.) umbrosa*. The eggs are laid singly, glued on the support by the ventral surface. According to Gasc *et al.* (1984), the presence or absence of perforations on the egg poles would be of ecological significance: the aquatic types have many aeropyles on both egg poles while terrestrial types have aeropyles only on the posterior egg pole. This distinction is exact for eggs of all sciomyzid studied to date. Unusually, the egg of *S. (P.) umbrosa* has several aeropyles at both poles although the larvae are the terrestrial type. These results differ from the classification given by Gasc *et al.* (1984) and probably indicate an apomorphic feeding behaviour for larvae of this species compared to the majority of *Sepedon*, which have a plesiomorphic diet. Likewise, Vala and Gasc (1990) recognized four ecological types of sciomyzid larvae according to the morphology of the posterior disk: (1) strictly aquatic (long peripheral lobes and interspiracular processes); (2) semi-aquatic (these two features moderately developed); (3) semi-terrestrial (the two features very reduced); (4) terrestrial (short spiracular lobes, interspiracular processes scale shape). Regarding *S. (P.) umbrosa*, the posterior disk with very small interspiracular processes and short peripheral lobes are consistent with the inclusion of this species in category 3. Two aspects support this: (a) the larval feeding behaviour type, i.e. parasitoid/predator of terrestrial snails; (b) the consumed prey living predominantly in slightly humid, terrestrial environments. Could it be a migration of a species with aquatic development which then evolved towards a terrestrial development while preserving these ancestral attributes?

Among the sciomyzids, Trelka and Foote (1970), report that larvae L1–L3 of *Tetanocera clara* and L3 of *T. valida* (two Nearctic species) enter by force into the oral cavity of their prey (slugs) and remain there for a few hours before killing and consuming them. The same scenario occurs with a few *S. (P.) umbrosa* larvae, which remain alive for at least approx. 3 days before killing their prey. In view of African Sciomyzidae studied, two other species show a larval parasitoid behaviour: (1) *Sepedon hispanica hispanica* whose first and young second instar larvae live mainly in the *Succinea* spp. and remain between the mantle and the shell (Vala 1989); but the aged second and third instar larvae (L2, L3) become real predators of *Planorbis* and other freshwater snails; (2) *Sepedon (P.) trichrooscelis*, whose larvae are specifically parasitoids of *Succinea* (as the American *S. campestris* Say reported by Knutson *et al.* (1967) and one African *Succinea* sp. collected in Benin by Vala *et*

al. (1995) to fulfil its life cycle). As briefly reported by Miller (1995), the larvae of *Salticella stuckenbergi* Verbeke, 1962 may have a saprophagous feeding behaviour on dead terrestrial snail *Archachatina marginata* (Swainson), at least during the end the larval development. Future biological studies on Afrotropical Sciomyzidae life-cycles may still surprise us, mainly by variations in the larval feeding behaviour as observed for those of *Sepedon (P.) ruficeps*, which may consume both small freshwater molluscs and tiny freshwater oligochaetes.

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